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Oryza Ceramide®, a rice-derived extract consisting of glucosylceramides and β-sitosterol glucoside, improves facial skin dehydration in Japanese subjects

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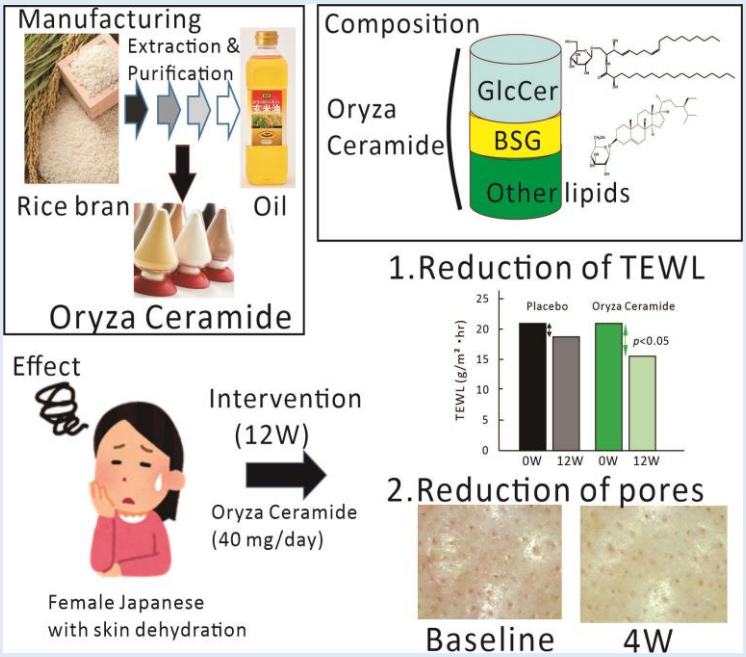
ABSTRACT

Background and objective: The ingestion of plant-derived glucosylceramides (GlcCer) has been reported to contribute to skin barrier function and hydration of the epidermis. β-sitosterol glucoside (BSG) colocalized with GlcCer in the rice hydrophobic fraction has been shown to increase ceramides in the stratum corneum *in vitro*. Although clinical studies demonstrated that GlcCer reduced transepidermal water loss (TEWL), the contribution of BSG to epidermal dehydration when applied with GlcCer remains unknown. Therefore, we herein conducted a clinical trial on the effects of a rice-derived mixed fraction of GlcCer and BSG (Oryza Ceramide®) on TEWL and other skin parameters.

Methods: A randomized, double-blind, placebo-controlled study design was used. Oryza Ceramide® (type PCD, 40 mg daily) containing 1.2 mg of GlcCer and 40 μg of BSG was used as the active sample. We enrolled 44 healthy Japanese women with epidermal dehydration. All subjects were randomly allocated to an active group (n=22) or placebo group (n=22) using a computerized random number generator. Capsules containing the active sample or placebo were administered for 12 weeks between August and December 2020. Cheek TEWL after 12 weeks was assessed as the primary outcome, and TEWL on a different part of the skin and various skin parameters, including epidermal moisture, pigmentation, pores, and elasticity, were measured before and after 4, 8, and 12 weeks of the intervention. Blood, urine, and body parameters were also examined to evaluate safety.



Results: Forty-four subjects completed the trial, and the per protocol set comprised 22 each in the active and placebo groups. Cheek TEWL significantly reduced after the Oryza Ceramide® intervention for 4 and 12 weeks. Among the secondary outcomes examined, lip moisture (12 weeks) and visible pore number (4 weeks) were improved by Oryza Ceramide®. Laboratory tests revealed no abnormalities to suggest any adverse effects of Oryza Ceramide®.

Conclusions: Oryza Ceramide® (40 mg/day) consisting of GlcCer and BSG improved facial TEWL, lip moisture, and visible pores, and these effects may be attributed to increases in epidermal ceramides. The combination of rice GlcCer and BSG appears to be beneficial for improving facial skin conditions.

Trial Registration: UMIN-CTR: UMIN000041295

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Keywords: rice; glucosylceramide; β-sitosterol glucoside; trans epidermal water loss; pore

INTRODUCTION

Glucosylceramides (GlcCer) derived from edible plant resources have been used as dietary supplements to promote skin hydration and barrier function [1]. Rice, wheat, pineapple, konjac potato, peach, beet, corn, and golden oyster mushroom are sources of GlcCer [2-9] and the composition of GlcCer differs among plants [10-11]. GlcCer is decomposed into glucose and a ceramide in intestinal mucosa and then transferred from the intestines through lipid-absorbing pathways and ceramides into the veins via the lymph ducts [8,12]. Ceramides reach the epidermis and exert moisturizing and barrier functions [2,13]. Recent studies revealed that GlcCer is directly absorbed from the intestines without glycolytic digestion and reaches the lymph nodes [5,14]. The findings of clinical studies indicate the potential of rice-derived GlcCer to reduce transepidermal water loss (TEWL) [15-16]. Increases in epidermal ceramide production may be one of the mechanisms by which GlcCer supplementation prevents epidermal dehydration [13].

We recently focused on β-sitosterol glucoside (BSG) [17], which is present with GlcCer in the rice GlcCer fraction. BSG is a sterol glucoside that is widely distributed in many plants and has been reported to exhibit analgesic [18], estrogenic [19], apoptogenic [20], and anti-hyperglycemic [21] activities. However, the skin-related activities of BSG currently remain unclear. BSG may contribute to epidermal hydration similar to GlcCer based on following predictions. Firstly, the sterol structure of BSG is similar to that of cholesterol, which is the second most dominant epidermal lipid in the stratum (s.) corneum. Therefore, BSG may be digested and absorbed from the gut and alter epidermal hydration similar to cholesterol and ceramides. Secondly, we recently demonstrated that BSG increased ceramides in the s. corneum of a human epidermal equivalent by enhancing the *de novo* ceramide synthesis pathway [22]. Based on these findings about dietary supplemented GlcCer and an *in vitro* study of BSG, we hypothesized that the combination of GlcCer and BSG is an appropriate composition for effectively improving epidermal dehydration. Our commercially available product (Oryza Ceramide®) contains rice-derived GlcCer and BSG, and this formulation appears to be appropriate for assessing the effects of GlcCer and BSG. Therefore, we herein conducted a clinical trial using healthy Japanese adults to investigate the effects of Oryza Ceramide® on TEWL and other skin parameters.

MATERIALS AND METHODS

Quantification and identification of GlcCer and BSG in Oryza Ceramide®: Oryza ceramide®-PCD (Lot. B-006) manufactured by Oryza Oil & Fat Chemical Co., Ltd. was used in the study. It is composed of 90% rice bran extract and 10% cyclodextrin. Total GlcCer and BSG contents were 3.0 and 0.01%, respectively, as confirmed by HPLC equipped with an evaporative light scattering detector (ELSD LT-II, Shimadzu, Japan) and ordinal phase HPLC column (Inertsil SIL 100-5, 4.6 mm *i.d.*×250 mm, GL Science Inc., Japan). Solvents were chloroform (solvent A) and 95% MeOH (solvent B) and the ratio of solvents A and B chronologically changed, namely, 1 to 25% solvent B from 0 to 15 min, 25 to 90% solvent B from 15 to 20 min, 90 to 100% solvent B from 20 to 21 min, and 100% solvent B from 21 to 26 min. The injection volume was 20 μL and the flow rate was 1 mL/min. The gain setting of ELSD was 6 and N_2 gas pressure was 350 kPa. A rice GlcCer mixture standard (NS170102) was purchased from Nagara Science (Japan) and BSG was prepared in our laboratory. Standard samples and Oryza Ceramide®-PCD were dissolved in a mixture of chloroform and MeOH (2:1). 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanol amine dissolved in a mixture of chloroform and MeOH (2:1) was added to each solution as an internal standard. The HPLC chromatogram obtained is shown in Figure 1.

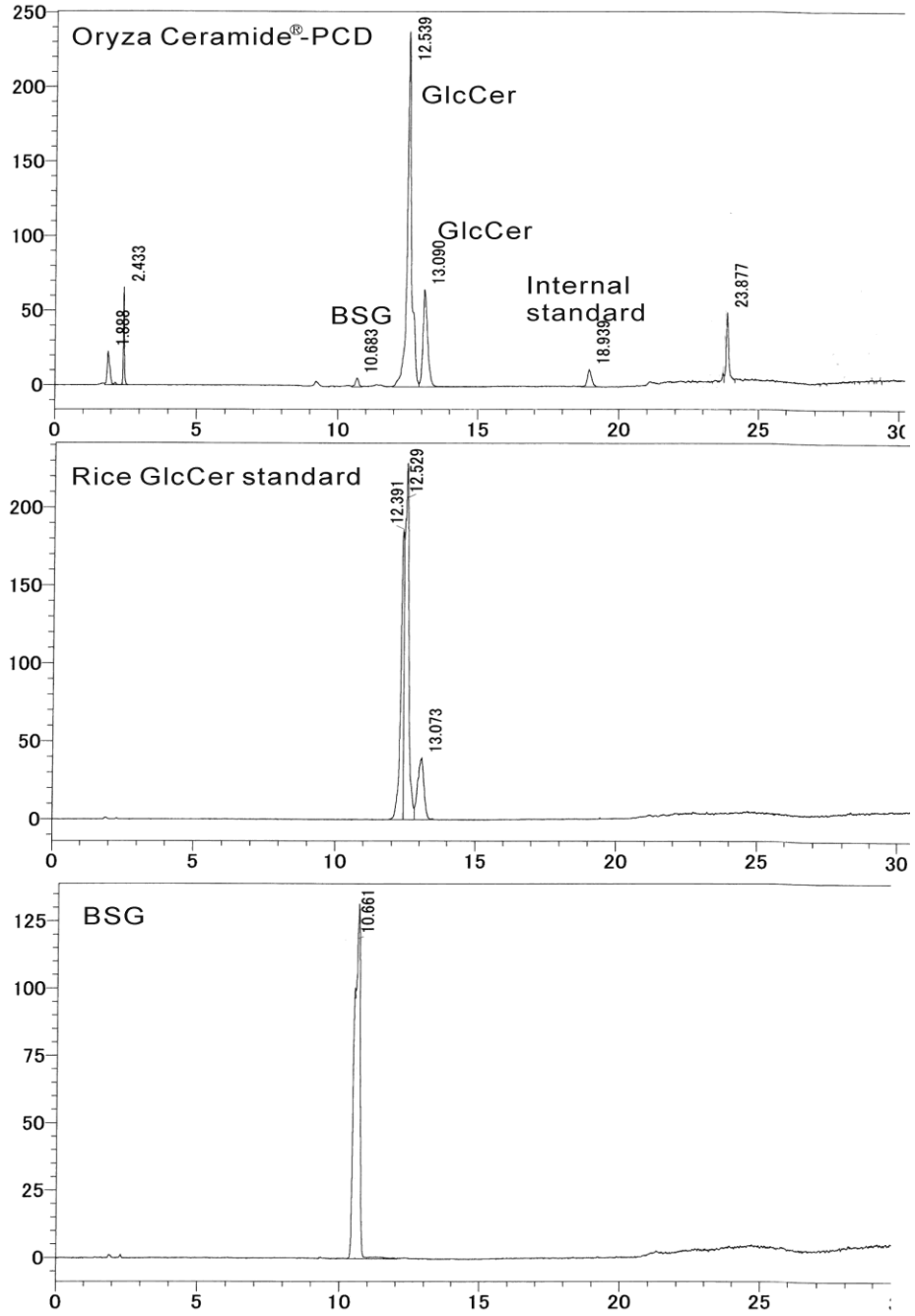

Figure 1. Silica gel HPLC chromatogram of Oryza ceramide®-PCD, the rice GlcCer standard, and BSG. Each sample was dissolved in MeOH and detected by ELSD.

To identify each GlcCer in Oryza Ceramide®-PCD, we used a previous HPLC-ELSD system equipped with an ODS column (Capcell pak C18 SG-120, 4.6 mm *i.d.*×250 mm, Osaka Soda, Japan) and the isocratic mode with MeOH was employed. Each GlcCer standard was purified and isolated from the intermediate product of Oryza Ceramide® and identified based on comparisons of NMR and MS spectra with referenced values [23-30]. HPLC charts of Oryza Ceramide®-PCD and each GlcCer are shown in Figure 2 with the chemical structures of GlcCer species.

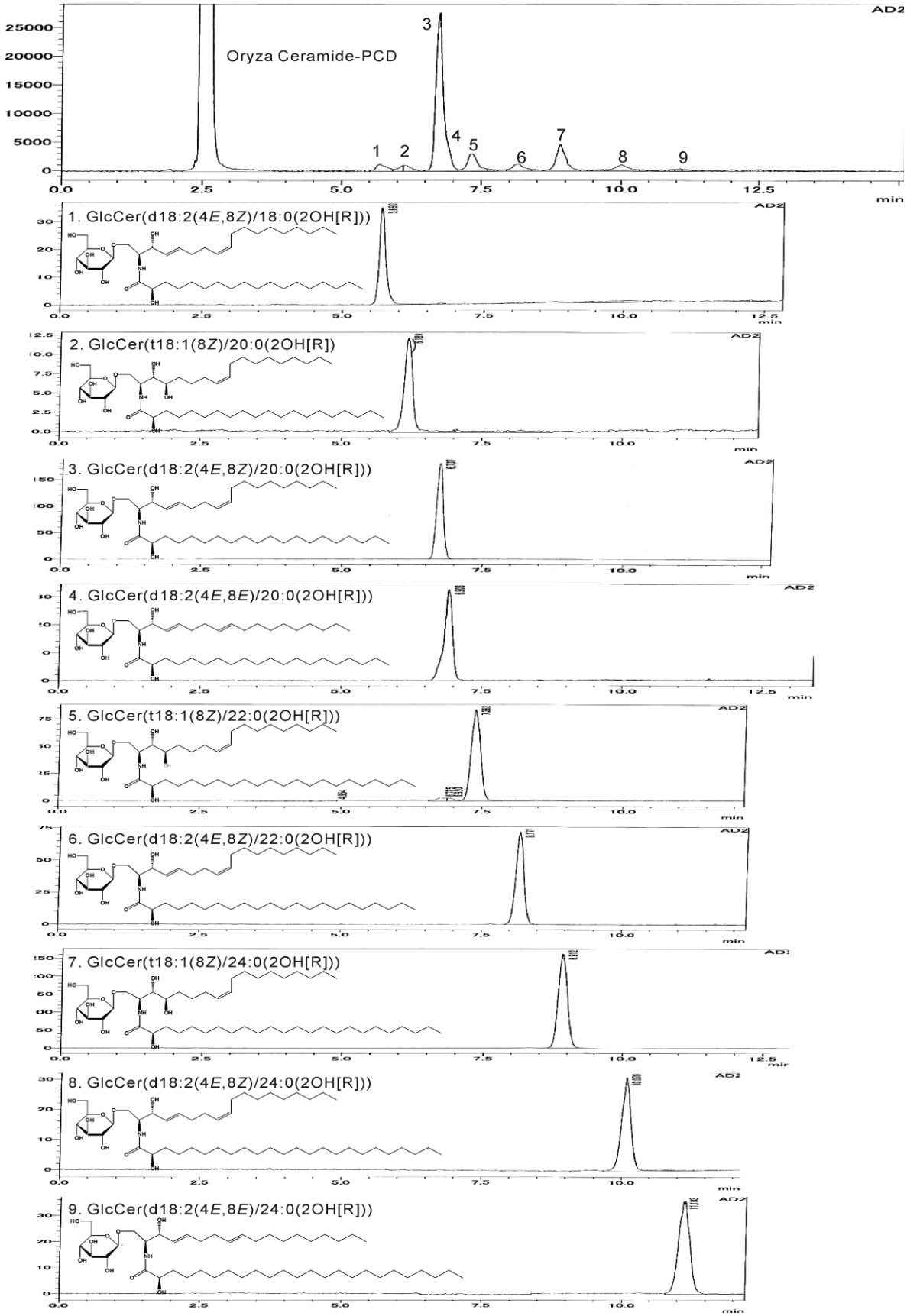

Figure 2. ODS HPLC chromatogram of Oryza ceramide®-PCD and GlcCer species.

Each sample was dissolved in MeOH and detected by ELSD.

Subjects and grouping: All subjects were recruited between August 4 and August 22, 2020 through the Go106 website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The number of subjects assigned was the maximum number of attendees within our budget, as supported by the research subsidy. Inclusion criteria were healthy Japanese female adults (20 years or older) with concerns about skin dehydration. Exclusion criteria were as follows:

1. Current or previous cancer, heart failure, or myocardial infarction.
2. Subjects with a cardiac pacemaker or implantable cardioverter defibrillator
3. Current treatment for arrhythmia, hepatitis, nephritis, rheumatoid arthritis, cerebrovascular disease, diabetes, hyperlipidemia, hypertension, or other chronic diseases.
4. Current use of medications or dietary supplements/beverages.
5. Subjects with allergic reactions to medicines and foods containing GlcCer.
6. Pregnancy, lactation, or expected/planned pregnancy during the study period.
7. Subjects currently participating in another clinical trial or who had participated within the previous 3 months.
8. Subjects who previously underwent plastic surgery.
9. Subjects who receive daily skin care therapy, such as esthetic treatments, or use facial beauty appliances.
10. Subjects who use skin care products daily, except for creams, serums, all-in-one cosmetics, facial packs, face lotions, face emulsions, and sunscreen.
11. Subjects previously diagnosed with atopic dermatitis.
12. Subjects considered to be inappropriate for the study for other reasons by the attending physician.
13. Selection criteria were individuals with relatively higher cheek TEWL who were considered to be appropriate for the study by the attending physician.

Forty-four female subjects with concerns about skin moisture were selected after confirmation of their suitability for the study by the attending physician (Figure 3). Subjects were asked to take the test samples in the designated manner and avoid excessive eating and drinking. Subjects were also requested to refrain taking dietary supplements/beverages and maintain a regular lifestyle during the study period. In terms of skin care, excessive sun burn, the use of ceramide creams, and receiving esthetic treatments were prohibited and subjects were asked not to change their usual make-up routine.

One day before testing, subjects were required to avoid the excessive consumption of alcohol and intensive exercise and fasted for 6 hours prior to blood collection, except for drinking water. On the screening day, the use of any types of cosmetics and taking a bath or shower were prohibited.

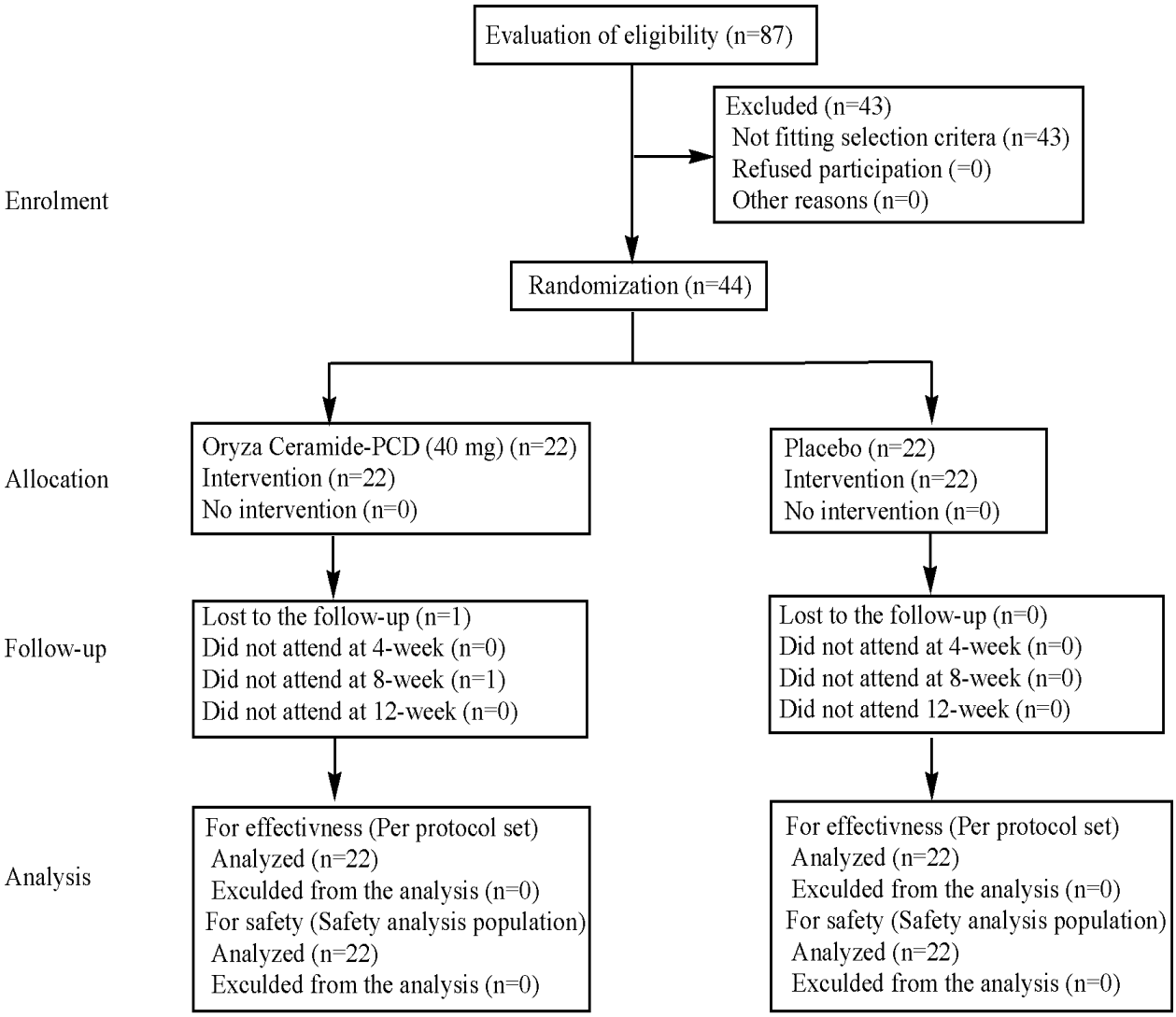

Figure 3. Flow chart showing subject characteristics.

Test samples and allocation: Test samples (indistinguishable brown capsules containing either Oryza Ceramide®-PCD or placebo) were provided by Oryza Oil & Fat Chemical Co., Ltd. as hard capsules. Active capsules contained 40 mg of Oryza Ceramide®-PCD (containing 1.2 mg of GlcCer and 40 μg of BSG) and 160 mg of γ-cyclodextrin. Oryza Ceramide®-PCD consisted of 40% purified rice extract and 60% γ-cyclodextrin. Placebo capsules contained 200 mg of γ-cyclodextrin.

Oryza Oil & Fat Chemical Co., Ltd. provided the test samples with red or blue markings on the packages. They kept sample information strictly concealed until the study period was complete. When the number of registered subjects reached 44, an allocation controller in ORTHOMEDICO Inc. generated an allocation sequence for test capsules according to the identification markers provided and made an allocation sheet and emergency key. Statlight #11 (Ver. 2.10, Yukms Inc.) was used to prepare a random number for the allocation sheet. The allocation sheet

was only provided to test sample distributers and then strictly concealed with the emergency key by the allocation controller. Test capsules were then allocated by class randomization to equalize the allocation ratio (1:1). Allocation was required in a manner to prevent significant differences in the means and standard deviations (SD) of cheek TEWL and ages between groups. Information on allocation was not disclosed to any other party until the subjects for analysis were selected at a clinical conference after study completion.

Study protocol and skin parameters: This randomized, placebo-controlled, double-blind, parallel-group study was performed at Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan), and statistical analyses were conducted by ORTHOMEDICO Inc. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000041295). Subjects took one appropriate capsule (Oryza Ceramide® or placebo) daily after breakfast for 12 weeks. All subjects recorded a daily report, including capsule ingestion and menstruation, and answered a questionnaire from a physician after 4, 8, and 12 weeks. Subjects were also asked to record a Calorie And Nutrition Diary from 3 days before to the day of screening.

The following items were examined at baseline and after 4, 8, and 12 weeks of the intervention: cheek TEWL after 12 weeks measured by Tewameter® TM300 (Courage+Khazaka Electronic GmbH, Köln, Germany) was set as the primary outcome. TEWL measured after 4 and 8 weeks were set as the secondary outcomes.

To assess other secondary outcomes, the following items were evaluated. Epidermal moisture and skin elasticity were measured using Corneometer CM825 (Courage+Khazaka Electronic GmbH) and Cutometer dual MPA580 (Courage+Khazaka Electronic GmbH). The Robo Skin Analyzer (Inforward, Inc., Tokyo, Japan) [31] was used to measure the number of pores, pigmentation, wrinkles, skin roughness, sebum, and brightness. 3R-Wmbtprime (3R Solution Corp, Fukuoka, Japan) was employed to evaluate skin conditions, including pores, melanin/pigmentation, wrinkles, porphyrin/sebum, and the s. corneum.

Laboratory tests: Body weight, BMI, the body fat ratio, blood pressure, and pulse rate were measured at all test periods.

Blood and urine were analyzed by LSI Medience Corporation (Tokyo, Japan). All items were examined at baseline and after 4, 8, and 12 weeks of the intervention. A venous blood sample was collected from an arm vein and the following tests were performed for a safety assessment.

Hematology components were as follows: hemoglobin, hematocrit, and red blood cell, leukocyte, platelet, lymphocyte, monocyte, eosinophil, and basophil counts. Biochemical components were as follows: total protein, total bilirubin, urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol,

triglycerides, hemoglobin (Hb) A1c, blood glucose, glycoalbumin, amylase, creatine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), chorine esterase, cholecystokinin, Na, K, Cl, Ca, Fe, inorganic phosphorus, and IgE. Urine samples were collected for a qualitative evaluation, including protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood.

Ethics, adherence, and compliance: The study was performed according to the Declaration of Helsinki (2013 revision) and conducted in conformity with ethical considerations. This protocol was approved by the Ethics Committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) on July 27, 2020 (Approved ID: 2007-00023-0020-21-TC), and substantial deviations from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before consenting to participate. No subject was part of the sponsoring or funding companies.

Statistical analysis: A per protocol set (PPS) was selected as the analysis dataset for the primary and secondary outcomes. Results were shown as the mean and SD. In statistical analyses, baseline data were analyzed using the Student's *t*-test. After the intervention, actual scores were analyzed using the linear mixed model, with baseline data utilized as covariates and time, groups, group–time interaction, and subjects as factors; changes from the baseline were analyzed using the linear mixed model, with time, groups, group–time interaction, and subjects as factors. The results of the physical examination and blood tests were reported as the mean and SD. The Student's *t*-test was used to evaluate the significance of differences between before and after the ingestion of the test sample. The χ^2-test was used for urinalysis parameters, with normal and abnormal values being coded as "1" and "0", respectively. We set the significance level to 5% with no adjustments for multiple comparisons. SPSS (Ver. 23.0, Japan IBM) or Microsoft Excel 2013 was used for statistical evaluations. Missing data were analyzed without storage.

RESULTS

Study performance: The study was performed between August 4, 2020 and December 4, 2020. During the study period, one subject in the Oryza Ceramide® group was unable to track the study from the 8-week period for personal reasons (Figure 3), and, thus, was excluded from the analysis from this time point. Accordingly, 21 or 22 subjects (50.2 ± 8.0 years) were available for analysis in the Oryza Ceramide® group, and 22 (49.3 ± 8.8 years) for the placebo group. The physical profiles of subjects included in the analysis are shown in Table 1. No significant differences were observed between the groups.

Table 1. Profiles of subjects.

	Baseline		12 W	
	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®
Age	49.3±8.8	50.2±8.0	–	–
Height (cm)	157.3±4.9	158.4±5.8	–	–
Body weight (kg)	53.0±7.6	55.6±9.2	52.3±7.7	54.4±9.6
BMI (kg/m^2)	21.4±2.9	22.1±3.4	21.1±3.0	21.7±3.6
Body fat ratio (%)	25.7±5.7	26.6±6.1	27.7±7.7	28.8±7.4
Systolic blood pressure (mmHg)	112.9±11.9	113.5±13.7	114.2±12.3	115.6±13.1
Diastolic blood pressure (mmHg)	69.9±9.1	73.8±10.5	72.4±9.1	76.2±11.4
Pulse rate (bpm)	70.9±9.3	74.5±6.9	69.1±8.9	74.0±7.7
Body temperature (°C)	34.6±0.7	34.8±0.7	33.0±0.7	33.2±0.8
IgE (IU/mL)	64.1±83.8	221.0±369.7	–	–

Data are represented as the mean ±SD (n=22 for placebo, n=22 for Oryza Ceramide®). The Student's *t*-test was used to assess the significance of differences, except for age (χ^2 squared test). No significant differences were detected between the placebo and Oryza Ceramide® groups.

Moisture parameters: Table 2 shows TEWL in several body parts and epidermal moisture in the cheeks and lips. In terms of the primary outcome of the study, cheek TEWL after 12 weeks of the Oryza Ceramide® intervention, as well as TEWL after 4 weeks, were significantly lower than in the placebo group. No significant differences were observed in TEWL in other parts between the groups. In terms of epidermal moisture, lip moisture was increased in the intervention with Oryza Ceramide® group for 12 weeks. Cheek moisture was also slightly higher in the Oryza Ceramide® group than in the placebo group.

Pores and Pigmentation: Pore parameters were measured and analyzed using the RoboSkin Analyzer. After the Oryza Ceramide® intervention for 4 weeks, the number of total conspicuous pores and obviously visible pores were significantly higher than placebo group (Table 3), while changes in these parameters and obviously opened and visible black pores exhibited significantly lower values during this period.

Regarding pigmentation parameters, the number and area of small pigmentation were significantly higher in the Oryza Ceramide® group than in the placebo group after 8 weeks (Table 3). Furthermore, there were slightly more areas of large pigmentation (Lv1) in the Oryza Ceramide® group after 8 weeks than in the placebo group.

Other skin parameters: As shown in Table 4, no significant differences were observed in wrinkles, brightness, or texture parameters between the Oryza Ceramide® and placebo groups. Only sebum was significantly lower in the Oryza Ceramide® group than in the placebo group.

Table 2. Changes in TEWL and epidermal moisture.

	Baseline		4 W		8 W		12 W	
	Placebo	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**
TEWL Cheek (g/m²·hr)	**21.0±6.3**	**21.0±5.8**	**15.0±4.6**	**11.1±3.8***	**19.6±5.7**	**16.9±6.1**	**18.8±4.4**	**15.6±5.1***
			(-6.0±7.5)	**(-9.9±7.0)**	**(-1.5±9.1)**	**(-4.3±7.2)**	**(-2.2±8.4)**	**(-5.5±5.4)**
Scalp	**6.0±4.7**	**7.4±6.4**	**4.6±2.8**	**5.3±3.8**	**10.9±6.8**	**12.9±6.1**	**10.7±2.3**	**13.3±4.1**
			(-1.5±4.6)	**(-2.1±5.5)**	**(4.9±8.8)**	**(5.4±4.9)**	**(4.7±6.7)**	**(5.8±7.5)**
Upper arm	**12.5±3.2**	**14.2±7.9**	**10.6±5.5**	**8.5±2.7**	**10.3±1.9**	**12.5±11.2**	**10.7±2.3**	**10.2±1.3**
			(-1.9±6.2)	**(-5.7±8.3)**	**(-2.2±3.4)**	**(-1.9±13.5)**	**(-1.7±4.0)**	**(-4.1±8.1)**
Back	**20.2±14.7**	**21.7±20.8**	**15.0±11.9**	**15.8±17.7**	**6.8±3.2**	**8.4±8.3**	**7.6±2.2**	**8.4±1.5**
			(-5.2±14.5)	**(-5.9±21.5)**	**(-13.4±14.5)**	**(-13.5±22.5)**	**(-12.6±14.8)**	**(-13.5±21.4)**
Lip	**32.4±21.3**	**37.6±16.1**	**26.0±15.2**	**26.8±13.3**	**44.8±19.7**	**37.7±13.4**	**42.7±16.6**	**39.9±11.7**
			(-6.4±26.3)	**(-10.8±22.8)**	**(12.4±33.4)**	**(-0.3±20.1)**	**(10.4±30.1)**	**(1.9±14.2)**
Back of the right hand	**12.5±2.9**	**12.8±3.2**	**8.6±2.7**	**10.5±7.6**	**9.1±2.9**	**10.0±3.9**	**10.1±2.2**	**11.3±2.4**
			(-3.9±2.9)	**(-2.3±7.2)**	**(-3.4±3.4)**	**(-3.0±5.1)**	**(-2.3±3.3)**	**(-1.7±4.4)**
Epidermal moisture	**38.0±13.1**	**41.8±11.1**	**45.8±11.2**	**47.7±12.7**	**48.7±16.1**	**53.7±10.6**	**41.1±11.7**	**43.0±10.5**
Cheek			**(7.8±11.0)**	**(6.0±12.9)**	**(10.8±18.2)**	**(11.2±15.9)**	**(3.2±12.4)**	**(0.5±12.9)**
Lip	**43.2±10.5**	**45.1±7.1**	**47.2±10.4**	**44.2±9.0**	**39.6±10.5**	**40.5±6.8**	**39.2±9.3**	**46.4±9.4***
			(4.0±6.0)	**(-0.9±8.9)**	**(-3.7±13.6)**	**(-4.6±9.3)**	**(-4.1±10.5)**	**(1.3±9.5)**

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). Baseline data were analyzed using the Student's *t*-test. After intervention data had been obtained, actual scores were analyzed using the linear mixed model, with baseline data utilized as covariates and time, groups, group–time interaction, and subjects as factors, and changes from baseline were examined using the linear mixed model, with time, groups, group–time interaction, and subjects as factors. An asterisk indicates a significant difference from the placebo group at *: $p<0.05$.

Table 3. Changes in pores and pigmentation parameters evaluated by the Robo Skin Analyzer.

	Baseline		4 W		8 W		12 W	
	Placebo	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**	**Placebo**	**Oryza Ceramide®**
Total conspicuous pores	**3712±1633**	**4800±2983**	**3555±1512**	**3778±2579***	**3476±483**	**3927±2789**	**3446±1429**	**4213±2587**
			(-156±681)	**(-1021±1332**)**	**(-236±1049)**	**(-339±1077)**	**(-266±1109)**	**(-258±981)**
Obviously visible pores	**2486±1015**	**3132±1764**	**2400±956**	**2509±1541***	**2358±943**	**2611±1665**	**2339±887**	**2800±1571**
			(-86±399)	**(-622±791**)**	**(-127±620)**	**(-202±647)**	**(-147±704)**	**(-148±601)**
Obviously opened pores	**69.4±45.4**	**103.4±107.1**	**66.7±47.9**	**77.5±96.9**	**62.1±45.1**	**68.8±66.8**	**67.8±47.6**	**84.2±68.5**
			(-2.7±32.6)	**(-25.9±34*)**	**(-7.3±38.1)**	**(-12.5±23.1)**	**(-1.6±33.1)**	**(0.3±26.8)**
Visible black pores	**1156±587**	**1564±1132**	**1088±518**	**1191±959***	**1055±504**	**1246±1062**	**1039±507**	**1328±956**
			(-68±265)	**(-329±514*)**	**(-101±401)**	**(-124±417)**	**(-117±387)**	**(-110±367)**
Small pigmentation	**143±31**	**144±37**	**144±32**	**140±4.3**	**141±32**	**151±42***	**148±29**	**153±41**
			(1.2±22.5)	**(-4.4±18.7)**	**(-1.7±22.3)**	**(12.8±22.5)**	**(5.2±22.7)**	**(12.5±21.6)**
Large pigmentation (Lv1)	**182±69**	**175±59**	**177±66**	**173±70**	**191±71**	**182±77**	**194±68**	**190±68**
			(-4.7±25.6)	**(-2.5±24.2)**	**(8.6±20.9)**	**(12.1±33.3)**	**(12.1±28.0)**	**(18.6±27.4)**
Small pigmented area (mm^2)	**69.1±16.8**	**66.7±17.5**	**67.9±17.3**	**65.9±20.8**	**66.9±17.8**	**70.0±21.4***	**69±14**	**71±20**
			(-1.2±9.7)	**(-0.9±9.5)**	**(-2.2±11.9)**	**(5.9±11.2)**	**(0.1±10.4)**	**(5.9±10.4)**
Large pigmented area (Lv1,mm^2)	**1153±529**	**1068±530**	**1039±471**	**1027±515**	**1159±526**	**1112±611**	**1119±550**	**1152±562***
			(-113±175)	**(-41±142)**	**(5.7±228)**	**(91±195)**	**(-34±230)**	**(107±189*)**

"Total conspicuous pores" include "obviously visible pores", "obviously opened pores" and "visible black pores". Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). Baseline data were analyzed using the Student's *t*-test. After intervention data had been obtained, actual scores were analyzed using the linear mixed model, with baseline data utilized as covariates and time, groups, group–time interaction, and subjects as factors, and changes from baseline were examined using the linear mixed model, with time, groups, group–time interaction, and subjects as factors. Asterisks indicate significant differences from the placebo group at *: $p<0.05$, **: $p<0.01$.

Table 4. Changes in wrinkles and several skin parameters evaluated by the Robo Skin Analyzer.

	Baseline		4 W		8 W		12 W	
	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®
All wrinkles	13.2±4.4	15.3±6.2	12.9±4.8	13.2±5.7	12.9±6.2	13.6±6.9	13.3±5.0	14.4±6.4
			(-0.3±3.0)	(-2.1±3.4)	(-0.3±3.8)	(-1.4±4.1)	(0.1±2.9)	(-0.9±3.5)
Right-sided wrinkle	6.5±2.2	7.6±3.0	6.6±2.6	6.5±2.8	6.6±3.6	6.7±3.5	6.7±2.5	7.0±3.6
			(0.0±2.6)	(-1.1±2.6)	(0.1±2.8)	(-0.7±2.9)	(0.1±1.8)	(-0.6±2.7)
Left-sided wrinkle	6.6±2.7	7.7±3.5	6.3±2.7	6.7±3.3	6.2±3.2	6.9±3.9	6.6±2.9	7.3±4.0
			(-0.4±1.6)	(-1.0±1.6)	(-0.4±1.7)	(-0.7±2.3)	(0.0±1.7)	(-0.3±2.8)
Total wrinkle length	89.3±47.0	90.4±40.1	93.1±53.4	90.1±48.2	93.5±44.3	94.8±41.3	90.3±50.6	93.0±29.9
(mm)			(-3.8±24.8)	(-0.2±26.8)	(4.2±30.9)	(8.6±35.1)	(1.0±29.4)	(5.4±29.0)
Total wrinkle area	120±47	139±56	125±53	138±54	139±52	147±56	138±55	145±41
(mm^2)			(4.6±39.8)	(-1.1±40.1)	(18.5±54.9)	(12.3±53.3)	(18.1±48.9)	(8.3±38.4)
Skin brightness	63.6±2.0	62.7±2.8	63.9±2.4	63.0±2.8	64.1±2.0	63.3±2.2	64.1±2.0	63.3±2.7
			(0.4±1.5)	(0.3±1.9)	(0.5±1.5)	(0.5±2.0)	(0.6±1.1)	(0.6±2.1)
Skin texture	12.1±7.6	14.0±5.9	13.8±7.9	13.8±7.1	16.8±10.9	12.0±10.0	8.0±4.1	9.6±6.7
			(1.7±12.2)	(-0.1±9.3)	(4.7±12.6)	(-2.2±12.2)	(-4.1±7.6)	(-4.3±10.2)
Sebum	0.5±0.8	2.7±4.7*	1.2±1.5	1.0±1.5	1.7±4.2	0.9±1.4	0.0±0.2	0.3±1.5
			(0.7±1.6)	(-1.7±4.8*)	(1.1±4.0)	(-1.9±4.8)	(-0.5±0.9)	(-2.3±5.1)

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). Baseline data were analyzed using the Student's *t*-test. After intervention data had been obtained, actual scores were analyzed using the linear mixed model, with baseline data utilized as covariates and time, groups, group–time interaction, and subjects as factors; changes from baseline were examined using the linear mixed model, with time, groups, group–time interaction, and subjects as factors. An asterisk indicates significant differences from the group placebo at *: $p<0.05$.

Table 5. Changes in elasticity and microscope parameters.

	Baseline		4 W		8 W		12 W	
	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®
Skin elasticity (R7 value)	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1
Pores	31.0±20.1	42.1±24.5	29.7±16.6 (-1.3±13.5)	37.5±18.7 (-4.7±14.5)	23.1±16.5 (-7.8±15.3)	28.5±15.0 (-11.4±16.2)	23.1±16.5 (-7.8±15.3)	28.5±15.0 (-11.4±16.2)
Melanin/stain	25.4±13.1	25.7±12.5	30.8±14.2 (5.4±11.7)	27.1±13.0 (1.4±10.5)	19.1±14.2 (-6.3±15.7)	20.5±9.7 (-4.7±10.4)	19.1±14.2 (-6.3±15.7)	20.5±9.7 (-4.7±10.4)
Wrinkles	5.0±6.9	6.6±5.1	6.7±5.8 (1.7±6.6)	6.3±4.8 (-0.4±4.2)	2.9±4.0 (-2.1±6.7)	5.0±3.6 (-1.4±5.5)	2.9±4.0 (-2.1±6.7)	5.0±3.6 (-1.4±5.5)
Porphyrin/Sebum	7.0±12.7	3.3±5.1	11.4±17.0 (4.4±10.1)	5.8±7.0 (2.5±3.9)	4.2±8.1 (-2.8±7.4)	3.7±5.2 (0.4±4.4)	4.2±8.1 (-2.8±7.4)	3.7±5.2 (0.4±4.4)
S. corneum	7.4±6.9	5.8±6.5	5.5±6.7 (-1.8±9.1)	7.5±8.3 (1.7±9.0)	4.8±5.0 (-2.5±8.5)	3.7±5.0 (-2.4±9.1)	4.8±5.0 (-2.5±8.5)	3.7±5.0 (-2.4±9.1)

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). Baseline data were analyzed using the Student's *t*-test. After intervention data had been obtained, actual scores were analyzed using the linear mixed model, with baseline data utilized as covariates and time, groups, group–time interaction, and subjects as factors; changes from baseline were examined using the linear mixed model, with time, groups, group–time interaction, and subjects as factors. No significant differences were detected between the placebo and Oryza Ceramide® groups.

Table 6. Changes in hematology parameters.

	Baseline		12 W		Standard value
	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	
Red blood cells (×10⁴ cells/μL)	428±31	445±28	435±3	452±33	380-500
Leukocytes (cells/μL)	4845±1310	5331±1377	4636±988	5461±1804	3300-9000
Hemoglobin (g/dL)	12.9±1.2	13.1±0.7	12.9±1.6	13.3±0.8	11.5-15.0
Hematocrit (%)	40.6±3.4	41.5±1.9	41.5±4.6	43.0±2.6	34.8-45.0
Platelets (×10⁴ cells/μL)	25.5±4.6	26.7±4.2	27.7±4.7	27.7±3.7	14.0-34.0
Neutrophils (cells/μL)	2877±1034	3254±1052	2843±785	3393±1486	
Lymphocytes (cells/μL)	1565±340	1619±376	1370±347	1579±401	
Monocytes (cells/μL)	236±82	284±88	249±80	302±133	
Eosinophils (cells/μL)	137±71	137±140	139±102	145±110	
Basophils (cells/μL)	28.6±20.4	36.4±15.3	34.0±24.3	41.5±31.6	

Each value is shown as the mean and SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). The Student's *t*-test was used for statistical analyses. No significant differences were detected between the placebo and Oryza Ceramide® groups.

Table 7. Changes in biochemical parameters.

	Baseline		12 W		Standard value
	Placebo	Oryza Ceramide®	Placebo	Oryza Ceramide®	
Total protein (g/dL)	6.8±0.4	7.1±0.3	6.9±0.4	7.2±0.4*	6.7-8.3
Total bilirubin (mg/dL)	0.85±0.32	0.79±0.31	0.79±0.27	0.80±0.31	0.2-1.2
Urea N (mg/dL)	13.2±3.3	14.0±3.6	13.4±3.6	13.1±3.4	8-20
Creatinine (mg/dL)	0.66±0.08	0.67±0.09	0.63±0.06	0.64±0.09	0.47-0.79
Uric acid (mg/dL)	4.4±0.8	4.5±0.9	4.4±0.8	4.2±0.7	2.5-7.0
Total cholesterol (mg/dL)	224±36	212±42	233±40	223±40	120-219
LDL cholesterol (mg/dL)	131±31	126±40	135±33	132±37	65-139
HDL cholesterol (mg/dL)	78±12	69±16	81±19	73±16	40-95
Triglyceride (mg/dL)	83±58	100±121	100±89	92±63	30-149
HbA1c (%)	5.3±0.3	5.4±0.3	5.3±0.2	5.4±0.3	4.6-6.2
Glycoalbumin (%)	14.4±1.2	14.4±0.9	13.8±1.0	13.9±0.8	12.3-16.5
Blood glucose (mg/dL)	82±6	85±7	85±7	83±11	70-109
Amylase (U/L)	77±20	81±25	84±20	92±36	40-122
CK (U/L)	87±54	86±28	84±21	86±30	40-150
AST (U/L)	19.8±4.5	18.9±2.8	20.3±3.8	19.9±3.8	10-40
ALT (U/L)	15.8±8.1	14.0±5.5	15.3±8.4	15.2±7.2	5-45
γ-GTP (U/L)	21.5±19.6	24.1±21.0	17.5±8.9	25.1±22.5	<30
ALP (U/L)	190±45	180±53	198±42	185±59	100-325
LAP(U/L)	49±9	52±16	48±8	53±20	37-61
LDH (U/L)	178±16	178±22	183±21	180±22	120-240
Na (mEq/L)	140±2	140±2	140±1	140±1	137-147
K (mEq/L)	3.8±0.2	3.9±0.2	4.0±0.2	4.2±0.3*	3.5-5.0
Cl (mEq/L)	101±2	102±1	101±	101±1	98-108
Ca (mg/dL)	9.1±0.3	9.2±0.3	9.2±0.3	9.3±0.3	8.4-10.4
Fe (μg/dL)	93±35	107±64	83±35	91±34	40-180
Inorganic P (mg/dL)	3.9±0.6	3.9±0.6	3.6±0.6	3.5±0.5	2.5-4.5

Each value is shown as the mean ± SD (n=22 for placebo and n=21-22 for Oryza Ceramide®). The Student's *t*-test was used for statistical analyses. An asterisk indicates a significant difference from the placebo group at $p<0.05$.

Table 8. Changes in urine parameters.

	Week	Placebo	Oryza Ceramide®	Standard value
Protein	**0**	**(nor):20, (ab):2**	**(nor):21, (ab):1**	**(-)**
	12	**(nor):20, (ab):2**	**(nor):21, (ab):0**	
Glucose	**0**	**(nor):22, (ab):0**	**(nor):22, (ab):0**	**(-)**
	12	**(nor):22, (ab):0**	**(nor):21, (ab):0**	
Urobilinogen	**0**	**(nor):21, (ab):1**	**(nor):22, (ab):0**	**(±)**
	12	**(nor):22, (ab):0**	**(nor):21, (ab):0**	
Bilirubin	**0**	**(nor):22, (ab):0**	**(nor):22, (ab):0**	**(-)**
	12	**(nor):22, (ab):0**	**(nor):21, (ab):0**	
pH	**0**	**(nor):21, (ab):0**	**(nor):21, (ab):1**	**(5.0-7.5)**
	12	**(nor):21, (ab):1**	**(nor):20, (ab):1**	
Occult blood	**0**	**(nor):18, (ab):4**	**(nor):19, (ab):3**	**(-)**
	12	**(nor):20, (ab):2**	**(nor):18, (ab):3**	
Ketone bodies	**0**	**(nor):21, (ab):1**	**(nor):22, (ab):0**	**(-)**
	12	**(nor):22, (ab):0**	**(nor):21, (ab):0**	

Data are shown as the number of subjects with normal values (nor) or abnormal values (ab). The χ^2 squared test was used for urinalysis parameters. No significant differences were detected between the placebo and Oryza Ceramide® groups.

Laboratory data and adverse effects: Blood pressure, pulse rate, and body temperature are shown in Table 1 and blood hematology parameters in Table 6. No significant changes were observed between the 2 groups. Biochemical parameters are shown in Table 7. After 12 weeks of the intervention, significant differences were observed in total protein and K levels between the 2 groups. However, these changes were all within reference ranges. Urinalysis parameters did not significantly change in either group (Table 8).

DISCUSSION

The epidermis comprises the s. basal, s. spinosum, s. granulosum, and s. corneum [32]. The s. corneum exerts barrier and hydration functions via ceramides [32]. Ceramides contain a long chain amino alcohol that binds to a long chain fatty acid by an amide bond, and they function as interstitial lipids that cement gaps between cornified keratinocytes [33]. Ceramide levels decrease with age [34], resulting in dry skin. The loss of epidermal moisture causes dry, itchy, sensitive skin [35]. Epidermal moisture levels are lower in patients with dry skin than in normal subjects [36]. Furthermore, cracks in the epidermis caused by dehydration of the s. corneum increases susceptibility to bacterial and viral infections, as well as sensitization by allergens, which are risk factors for infectious diseases [37] and skin allergies [38]. Therefore, ceramide supplementation to prevent skin dehydration or replace lost natural ceramides may enhance skin barrier functions. Therefore, we examined the effects of daily ingestion of 40 mg of Oryza Ceramide®-PCD (40 mg) containing GlcCer and BSG in Japanese women with high TEWL.

Assessing the primary outcome, cheek TEWL after 4 weeks and after 12 weeks of the Oryza Ceramide® intervention was significantly reduced (Table 2). However, Oryza Ceramide® did not exert beneficial effects on TEWL after 8 weeks, which may have been due to stress-induced high TEWL. One possible reason for the increase in TEWL is anxiety. During this period (October 27 to November 6, 2020), restrictions were placed on daily activities under the stay at home order because of the third wave of COVID-19. The number of newly infected patients with COVID-19 was higher at the 8-week period of the present study than at 0 or 4 weeks, but then plateaued at 12 weeks. Skin TEWL has been reported to increase due to mental stress [39]. In the study, cheek TEWL was higher after 8 weeks than after 4 weeks in both groups, and similar results were obtained for lip and scalp TEWL. In addition, the intervention period was in autumn when the humidity was getting lower toward winter. Therefore, the hydrating effects of Oryza ceramide® appeared to be diminished by stress and lowering humidity-induced increases in TEWL, and, thus, significant differences were not observed.

Regarding the effects of GlcCer on cheek TEWL, the number of clinical studies that satisfy specific conditions, including healthy subjects and a 12-week intervention period, is limited. Previous studies did not obtain any evidence to show that cheek TEWL was reduced by GlcCer derived from corn [40], beets [41], and peaches [6]. On the other hand, only a single clinical study has reported the beneficial effects of rice GlcCer [16]. The ingestion of rice-derived GlcCer (1.8 mg/day) for 12 weeks reduced cheek TEWL. The difference in TEWL between the placebo and GlcCer groups during the intervention period was −2.2 g/m^2·hr. This value was slightly lower than that obtained in the present study (−3.3 g/m^2·hr), indicating the weaker effects of rice GlcCer on TEWL even with the ingestion of 1.8 mg of GlcCer. In contrast, the combination of GlcCer (1.2 mg) and BSG (40 μg) more effectively reduced cheek TEWL. BSG has been shown to enhance skin ceramide production [22], particularly ceramide [EOS], which exerts stronger hydrating effects among ceramide species [42]. As the underlying mechanism, we confirmed that BSG enhanced the production of ceramide synthase-3 and glucosylceramide synthase [22]. Therefore, the combination of GlcCer (1.2 mg) and BSG (40 μg) was more effective for epidermal hydration than GlcCer alone (1.8 mg).

Three studies examined changes in cheek TEWL with interventions using GlcCer derived from konjac potato [15, 43] and pineapple [44]. The study using konjac GlcCer [15] examined subjects with high TEWL of approximately 25 to 30 g/m^2·hr, which was regarded as dry skin because non-lesional skin in atopic dermatitis patients exhibited TEWL of approximately 30 g/m^2·hr [45]. Another study used healthy subjects with TEWL of approximately 20 g/m^2 hr [43]. Therefore, we compared the efficacy of 40 mg of Oryza Ceramide®-PCD [rice-derived GlcCer (1.2 mg) plus BSG (40 μg)] with konjac GlcCer (0.6 or 1.2 mg) after a 12-week intervention. The results obtained showed a difference of −3.3 g/m^2·hr in TEWL after 12 weeks between the placebo and Oryza Ceramide® groups. On the other hand, konjac GlcCer showed differences of −3.6 and −4.2 g/m^2·hr in TEWL for 0.6 and 1.2 mg of GlcCer, respectively. Therefore, the ability of Oryza Ceramide®-PCD to promote epidermal water retention is similar to or slightly less effective than that of konjac GlcCer. The GlcCer composition of konjac GlcCer differs from that of rice GlcCer [43]. Konjac GlcCer is rich in GlcCer with a shorter length of free fatty acids, such as GlcCer(d18:2/C16:0) and GlcCer(d18:2/C18:0) [43,46]. The major components of rice GlcCer are GlcCer(d18:2/C20:0) and GlcCer(t18:1/C24:0), as shown in Figure 2 and [43]. The length of fatty acids has been associated with epidermal barrier function [47]. In the skin of patients with atopic eczema, ceramides with short free fatty acids, such as ceramide [AS] and ceramide [NS], increase, while those with very long chain fatty acids, including ceramides [EOH] and [EOP], decrease. Since

Oryza ceramide®-PCD contains GlcCer with longer free fatty acids than that of konjac GlcCer, the effects of Oryza ceramide®-PCD were expected to be stronger than those of konjac GlcCer; however, the opposite result was obtained. The length of free fatty acids is C24 for ceramide [AS] and [NS] and C41 for ceramide [EOH] and [EOP]. This difference in the free fatty acid length of 16 carbons is markedly longer than our comparison case (4 to 6 carbons between rice and konjac GlcCer). Therefore, a small difference in fatty acid lengths may not affect the water retention ability of GlcCer when ingested by humans. BSG may contribute to the water retention ability of GlcCer. The contents of BSG in konjac GlcCer have not yet been examined; however, its presence has been already confirmed [48] and the contents appear to be higher than that of in the rice-derived GlcCer fraction (supplementary file 1, https://www.daicelchiral.com/img/mainpage/column/pdf/dcpak_p_series.pdf?3). Therefore, konjac GlcCer may contain more BSG than Oryza Ceramide®, which may contribute to its efficacy.

In a 12-week intervention, the ability of pineapple GlcCer (1.2 mg/day) to reduce TEWL was weaker than that of Oryza Ceramide®. The GlcCer composition and BSG content of pineapple GlcCer have not yet been reported in detail. Therefore, further studies are warranted.

Regarding cheek TEWL, Oryza Ceramide® significantly reduced TEWL after 4 weeks (−3.9 g/m^2·hr). A previous study examined the effects of pineapple GlcCer (0.6 and 1.2 mg) on TEWL after 4 weeks [44]. Pineapple GlcCer at 0.6 mg reduced TEWL by −3.3 g/m^2·hr, and −5.0 g/m^2·hr for 1.2 mg of GlcCer, compared to the baseline. These effects appear to be stronger than those of Oryza Ceramide®; however, TEWL in these subjects was approximately 13 to 15 g/m^2 hr. Therefore, the findings obtained cannot be compared to this study's results because the epidermis of their subjects was well hydrated and, as a consequence, hydration by interventions was not required.

Regarding other moisture parameters, Oryza Ceramide® increased lip moisture after 12 weeks. During the intervention period, including at 8 weeks, changes in lip moisture in both groups were not as prominent as those in TEWL. This may be due to the wearing of masks, which retains lip moisture, due to the COVID-19 pandemic. Since the application of artificial ceramides as lip balm has been reported to reduce dry lips [49], the ingestion of Oryza Ceramide® may increase lip surface ceramides and, thus, lip moisture. Moreover, to prevent oral dehydration, the application of lip balm every 8 hrs is recommended [50]. Since Oryza ceramide® enhanced lip moisturization following its single ingestion a day, an oral treatment with Oryza Ceramide® is considered to be more beneficial and convenient. In the present study, no significant differences were observed in lip TEWL with the Oryza Ceramide® intervention, which was attributed to the higher TEWL of the lip than in other parts. After 4 and 8 weeks, lip TEWL was approximately 40 g/m^2·hr, which may have been due to the stress caused by the COVID-19 pandemic. In addition, the number of corneum layers on the lips is lower than in other parts of the skin surface [51]. Physical damage to the lips, such as that caused by unconscious biting due to stress, may also increase lip TEWL [52]. Therefore, GlcCer supplementation with Oryza Ceramide® did not provide a sufficient supply of lip ceramides to reduce TEWL due to the thin corneum layer and its quick turnover cycle.

Regarding other skin parameters, Oryza Ceramide® facilitated reduction of various pore parameters in changed values, such as the numbers of obviously visible pores, opened pores, and black pores, after 4 weeks (Table 3). Comparing values obtained at baseline and after 4 weeks, baseline values on pores were higher in the Oryza Ceramide® group than in the placebo group. Therefore, comparisons of changes are preferable for evaluating the efficacy of an intervention. Reductions in pore values may have been enhanced by increases in ceramides on the skin surface. Moisture in the s. corneum and the condition of the skin surface markedly affect skin transparency and a higher transparency ratio against incident light increases

skin transparency [53]. A highly moisturized s. corneum generally shows stronger scattered reflected light [53] and surface reflection is stronger in bright skin [54]. Increased skin transparency may contribute to the visibility of pores. Therefore, the effects of Oryza Ceramide® on pores may be caused by increases in ceramides in the s. corneum. Four-week ingestion of Oryza ceramide decreased visibility of pores. However, further increases in ceramide may increase the transparency of skin surface, which may enhance pore visibility. In addition, it is not yet clear why slight and significant increases in small pigmentation and small pigmented area were observed in the Oryza Ceramide® group at 8-week period. Thus, further detailed studies targeting skin pigmentation and skin brightness are required.

We had predicted that Oryza Ceramide® might improve wrinkle condition and skin brightness, because water retention in skin surface could reduce shallow wrinkle and increase skin brightness. However, both parameters, as well as microscopic observation parameters, did not change with Oryza Ceramide® intervention.

Regarding the safety of Oryza Ceramide®, no abnormal changes associated with the Oryza Ceramide® intervention were observed in blood pressure, pulse rate, or blood and urine parameters. Adverse effects were not observed and the deterioration of health in several subjects during the intervention period was successfully treated with medication, and, thus, not related to the ingestion of Oryza Ceramide®.

In conclusion, the ingestion of Oryza Ceramide® (40 mg) reduced cheek TEWL and the combination of GlcCer (1.2 mg) and BSG (40 μg) appeared to contribute to ceramide supplementation in the s. corneum. These effects may be beneficial for skin barrier and moisturizing functions, and may suppress inflammation associated with atopic dermatitis and the symptoms of xerosis. Further studies are warranted.

CONCLUSIONS

The study demonstrated that Oryza Ceramide® (40 mg/day for 12 weeks) containing 1.2 mg of GlcCer and 40 μg of BSG ameliorated cheek TEWL and increased lip moisture in healthy female subjects. In addition, several pore and pigmentation parameters were improved by the Oryza Ceramide® intervention, suggesting increases in epidermal ceramides. Therefore, the ingestion of rice-derived GlcCer and BSG may increase skin ceramides and prevent skin dehydration. The intake of GlcCer and BSG was safe under the conditions of the present study.

Abbreviations: ANOVA: one-way analysis of variance, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate aminotransferase, BMI: body mass index, BSG: β-sitosterol glucoside, CK: creatinine kinase, ELSD: evaporative light scattering detector, GlcCer: glucosylceramide, GTP: glutamyltransferase, HDL: high-density lipoprotein, Hb: hemoglobin, LAP: leucine aminopeptidase, LDH: lactate dehydrogenase, LDL: low-density lipoprotein, PPS: per protocol set, S.: stratum, SD: standard deviation, TEWL: transepidermal water loss

Competing Interests: The sponsor of the present study, Oryza Oil & Fat Chemical Co., Ltd., assigned ORTHOMEDICO Inc. to conduct the study. S.T., W.Y. and H.S. (Ph.D.) are affiliated with Oryza Oil & Fat Chemical Co., Ltd., and K.Y., N.S., S.Y., S.I., H.N., T.K., and A.S. are members of ORTHOMEDICO Inc. This study was conducted by both Oryza Oil & Fat Chemical Co., Ltd. and ORTHOMEDICO Inc. T.T. (MD) was the principal investigator who monitored all subjects' conditions. H.S., S.T., W.Y., S.S., Y.M., and T.M (Professor). isolated and identified the chemical structures of GlcCer and BSG.

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REFERENCES

1. Tessema EN, Gebre-Mariam T, Neubert RHH, Wohlrab J: Potential applications of phyto-derived ceramides in improving epidermal barrier function. Skin Pharmacol. Physiol. 2017, 30:115-138. DOI: https://doi.org/10.1159/000464337
2. Tsuji K, Mitsutake S, Ishikawa J, Takagi Y, Akiyama M, Shimizu H, Tomiyama T, Igarashi Y: Dietary glucosylceramide improves skin barrier function in hairless mice. J. Dermatol. Sci. 2006, 44:101-107. DOI: https://doi.org/10.1016/j.jdermsci.2006.08.005
3. Tessema EN, Gebre-Mariam T, Lange S, Dobner B, Neubert RHH: Potential application of oat-derived ceramides in improving skin barrier function: Part 1. Isolation and structural characterization. Skin Pharmacol. Physiol 2017, 115-138. DOI: https://doi.org/10.1159/000464337
4. Murakami M, Nishi Y, Harada K, Masuzaki T, Minemoto Y, Yanagisawa T, Shimizu T, Tsuboi A, Hamada T, Nishimura M: Impact of oral intake of glucosylceramide extracted from pineapple on xerostomia: A double-blind randomized cross-over trial. Nutrients 2019, 11:2020. DOI: https://doi.org/10.3390/nu11092020
5. Mikami D, Sakai S, Nishimukai M, Yuyama K, Mukai K, Igarashi Y: Structure-dependent absorption of atypical sphingoid long-chain bases from digestive tract into lymph. Lipids Health Dis. 2021, 20:24. DOI: https://doi.org/10.1186/s12944-021-01448-2
6. Koikeda T, Tokudome Y, Okayasu M, Kobayashi Y, Kuroda K, Yamakawa J, Niu K, Masuda K, Saito M: Effects of peach (*Prunus persica*)-derived glucosylceramide on the human skin. Immunol. Endocrin. Metab. Agent. Medicinal Chem. 2017, 17:56-70. DOI: https://doi.org/10.2174/1871522217666170906155435
7. Tokudome Y, Masutani N, Uchino S, Fukai H: Recovery effects of oral administration of glucosylceramide and beet extract on skin barrier destruction by UVB in hairless mice. Nutrients 2017, 9:1178. DOI: https://doi.org/10.3390/nu9111178
8. Sugawara T, Tsuduki T, Yano S, Hirose M, Duan J, Aida K, Ikeda I, Hirata T: Intestinal absorption of dietary maize glucosylceramide in lymphatic duct cannulated rats. J. Lipid Res. 2010, 51:1761-1769. DOI: 10.1194/jlr.M002204
9. Guillou S, Ghabri S, Jannot C, Gaillard E, Lamour I, Boisnic S: The moisturizing effect of a wheat extract food supplement on women's skin: a randomized, double-blind placebo-controlled trial. Int. J. Cosmet. Sci. 2011, 33:138-143. DOI: https://doi.org/10.1111/j.1468-2494.2010.00600.x
10. Sugawara T, Aida K, Duan J, Hirata T: Analysis of glucosylceramides from various sources by liquid chromatography-ion trap mass spectrometry. J. Oleo Sci. 2010, 59:387-394. DOI: https://doi.org/10.5650/jos.59.387
11. Shirakura Y, Kikuchi K, Matsumura K, Mukai K, Mitsutake S, Igarashi Y: 4,8-Sphingadienine and 4-hydroxy-8-sphingenine activate ceramide production in the skin. Lipid. Health Disease. 2012, 11:108. DOI: https://doi.org/10.1186/1476-511X-11-108
12. Duan RD, Bergman T, Xu N, Wu J, Cheng Y, Duan J, Nelander S, Palmberg C, Nilsson Å: Identification of human intestinal alkaline sphingomyelinase as a novel ecto-enzyme related to the nucleotide phosphodiesterase family. J. Biol. Chem. 2003, 278:38528-38536.DOI: https://doi.org/10.1074/jbc.M305437200
13. Shimoda H, Terazawa S, Hitoe S, Tanaka J, Nakamura S, Matsuda H, Yoshikawa M: Changes in ceramides and glucosylceramides in mouse skin and human epidermal equivalents by rice-derived glucosylceramide. J. Med. Food 2012, 15:1064-1072. DOI: https://doi.org/10.1089/jmf.2011.2137
14. Ohta K, Hiraki S, Miyanabe M, Ueki T, Aida K, Manabe Y, Sugawara T: Appearance of intact molecules of dietary ceramides prepared from soy sauce lees and rice glucosylceramides in mouse plasma. J. Agric. Food Chem. 2021, 69:9188-9189. DOI: https://doi.org/10.1021/acs.jafc.0c07259
15. Uchiyama T, Nakano Y, Ueda O, Mori H, Nakashima M, Noda A, Ishizaki C, Mizoguchi M: Oral intake of glucosylceramide improves relatively higher level of transepidermal water loss in mice and healthy human subjects. J. Health Sci. 2008, 54:559-566. DOI: https://doi.org/10.1248/jhs.54.559

16. Hirakawa S, Sato A, Hattori Y, Matsumoto T, Yokoyama K, Kanai A: Dietary rice bran extract improves TEWL of whole body. Jpn. Pharmacol. Ther. 2013, 41:1051-1059. DOI: https://dx.doi.org/10.3390%2Fijms22137052
17. Aylward F, Nichols BW: Isolation of β-sitosterol-D-glucoside from ground nut phospholipids. Nature 1958, 181:1064. DOI: https://doi.org/10.1038/1811064a0
18. Villaseñor IM, Angelada J, Canlas AP, Echegoyen D: Bioactivity studies on β-sitosterol and its glucoside. Phytother. Res. 2002, 16:417-421. DOI: https://doi.org/10.1002/ptr.910
19. Ju YH, Clausen LM, Allred KF, Almada AL, Helferich WG: β-Sitosterol, β-sitosterol glucoside, and a mixture of β-sitosterol and β-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells *in vitro* and in ovariectomized athymic mice. J. Nut. 2004, 134:1145-1151. DOI: https://doi.org/10.1093/jn/134.5.1145
20. Dolai N, Kumar A, Islam A, Haldar PK: Apoptogenic effects of β-sitosterol glucoside from *Castanopsis indica* leaves. Nat. Prod. Res. 2016, 30:482-485. DOI: https://doi.org/10.1080/14786419.2015.1023201
21. Ivorra MD, D'Ocon MP, Paya M, Villar A: Antihyperglycemic and insulin-releasing effects of β-sitosterol 3-β-D-glucoside and its aglycone, β-sitosterol. Arch. Int. Pharmacodyn. Ther. 1988, 296:224-231.
22. Takeda S, Terazawa S, Shimoda H, Imokawa G: β-Sitosterol 3-*O*-D-glucoside increases ceramide levels in the stratum corneum via the up-regulated expression of ceramide synthase-3 and glucosylceramide synthase in a reconstructed human epidermal keratinization model. PLoS One 2021, 16:e0248150. DOI: https://doi.org/10.1371/journal.pone.0248150
23. Liu H, Orjala J, Rali T, Sticher O: Glycosides from *Stenochlaena palustris*. Phytochemistry 1998, 49:2403-2408. DOI: https://doi.org/10.1016/S0031-9422(98)00352-5
24. Huang Q, Tezuka Y, Hatanaka Y, Kikuchi T, Nishi A, Tubaki K: Studies on metabolites of mycoparasitic fungi. III. New sesquiterpene alcohol from *Trichoderma koningi*. Chem. Pharm. Bull. 1995, 43:1035-1038. DOI: https://doi.org/10.1248/cpb.43.1035
25. Kang SS, Kim JS, Son KH, Kim HP, Chang HW: Cyclooxygenase-2 inhibitory cerebrosides from Phytolaccae Radix. Chem. Pharm, Bull. (Tokyo) 2001, 49:321-323. DOI: https://doi.org/10.1248/cpb.49.321
26. Inagaki M, Oyama A, Arao K, Higuchi R: Constituents of Crinoidea, 4. Isolation and structure of ceramides and glucocerebrosides from the feather star *Comanthus japonica*. Chem. Pharm. Bull. (Tokyo) 2004, 52:1307-1311. DOI: https://doi.org/10.1248/cpb.52.1307
27. Luoa Y, Yi J, Lia B, Zhanga G: Novel ceramides and a new glucoceramide from the roots of *Incarvillea arguta*. Lipids 2004, 39: 907-913. DOI: https://doi.org/10.1007/s11745-004-1313-4
28. Jung JH, Lee CO, Kim YC, Kang SS: New bioactive cerebrosides from *Arisaema amurense.* J. Nat. Prod. 1996, 59:319-322. DOI: https://doi.org/10.1021/np960201+
29. Ryu J, Kim, JS, Kan SS: Cerebrosides from *Longan arillus*. Arch. Pharm. Res. 2003, 26:138-142. DOI: https://doi.org/10.1007/bf02976659
30. Tuntiwachwuttikul P, Pootaeng-on Y, Phansa P, Taylor WS: Cerebrosides and monoacylmonogalactosylglycerol from *Clinacanthus nutans*. Chem. Pharm. Bull. (Tokyo) 2004, 52:27-32. DOI: https://doi.org/10.1248/cpb.52.27
31. Kakudo N, Kushida S, Tanaka N, Minakata T, Suzuki K, Kusumoto K: A novel method to measure conspicuous facial pores using computer analysis of digital-camera-captured images: the effect of glycolic acid chemical peeling. Skin. Res Technol. 2011, 17: 427-433. DOI: https://doi.org/10.1111/j.1600-0846.2011.00514.x
32. Norlén L: Skin barrier formation: the membrane folding model. J. Invest. Dermatol. 2001, 117:823-829. DOI: https://doi.org/10.1046/j.0022-202x.2001.01445.x
33. Meisen I, Mormann M, Müthing J: Thin-layer chromatography, overlay technique and mass spectrometry: a versatile triad advancing glycosphingolipidomics. Biochim. Biophys. Acta. 2011, 1811: 875-896. DOI: https://doi.org/10.1016/j.bbalip.2011.04.006
34. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A: Decreased level of ceramides in stratum corneum of atopic dermatitis: An etiologic factor in atopic dry skin? J.

Invest. Dermatol. 1991, 96:523-526. DOI: https://doi.org/10.1111/1523-1747.ep12470233

35. Yosipovitch G: Dry skin and impairment of barrier function associated with itch - new insights. Int. J. Cosmet. Sci. 2004, 26:1-7. DOI: https://doi.org/10.1111/j.0142-5463.2004.00199.x
36. Wang B, Tai M, Zhang K, Chen H, Gan X, Che B, Abudukelimu N, Wang G, Xin X, Lin L, Han P, Peng Y, Du Z, Aker Aisa H: *Elaeagnus L* gum polysaccharides alleviate the impairment of barrier function in the dry skin model mice. J. Cosmet. Dermatol. 2021, 20:647-656. DOI: https://doi.org/10.1111/jocd.13541
37. Imokawa G: Cutting edge of the pathogenesis of atopic dermatitis: Sphingomyelin deacylase, the enzyme involved in its ceramide deficiency, plays a pivotal role. Int. J. Mol. Sci. 2021, 22:1613. DOI: https://doi.org/10.3390/ijms22041613
38. Sweeney A, Sampath V, Nadeau KC: Early intervention of atopic dermatitis as a preventive strategy for progression of food allergy. Allergy Asthma Clin. Immunol. 2021, 17:30. DOI: https://doi.org/10.1186/s13223-021-00531-8
39. Fukuda S, Baba S, Akasaka T: Psychological stress has the potential to cause a decline in the epidermal permeability barrier function of the horny layer. Int. J. Cosmet. Sci. 2015, 37:63-69. DOI: https://doi.org/10.1111/ics.12169
40. Asai S, Miyachi H: Evaluation of skin-moisturizing effects of oral or percutaneous use of plant ceramides. Rinsho Byori Jpn. J. Clin. Pathol. 2007, 55:209-215.
41. Hori M, Kishimoto S, Tezuka Y, Nishigori H, Nomoto K, Hamada U, Yonei Y: Double-blind study on effects of glucosyl ceramide in beet extract on skin elasticity and fibronectin production in human dermal fibroblasts. Anti Aging Med. 2010, 7:129-142. DOI: https://doi.org/10.3793/jaam.7.129
42. Uche LE, Gooris GS, Bouwstra JA, Beddoes CM: High concentration of the ester-linked ω-hydroxy ceramide increases the permeability in skin lipid model membranes. Biochim. Biophys. Acta. Biomembr. 2021, 1863:183487. DOI: https://doi.org/10.1016/j.bbamem.2020.183487
43. Mukai K, Shirai H, Koikeda T, Masuda Y, Saito M: Skin symptom improvement test including skin moisturizing property by intake of konjac ceramide-containing food. Jpn. Pharmacol. Ther. 2018, 46:781-799.
44. Yoshino S, Iwasaki T, Nojima J, Ohto N, Kuwahara H, Nakata S: Effect of glucosylceramide extracted from pineapple on healthy Japanese males and females with dullness and dry skin. Jpn. Pharmacol. Ther. 2015, 43:1593-1599.
45. Mócsai G, Gáspár K, Dajnoki Z, Tóth B, Gyimesi E, Bíró T, Maródi L, Szegedi A: Investigation of skin barrier functions and allergic sensitization in patients with hyper-IgE syndrome. J. Clin. Immunol. 2015, 35:681-688. DOI: https://doi.org/10.1007/s10875-015-0200-2
46. Usuki S, Yasutake Y, Tamura N, Tamura T, Tanji K, Saitoh T, Murai Y, Mikami D, Yuyama K, Monde K, Mukai K, Igarashi Y: Nrp1 is activated by konjac ceramide binding-induced structural rigidification of the a1a2 domain. Cells 2020, 9:517. DOI: https://dx.doi.org/10.3390%2Fcells9020517
47. Janssens M, Smeden J, Gooris G, Bras W, Portale G, Caspers P, Vreeken R, Hankemeier T, Kezic S, Wolterbeek R, Lavrijsen A, Bouwstra J: Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J. Lipid Res. 2012, 53(12):2755–2766. DOI: https://doi.org/10.1194/jlr.p030338
48. Takeda S, Shimoda H: Effect of β-sitsterol 3-*O*-glucoside included in rice-derived glucosylceramide fraction on expression of ceramide synthesizing enzymes. New Food Ind. 2017, 59:39-43.
49. Isoda K, Nakamura T, Yoshida K, Tamura E, Atsuta N, Ishida K, Takagi Y, Mizutani H: The efficacy of a lip balm containing pseudo-ceramide on the dry lips of sensitive skin-conscious subjects. J. Cosmet. Dermatol. 2018, 17:84-89. DOI: https://doi.org/10.1111/jocd.12351
50. Trookman NS, Rizer RL, Ford R, Mehta R, Gotz V: Clinical assessment of a combination lip treatment to restore moisturization and fullness. J. Clin. Aesthet. Dermatol. 2009, 2:44-48.
51. Ya-Xian Z, Suetake T, Tagami H: Number of cell layers of the stratum corneum in normal skin - relationship to the anatomical location on the body, age, sex and physical

parameters. Arch. Dermatol. Res. 1999, 291:555-559. DOI: https://doi.org/10.1007/s004030050453

52. Tomoki E, Kanto H, Tsuji Y: Objective evaluation of lip dryness and its associated factors. J. Jpn. Cosme. Sci. Soc. 2018, 42:1-8.
53. Masuda Y, Kunizawa N, Takahashi M: Methodology for evaluation of skin transparency and the efficacy of an essence that can improve skin transparency. J. Soc. Cosmet. Chem Jpn. 2005, 39: 201-208.
54. Minami K, Kaneko T, Nabeshima H, Iwamoto H, Kojima N, Nagatani N, Hori K: Evaluation of glossy appearance of facial make-up using CG images. J. Soc. Cosmet. Chem. Jpn. 2005, 39:16-25.