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RESEARCH ARTICLE

Effects of Peach (*Prunus persica*)-Derived Glucosylceramide on the Human Skin

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Abstract: ***Background*:** Plant-derived ceramides are functional natural products with various physiological effects that improve water retention and skin permeability functions.

***Objective*:** Herein, we isolated peach (*Prunus persica*)-derived glucosylceramide with high purity and evaluated its effects on moisture and permeability of skin.

***Methods*:** Three separate experiments were performed: evaluations of the effect of peach-derived glucosylceramide application on the ceramide content in a three-dimensional human cultured skin model, water retention effect of the oral administration of the peach-derived glucosylceramide on human skin functionality, and safety of the long-term administration of the peach-derived glucosylceramide in human subjects.

***Results*:** Treatments of three-dimensional cultures of human skin with peach-derived glucosylceramide led to dose-dependent increases in human ceramide contents. Additionally, after oral administration of ceramide to humans, dose-dependent improvements in water retention functions of skin, suppression of trans-epidermal water loss and improvements in skin texture were observed. Furthermore, no significant changes in subjective/objective symptoms, physical characteristics, or laboratory test values were observed in human subjects following long term oral administration of ceramide at doses that were 4.5-9-times greater than recommended. Taken together, the present data indicate the positive effects of peach ceramide on skin function and no adverse effects.

***Conclusion*:** Ingestion of peach ceramide safely improved the state of the skin.

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1. INTRODUCTION

Ceramides are the members of the sphingolipid family, which comprises fatty acids bound to

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sphingoid bases *via* amide bonds (Fig. **1**) [1]. Ceramides are the major components in human skin, and account for approximately 50% of intercellular lipids in the stratum corneum. Although ceramides are lipids, they have hydrophilic groups that allow the formation of lamellar structures in the stratum corneum, in which water molecules are retained with ceramide, cholesterol and fatty acids. These structures play important roles in water-retention and the function of skin permeability barriers [2-4].

Previous studies of ceramide supplements in food and/or cosmetics have shown that ceramide contents decrease with age, leading to improved water retention and permeability barrier function [3-6]. In particular, plant-derived ceramides (glycosphingolipid) are characterized by glucosylceramide structures in which sugar moieties are bound to sphingolipids through β-bonds (Fig. **2**) [7]. Sphingoid base and fatty acid compositions reportedly vary between plant species [8], and various ceramides are produced commercially from plants such as rice, corn, wheat and *Amorphophallus konjac* (konjac).

Because plants contain only trace amounts of ceramide, stable supply of plant-derived ceramides is hampered by large quantities of raw material and the requirement of advanced purification technologies, leading to high production costs. However, ceramide contents of peaches are greater than in other plants [7] and large peach-derived glucosylceramide yields have been achieved [9].

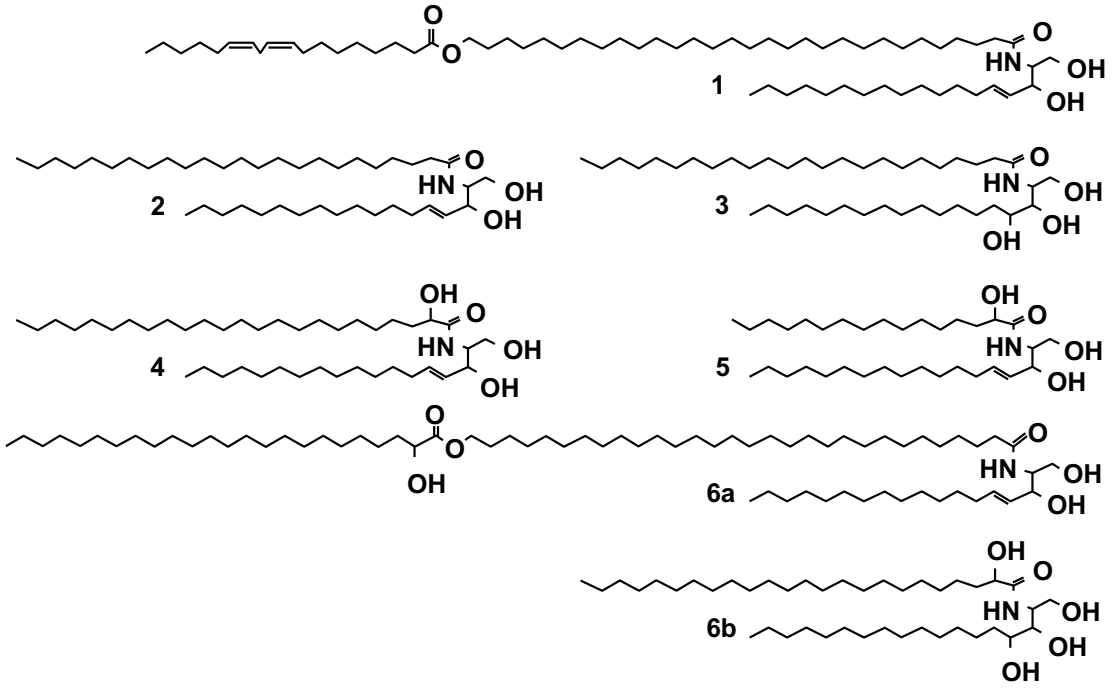

Fig. (1). Types of chemical structures of ceramide in stratum corneum. Wertz PW, Downing DL: Ceramides of pig epidermis; structure determinations. J Lipid Res, 24.759-63 (1983).

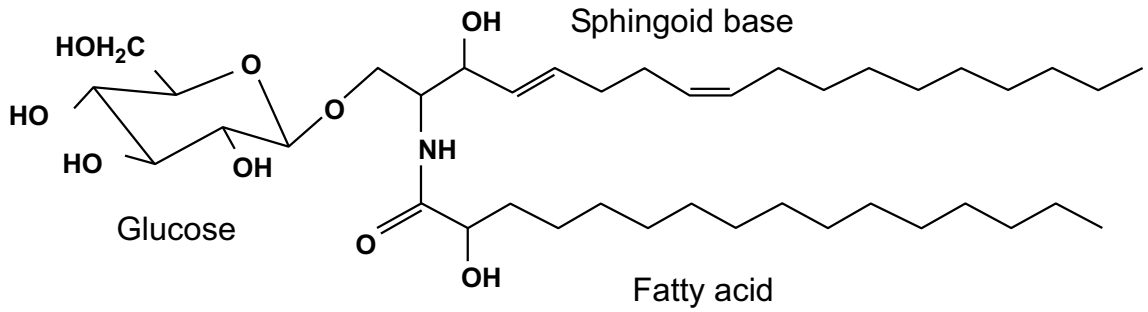

Fig. (2). Chemical structure of main component in peach-derived glucolsylceramide. Sugimoir, D.; Takase T.; Takakuwa, N. Study on peach ceramide, General Presentation O-11, First Academic Meeting, Ceramide Study Group: 2008, Sapporo.

In this study, we investigated changes in ceramide contents following the treatment of three-dimensional human cultured skin with peach-derived glucosylceramide. We also assessed the effects of orally administered ceramide on skin functions, including water retention and permeability barrier functions, and demonstrated the safety of long-term supplementation with high doses of peach-derived ceramide.

2. MATERIAL AND METHODS

To investigate the clinical properties of peach-derived glucosylceramide, we determined **2.1**) ceramide contents in a human three-dimensional human cultured skin model, **2.2**) water retention and skin function effects following oral administration to humans, and **2.3**) long term safety of overdoses.

2.1. Increases in Ceramide Contents in Three-dimensional Human Cultured Skin

The effects of the peach-derived glucosylceramide on ceramide contents of skin were determined using the previously described methods [10].

2.1.1. Materials

Purified peach-derived glucosylceramide was purchased from Okayasu Shoten Co., Ltd. (Koshigaya, Saitama, Japan). Three-dimensional human epidermal models, including Lab Cyte EPI-MODEL Air Lift 6 Days (12 well) and assay cultures for Lab Cyte were purchased from Japan Tissue Engineering Co., Ltd. (Gamagori, Aichi, Japan). Ceramide-2 and ceramide-5 were purchased from MATREYA LLC (Pleasant Gap, PA, USA), and ceramide-3 and ceramide-6 were purchased from Evonik (Goldschmidtstrabe, Essen, Germany). L-ascorbic acid, phosphate-magnesium salt n-hydrate, chloroform, methanol, and sodium benzoate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Phosphate-buffered saline (PBS) tablets were purchased from Takara Bio Inc. (Otsu, Shiga, Japan). Sunsoft A-121 (penta-glycerol monolaurate) was purchased from Taiyo Kagaku Co., Ltd. (Tokyo, Japan), and sodium oleate was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan).

2.1.2. Three-dimensional Cultures of Human Epidermal Skin Cells and Application of Peach-derived Glucosylceramide

Peach-derived glucosylceramide test solutions were prepared with 1% ceramide and were added to human epidermal cells in three-dimensional cultures. Test solutions were prepared by 1) sonicating solutions containing the purified peach-derived glucosylceramide, 0.2% Sunsoft A, 0.07% sodium benzoate, and purified water (-OA solution), or 2) 2% sodium oleate, 0.2% Sunsoft A, and 0.07% sodium benzoate (+OA solution) until constituents were evenly dissolved. PBS was used as a control solution, and 200-μL samples were applied to a Lab Cyte EPI-MODEL from the stratum corneum side and were incubated at 37°C in 5% CO_2. Seven days later, culture media were replaced with fresh media containing L-ascorbic acid and phosphate-magnesium salt n-hydrate (25 μg/mL), and the cells were incubated for 24 h. Culture media were then replaced every day or every other day, and samples were applied every other day.

2.1.3. Lipid Extraction from Three-dimensional Cultured Epidermis

After culture, cultured skin samples were carefully removed from scaffolds with the membrane filters of the cultivation cups in chloroform: methanol (2:1) using a surgical knife. Skin samples were then homogenized for 10 min using an Ultrasonic homogenizer (Advanced Sonifier 250; Branson Ultrasonics, Danbury, CT, USA) and lipids were extracted into the solution. Extraction solutions were then filtered through 0.2-μm filters (Millex-GN; Millipore, Billerica, Massachusetts, USA) using a glass syringe (VAN; TSUBASA INDUSTRY CO., LTD. Tokyo, Japan) and cellular debris was removed. Filtrates were exsiccated using a dry block bath (Dry Thermo Unit DTU-1C; Taitec, Koshigaya, Saitama, Japan), and lipid exsiccates were re-dissolved in 100 μL of chloroform:methanol (2:1).

2.1.4. Quantification of Ceramide

Ceramide contents were determined in 10-μL sample aliquots after spotting onto a (HPTLC Silica Gel 60 F_{254}; MERCK, Darmstadt, Hessen, Germany) using a glass capillary tube (Ringcaps; Hirschman Laborgerate, Eberstadt, Postfach, Germany). Identical volumes of ceramide-2 (NS), ce-

ramide-3 (NP), ceramide-5 (AS), and ceramide-6 (AP) were spotted as standards, and spots were developed using a mixture of chloroform:methanol: acetic acid (190:9:1). Spots were developed upward to 1 cm from the top of the HPTLC plate and were then dried and developed again. After development, 8% aqueous phosphoric acid containing 10% copper sulfate was sprayed onto the plates and the plates were then incubated at 180°C for 10 min using TLC plate heater III (Camag, Sonnenmattstrasse, Muttenz, Switzerland). Resulting spots were imaged using a Luminoimage Analyzer System (LAS-1000 Plus; Fujifilm Corporation, Tokyo, Japan), and ceramide types and contents in resulting images were quantified using a Multi Gauge (Fujifilm Corporation, Tokyo, Japan).

2.1.5. Data Analysis

Data are presented as mean ± standard deviations and were analyzed using Tukey's test with SAS 9.2 software (SAS Institute, Cary, NC, USA).

2.2. Water Retention Effects of Orally Administered Peach Glucosylceramide

Skin water retention effects of peach-derived glucosylceramide were determined following oral administration to humans.

2.2.1. Ethics

This study was conducted by Okayasu Shoten Co., Ltd.. The investigator explained the contents of the study, using the informed consent form to the subjects before conducting the study. Each subject who fully understood the contents of the study voluntarily participated in the study.

2.2.2. Materials

Jelly containing 3% peach ceramide PF3 was purchased from Okayasu Shoten Co., Ltd. and was administered at 20 and 40 mg/day doses (peach-derived glucosylceramide at 0.6-and 1.2 mg/day). Control jelly was prepared without the peach ceramide PF3. Prior to preparation of jellies, all materials and peach ceramide were assessed using three-dimensional primary skin irritation experiments (in-house document) and reverse mutation tests in bacteria, and no safety issues were identified.

2.2.3. Methods

2.2.3.1. Subjects and Methods of Administration

Twenty six healthy adult volunteers (13 males and 13 females; age, 27-67 years) agreed to get enrolled in this study after reading a study description

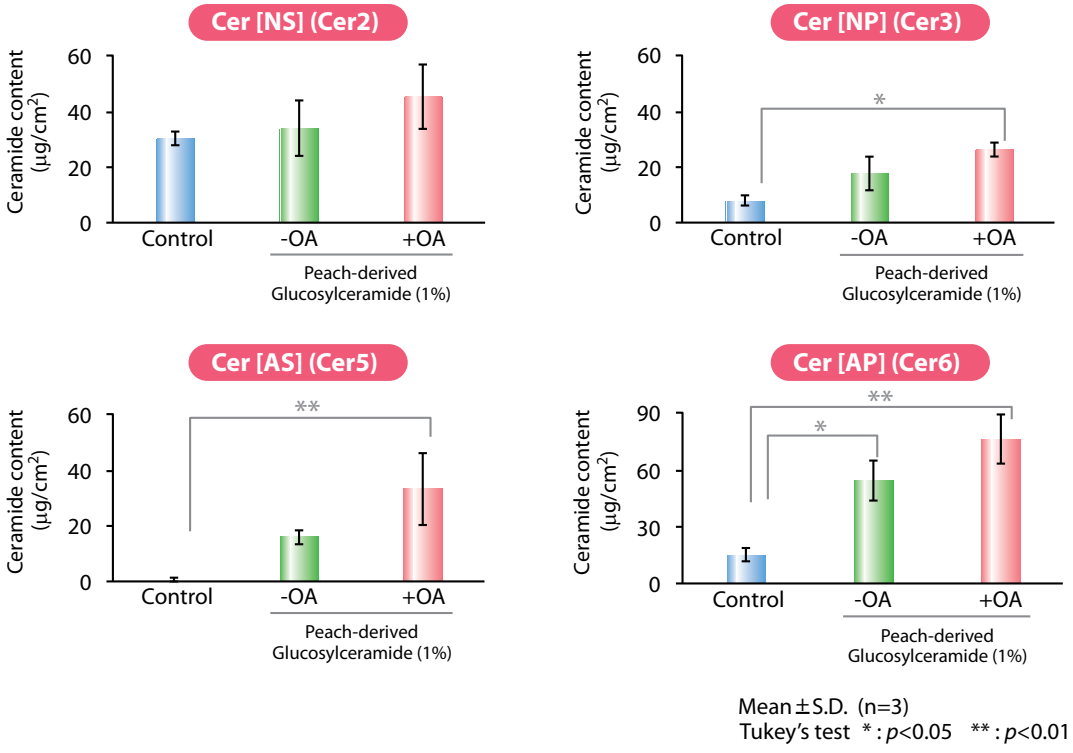

Fig. (3). Changes in ceramide in three-dimensional human cultured skin after administration of peach-derived glucosylceramide.

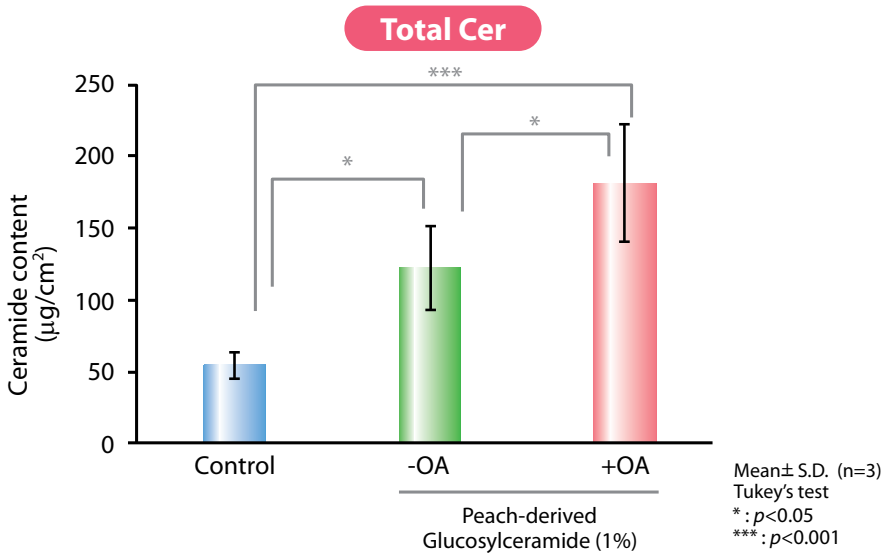

Fig. (4). Changes in total ceramide in three-dimensional human cultured skin after administration of peach-derived glucosylceramide.

Table 1.1. Subjects.

Item	Total	Male	Female
Number of Subjects	26	13	13
Age (Years)	47.9 ± 11.7	47.7 ± 12.3	48.0 ± 12.8

Mean ± standard deviation.

Table 1.2. Allocations of subjects.

Item	Control	0.6 mg Arm	1.2 mg Arm
Numbers of Subjects	3	11	12
Age (Years)	37.0 ± 5.4	51.5 ± 11.1	47.3 ± 13.4

Mean ± standard deviations.

(Table **1.1**). Subjects were assigned to groups with similar age and sex distributions (as much as possible). Subsequently, 3 subjects were included in the control group, 11 were treated with 20 mg/day PF3 (0.6 mg/day ceramide group), and 12 were treated with 40 mg/day PF3 (1.2 mg/day ceramide group; Table **1.2**). Jellies with and without ceramide were orally administered once daily with sufficient amounts of water within 30 min after breakfast.

2.2.3.2. Study Design

This open-label study was performed according to the schedule shown in Table **2**.

2.2.3.3. Duration of Study

Study materials were administered for 20 days from January 21 to February 9, 2015. During the study, subjects were instructed to live as they are accustomed and to avoid eating, drinking, and exercising excessively.

2.2.3.4. Study Items

Trans-Epidermal Water Loss (TEWL) was measured using a portable atomometer (VAPOMETER SWL4001JT; Delfin Technologies), and moisture contents of the stratum corneum at left brachial regions and left calves were measured using a Derma Unit SSC3/Corneometer

Table 2. Study schedule.

End Point	Before Administration	After 10-day Administration	After 20-day Administration
Trans-epidermal water loss	○	○	○
Water content	○	○	○
Skin surface photograph	○	—	○
Follow-up	◀———	———	———▶

Table 3. Ingredients of peach extract capsules.

Ingredient	Content
Peach ceramide (peach extract, peach-derived glucosylceramide-containing fraction)	18 mg
γ-cyclodextrin	182 mg
Hard capsule (pig gelatin)	62 mg

CM825 (Integral Corporation) before the start of the study and at 10 and 20 days after the start of the study. Skin textures at left brachial regions were observed by taking pictures of epidermis using a USB digital microscope (DinoCapture2.0/DINOAM2001; Sanko Co., Ltd. × 200) two times before the study and 20 days after the start of the study.

2.2.3.5. Measurement Conditions

Subjects were to stay in a temperature-controlled room (22 ± 2°C at 40%RH) before examination to allow for acclimation.

2.2.3.6. Statistical Processing

TEWL and moisture volumes in the stratum corneum were expressed as percentages relative to initial values before administration (100%) and are presented as mean ± standard error. Differences between before and after treatments were identified using two-sided Dunnett's tests (t-test) and were considered significant when $p < 0.01$ and $p < 0.05$.

2.3. Safety Evaluation of Long-term Treatments with Peach Ceramide

The efficacy of peach-derived glucosylceramide was confirmed in clinical studies, as described in the previous section. Subsequently, long-term safety was determined using capsules containing approximately 18 mg of peach ceramide (glucosylceramide 5.4 mg; 4.5-9 times the recommended dose) and approximately 30% glucosylceramide. In this open label study, subjects of 20-65 years received capsules once daily for 12 weeks.

2.3.1. Methods

2.3.1.1. Materials

Peach ceramide (30%) solutions were prepared from peach extracts containing peach-derived glucosylceramide (Okayasu Shoten). The ensuing glucosylceramide capsules contained γ-cyclodextrin and pig gelatin (Table **3**).

2.3.1.2. Subjects

Study candidates were required to answer preliminary questions, and those who met selection criteria A and did not meet exclusion criteria based on their answers passed the first selection. These candidates were screened and those who met selection criteria B were considered eligible. A total of 25 subjects were selected.

To ensure safety and protect human rights, subjects were informed of the study objectives in detail and provided written informed consent. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki, and was approved by the Ethics Review Board at Shiba Palace Clinic (President: Motonori Sano, MD).

2.3.1.3. Selection Criteria A

1) Japanese males and females of 20-60 years-of-age upon provision of consent.
2) Healthy adults without serious organ damage or disease and who were not receiving related treatments or drug therapies.

2.3.1.4. Selection Criteria B

1) Total cholesterol (T-Cho) level < 260 mg/dL.
2) Triglyceride (TG) level < 350 mg/dL.
3) Blood sugar (BS) level < 120 mg/dL.
4) γ-GT (γ-GTP) level < 2 times the standard value.
5) AST (GOT) level < 2 times the standard value.
6) ALT (GPT) level < 2 times the standard value.
7) Uric acid (UA) level < 8.0 mg/dL.
8) Creatinine (CRE) level < 1.5 mg/dL.
9) Systolic blood pressure between 101 mmHg and 139 mmHg.
10) Diastolic blood pressure between 61 mmHg and 89 mmHg.

2.3.1.5. Exclusion Criteria

1) Subjects who were considered inappropriate for the study for various reasons as judged by the investigator.
2) Subjects who were receiving a drug that might have affected the study results.
3) Pregnant women, women who may have been pregnant, and women who were breastfeeding.
4) Subjects who could have been allergic to the ingredients of the study products.
5) Subjects who were participating in another clinical study.
6) Subjects who had pre-existing conditions of serious hepatic impairment, renal impairment, or heart disease.
7) Subjects who had pre-existing or current hepatitis.
8) Subjects who had severe anemia.
9) Subjects who were hypertensive.
10) T-Cho level ≥ 260 mg/dL.
11) TG level ≥ 350 mg/dL.
12) BS level ≥ 120 mg/dL.
13) γ-GTP level ≥ 2 times the standard value.
14) AST (GOT) level ≥ 2 times the standard value.
15) ALT (GPT) level ≥ 2 times the standard value.
16) UA level ≥ 8.0 mg/dL.
17) CRE level ≥ 1.5 mg/dL.

2.3.1.6. Study Design

This open-label study was conducted according to the schedule shown in Table **4**.

2.3.1.7. Study Duration

The study material was administered for 12 weeks from August 19 to November 11, 2015. During the study, subjects were instructed to live as they were accustomed and to avoid excessive eating, drinking, and exercising.

2.3.1.8. Method of Administration

Single capsules of the study material (Table **3**) were administered to subjects once daily after breakfast or supper with water or lukewarm water. When subjects skipped meals and suffered difficulty taking the capsule in the morning or evening, they were instructed to take predetermined doses anytime within that day.

2.3.1.9. Examination Items

2.3.1.9.1. Physical Examination (Body Weight)

Body weights were measured using a digital weight scale (WB-110; TANITA Corporation, Tokyo, Japan) at 0, 4, 8, and 12 weeks after the start of the administration period.

Table 4. Study schedule.

Endpoint	Screening	Before Administration	4 Weeks after Administration	8 Weeks After Administration	12 Weeks after Administration
Physical examination	-	○	○	○	○
Clinical laboratory test	○	○	○	○	○
Blood pressure/pulse	○	○	○	○	○
Investigation of adverse event	-	◄———	———	———	———►

2.3.1.9.2. Laboratory Tests

Clinical laboratory tests were performed at 0, 4, 8, and 12 weeks after the start of the administration period.

Blood biochemical test included white blood cell counts, red blood cell counts, hemoglobin level, hematocrit, MCV, MCH, MCHC, platelet count, A/G ratio, total protein, albumin, UA, urea nitrogen, LDL cholesterol, CRE, sodium, potassium, chlorine, total cholesterol, TG, HDL cholesterol, total bilirubin, AST (GOT), ALT (GPT), ALP, γ-GT (γ-GTP), LD (LDH), and blood sugar levels.

Qualitative urinalyses comprised qualitative analyses of protein (score), sugar (score), urobilinogen (score), bilirubin (score), and ketone body (score) levels and occult blood reaction (score), specific gravity (numerical value), and reaction pH (numerical value) values.

Subjects fasted for 5 h before the tests (only water intake was allowed). All laboratory tests were performed at BML Inc. (Tokyo, Japan).

2.3.1.9.3. Blood Pressure (Seated Position) and Pulse Rate (Seated Position)

Blood pressures and pulse rate were measured five times in the seated position at the time of screening and at 0, 4, 8, and 12 weeks after the start of the administration period.

Blood pressures were measured after resting in the seated position for ≥ 10 min after arrival using an automated sphygmomanometer (HBP-9020; Omron Health Care Co., Ltd. Kyoto, Japan) and the second of two values were recorded and analyzed.

2.3.1.9.4. Investigation of Adverse Events

Adverse events were investigated in interviews of subjects on the basis of all unfavorable and unintended medical events, including signs, symptoms, and illnesses that took place between the start and end of the treatment period.

2.3.1.9.5. Statistical Processing

Continuous variables are expressed as mean ± standard deviations. Differences between blood pressure (seated position) and pulse rate (seated position), physical examination (body weight), blood biochemical test, and urinalysis (numerical value) results at the start and at 4, 8, and 12 weeks were identified using Dunnett's test. Urinalysis (score) results were analyzed using Wilcoxon signed-rank multiple comparison tests with Bonferroni's test for inequality. Two sided statistical analyses were performed using SPSS II for Windows (SPSS Co. Ltd.) and differences were considered significant when $P < 0.05$.

3. RESULTS

3.1. Increased in Ceramide Production

Peach-derived glucosylceramide (1%) was applied to three-dimensional human cultured skin models for 8 days, and ceramide was isolated and quantified using HPTLC (Fig. **3** and **4**). Treatment with peach-derived glucosylceramide tended to increase ceramide-2 (NS) contents, whereas ceramide-6 (AP) concentrations were significantly increased in the-OA group and ceramide-3 (NP), -5 (AS), and -6 (AP) contents were significantly increased in the +OA group. Total ceramide contents clearly increased following treatment with peach-derived glucosylceramide, and were particularly elevated in the +OA group.

3.2. Water Retention Effects

No subjects dropped out during the study and no particular abnormalities were observed following administration of the present treatments and controls.

3.2.1. TEWL

Tendencies toward slight decreases in TEWL in brachial regions were observed in both 20- and 40-mg/day ceramide PF3 groups at 10 days after administration. After 20 days, TEWL were decreased by approximately 15% and 27% in 20- and 40-mg/day groups, respectively (Fig. **5**). Similarly, TEWL decreased by 20%-30% in 20- and 40-mg/day groups at 10 days after treatment, and significant differences between these and baseline values were observed at 10 and 20 days (Fig. **6**).

3.2.2. Water Content in the Stratum Corneum

Water content of the stratum corneum tended to increase gradually in brachial regions of the 20-mg/day group, and were increased by 25% in the 40-mg/day group at 10 days and remained constant until the 20th day (Fig. **7**). Water content of skin at calves in 20- and 40-mg/day PF3 treated subjects tended to increase by approximately 20% on the 10th day. On the 20th day, Water content were maintained in the 20-mg/day group but were increased by approximately 30% in the 40-mg/day group (Fig. **8**).

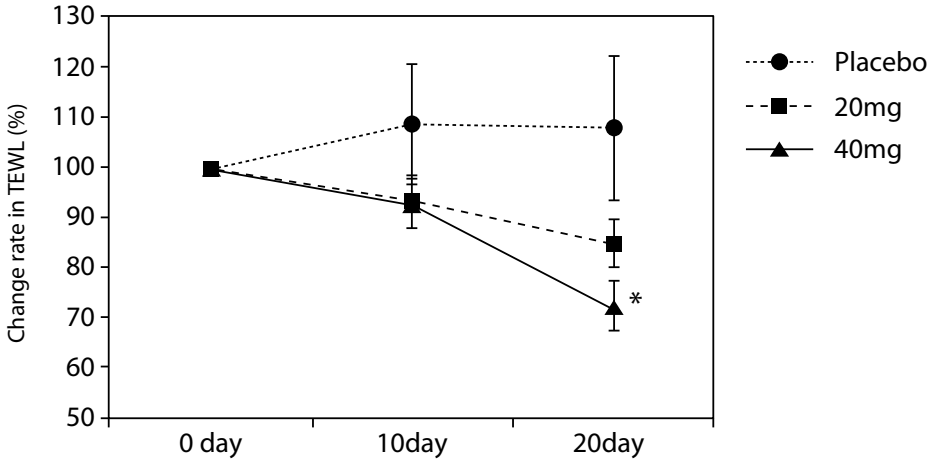

Fig. (5). Changes in TEWL on upper left arm after oral intake of peach-derived glucosylceramide. The data are indicated by the ratio at the start time as 100%. The data represent the mean ± SE, * P<0.05 (Tukey's test *versus* placebo).

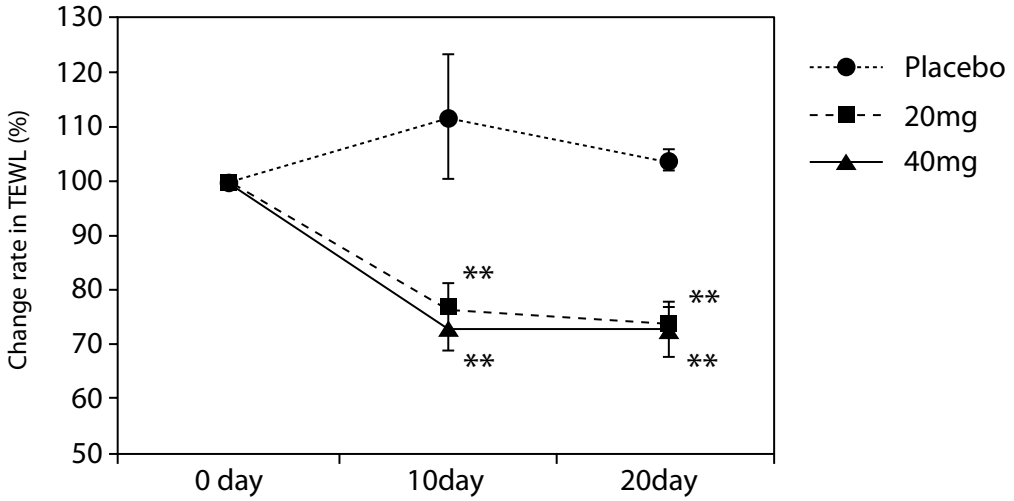

Fig. (6). Changes in TEWL on left calves after oral intake of peach-derived glucosylceramide. The data are indicated by the ratio at the start time as 100%. The data represent the mean ± SE, ** P<0.01 (Tukey's test *versus* placebo).

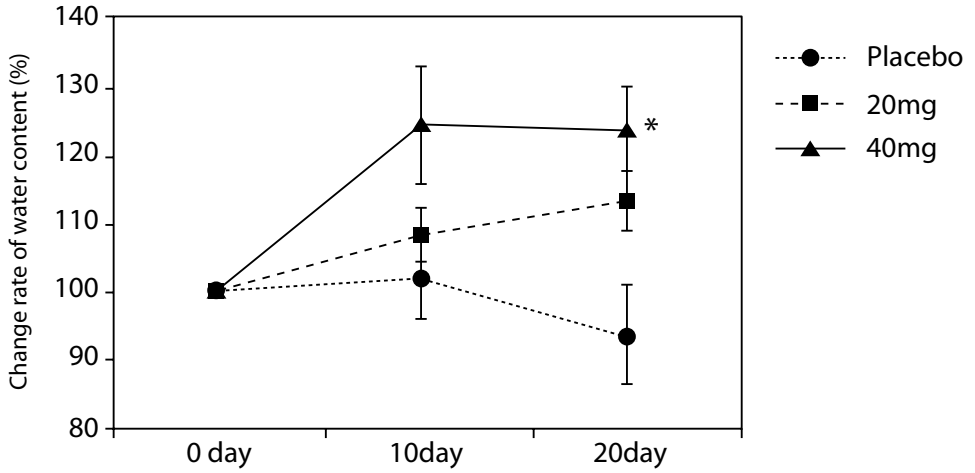

Fig. (7). Changes in water content on upper left arm after oral intake of peach-derived glucosylceramide. The data are indicated by the ratio at the start time as 100%. The data represent the mean ± SE, *P<0.05 (Tukey's test *versus* placebo).

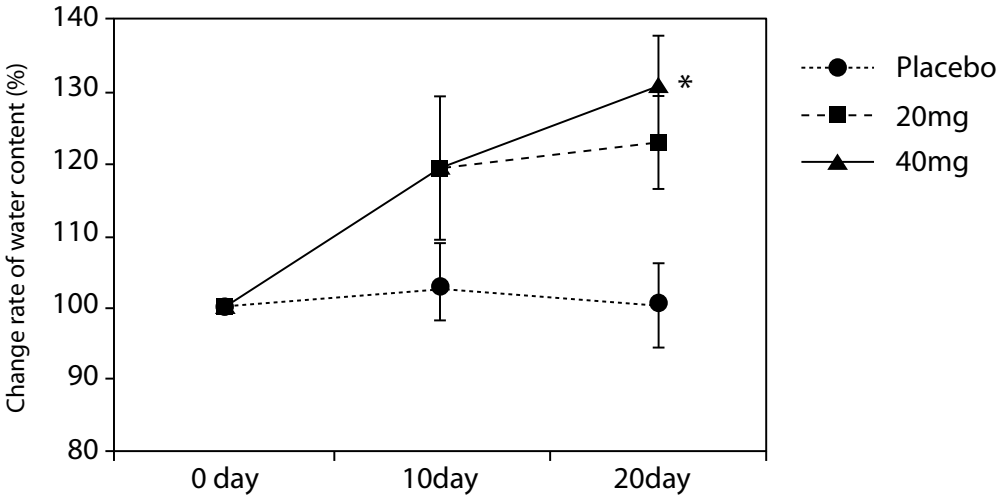

Fig. (8). Changes in water content on left calves after oral intake of peach-derived glucosylceramide. The data are indicated by the ratio at the start time as 100%. The data represent the mean ± SE, *P<0.05 (Tukey's test *versus* placebo).

Table 5. Background of subjects.

Item	Total	Male	Female
Number of Subjects	25	14	11
Age (Years)	46.0 ± 8.8	44.9 ± 8.7	47.5 ± 9.1

Mean ± standard deviation.

3.2.3. Skin Texture

Skin textures tended to improve in the 20-mg/day peach ceramide PF3 treated group (fine-textured skin; Fig. **9**, middle). Similarly, 7 of 12 subjects in the 40-mg/day group showed improvements in skin texture. In particular, distinct cristae cutis and sulci cutis were observed in two subjects who showed substantial decreases in TEWL and had strong linear scars before administration. In these subjects, roughly textured skin became finely textured, indicating substantial improvements in skin conditions (Fig. **9**, lower).

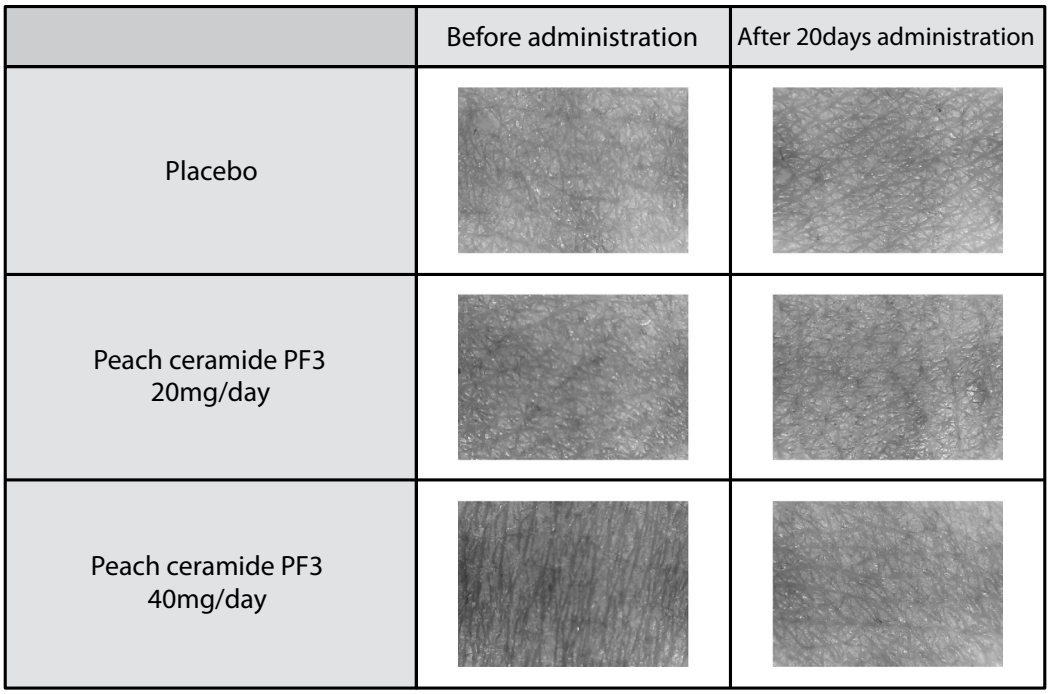

Fig. (9). Changes in skin texture on upper left arm after oral intake of peach-derived glucosylceramide.

3.3. Safety Evaluations

Because no changes in the study protocol were made, the study was conducted according to the original schedule. No subjects dropped out or were excluded from the study and data analyses of all 25 subjects were performed according to details that were discussed at a clinical conference.

3.3.1. Subjects

The mean age of the 25 subjects (14 males and 11 females) was 46.0 ± 8.8 years (males, 44.9 ± 8.7 years and females, 47.5 ± 9.1 years; Table **5**).

3.3.2. Physical Examination

Body weights at the start of the treatment period and at 4, 8, and 12 weeks after the start are shown in Table **6**. Body weights did not differ between any of the time points.

3.3.3. Laboratory Tests

The hematological and blood biochemical test results at the start of treatments and at 4, 8, and 12 weeks after the start are shown in Table **7**. However, significant differences in MCV, MCH, MCHC, TP, Alb, LDL cholesterol, creatinine, Cl, total cholesterol, HDL cholesterol, γ-GT (γ-GTP), and BS levels were identified between the start of the treatment period and at 4-, 8-, and 12-week follow-up examinations. No clinically problematic variations were observed in these values or in urinalysis (qualitative) results.

3.3.4. Blood Pressure and Pulse Rates

Changes in blood pressures (seated position) and pulse rates (seated position) between the start of administration and 4, 8, and 12 weeks are shown in Table **8**. Although decreases in systolic and diastolic blood pressures were statistically significant, these were not sufficient to cause hypotension and were not clinically problematic.

3.3.5. Adverse Events

No side effects were observed during the entire study period. However, 25 adverse events were recorded, and 10 of these (40%) were classified as cold (mild, non-serious), abdominal pain (mild, non-serious), diarrhea (mild, non-serious), abdominal bloating (mild, non-serious), constipation (mild, non-serious), pruritus (mild, non-serious), and insomnia (mild, non-serious). However, because all events were mild and were resolved within the study period, the study material was not considered a cause.

Table 6. Physical examination (body weight).

Item	Unit	Before Administration	4 Weeks after Administration	Passage p-value from Start	8 Weeks after Administration	Passage p-value from Start	12 Weeks after Administration	Passage p-value from Start
Body Weight	kg	60.5 ± 12.8	60.6 ± 12.6	P = 0.737	60.5 ± 12.6	P = 1.000	60.5 ± 13.0	P = 0.990

Mean ± standard deviations; n = 25.

Table 7. Clinical laboratory test values.

Item	Unit	Normal	Before Admin.	4 Weeks after	Overtime P-Value	8 Weeks after	Overtime p-Value	12 Weeks after	Overtime p-Value
White Blood Cell Count (WBC)	/μL	3500-9700	5177±1394	4902±1232	P = 0.465	4786±1219	P = 0.198	5279±1357	P = 0.940
Red Blood Cell Count (RBC)	$\times 10^4$ μL	M: 438-577 F: 376-516	471±39	466±45	P = 0.524	465±39	P = 0.351	474±42	P = 0.728
Hemoglobin (Hb)	g/dL	M: 13.6-18.3 F: 11.2-15.2	14.1±1.4	14.1±1.7	P = 1.000	13.9±1.6	P = 0.381	14.1±1.7	P = 1.000
Hematocrit (Ht)	%	M: 40.4-51.9 F: 34.3-45.2	42.3±3.6	42.0±4.2	P = 0.567	42.2±3.7	P = 0.963	42.4±4.1	P = 1.000
MCV	fL	M: 83-101 F: 80-101	90.1±4.5	90.2±4.4	P = 0.998	90.9±4.5	P = 0.033*	89.3±4.3	P = 0.023*
MCH	pg	M: 28.2-34.7 F: 26.4-34.3	29.9±2.0	30.2±1.9	P = 0.045*	29.8±2.1	P = 0.932	29.6±2.1	P = 0.045*
MCHC	%	M: 31.8-36.4 F: 31.3-36.1	33.2±1.2	33.5±1.1	P = 0.105	32.8±1.2	P = 0.029*	33.1±1.2	P = 0.982
Platelet Count	$\times 10^4$ /μL	14.0-37.9	25.6±5.5	25.8±5.1	P = 0.947	26.1±6.0	P = 0.696	26.9±4.9	P = 0.051
Total Protein (TP)	g/dL	6.5-8.2	7.06±0.34	7.01±0.36	P = 0.879	7.01±0.39	P = 0.879	7.23±0.30	P < 0.050*
A/G Ratio	-	1.30-2.00	1.70±0.23	1.74±0.21	P = 0.160	1.73±0.24	P = 0.361	1.75±0.24	P = 0.080
Albumin (Alb)	g/dL	3.7-5.5	4.42±0.29	4.44±0.32	P = 0.947	4.42±0.26	P = 1.000	4.58±0.28	P = 0.002**
Uric Acid (UA)	mg/dL	M: 3.6-7.0 F: 2.7-7.0	5.13±1.33	5.05±1.35	P = 0.835	4.95±1.30	P = 0.312	5.24±1.29	P = 0.657
Urea Nitrogen (UN)	mg/dL	8.0-20.0	11.8±3.2	12.2±3.1	P = 0.709	12.8±3.3	P = 0.100	12.0±3.1	P = 0.952
LDL Cholesterol	mg/dL	70-139	112±36	114±39	P = 0.881	114±37	P = 0.930	121±39	P = 0.011*
Creatinine (CREA)	mg/dL	M: 0.65-1.09 F: 0.46-0.82	0.725±0.154	0.690±0.145	P = 0.028*	0.724±0.156	P = 1.000	0.759±0.153	P = 0.035*
Sodium (Na)	mEq/L	135-145	141±2	141±1	P = 0.967	141±1	P = 0.555	141±2	P = 0.640
Chlorine (Cl)	mEq/L	98-108	104±1	105±2	P = 0.007**	104±2	P = 0.906	103±2	P = 0.716
Total Cholesterol	mg/dL	150-219	192±36	198±41	P = 0.236	202±40	P = 0.014*	209±38	P < 0.001***
Triglyceride (TG)	mg/dL	50-149	85.0±57.5	84.0±43.3	P = 0.997	87.9±51.9	P = 0.936	81.9±39.0	P = 0.921
HDL Cholesterol	mg/dL	M: 40-80 F: 40-90	65.2±16.0	67.4±21.5	P = 0.324	67.2±19.3	P = 0.412	72.0±21.4	P < 0.001***
Total Bilirubin	mg/dL	0.3-1.2	0.796±0.336	0.736±0.258	P = 0.364	0.744±0.289	P = 0.478	0.752±0.318	P = 0.605
AST (GOT)	U/L	10-40	20.8±4.8	20.7±4.0	P = 0.999	21.4±5.0	P = 0.911	22.8±8.0	P = 0.111
ALT (GPT)	U/L	5-45	19.8±12.0	19.9±10.0	P = 1.000	21.7 ±11.9	P = 0.301	22.8±13.9	P = 0.054
ALP	U/L	104-338	184±54	181±42	P = 0.911	189±51	P = 0.588	193±52	P = 0.188

(Table 7) Contd….

Item	Unit	Normal	Before Admin.	4 Weeks after	Overtime P-Value	8 Weeks after	Overtime p-Value	12 Weeks after	Overtime p-Value
γ-GT (γ-GTP)	U/L	M: ≦79 F: ≦48	23.6±13.8	25.0±16.5	P = 0.847	28.8±23.5	P = 0.031*	30.8±21.5	P = 0.002**
LD (LDH)	U/L	120-245	172±19	172±23	P = 0.987	170±24	P = 0.729	176±23	P = 0.356
Blood Sugar (fasting)	mg/dL	70-109	91.0±7.2	92.4±6.1	P = 0.607	96.1±8.8	P < 0.001***	91.1±7.5	P = 1.000
Protein Qualitative-Urine	-	-	0.440 ±1.003	0.080±0.400	P = 0.034	0.240±0.723	P = 0.129	0.520±0.963	P = 0.608
Sugar Qualitative-Urine	-	-	0.000±0.000	0.000±0.000	P = 1.000	0.040±0.200	P = 0.317	0.000±0.000	P = 1.000
Urobilinogen Qualitative-Urine	-	-	1.000±0.000	1.000±0.000	P = 1.000	1.000±0.000	P=1.000	1.040±0.200	P = 0.317
Bilirubin Qualitative-Urine	-	-	0.000±0.000	0.000±0.000	P = 1.000	0.000±0.000	P=1.000	0.000±0.000	P = 1.000
Ketone Body Qualitative-Urine	-	-	0.000±0.000	0.000±0.000	P = 1.000	0.080±0.400	P=0.317	0.120±0.600	P = 0.317
Occult Blood-Urine	-	-	0.280±0.891	0.320±0.945	P = 0.317	0.320±0.945	P=0.317	0.560±1.083	P = 0.020
Specific Gravity-Urine	-	-	1.018±0.008	1.013±0.008	P = 0.008**	1.017±0.008	P=0.972	1.017±0.010	P = 0.978
Reaction pH-Urine	-	-	6.060±0.712	6.080±0.640	P = 0.998	6.160±0.688	P = 0.854	5.940±0.565	P = 0.777

*:P < 0.05; **:P < 0.01; ***:P < 0.001; mean ± standard deviations; n = 25

Table 8. Blood pressures (seated position) and pulse rates (seated position).

Item	Unit	Before Administration	4 Weeks after Administration	Passage p-value from Start	8 Weeks after Administration	Passage p-value from Start	12 Weeks after Administration	Passage p-value from Start
Systolic Blood Pressure	mmHg	125 ± 11	116 ± 9	p < 0.001***	114 ± 11	p < 0.001***	119 ± 11	p = 0.002**
Diastolic Blood Pressure	mmHg	76.5 ± 8.0	70.6 ± 8.8	p = 0.001**	70.3 ± 10.2	p < 0.001***	72.0 ± 10.8	p = 0.019*
Pulse Rate	beats/min	72.5 ± 11.9	73.1 ± 13.3	p = 0.975	73.1 ± 13.4	p = 0.975	75.3 ± 10.7	p = 0.271

*P < 0.05; **P < 0.01; ***P < 0.001; mean ± standard deviation; n = 25

4. DISCUSSION

Human skin protects the body from physical impacts and prevents bacteria and viruses from entering the body. Skin has a three-layer structure comprising epidermis, dermis, and hypodermal fatty layer. The epidermis is approximately 0.2 mm thick, and includes the outermost layer of the skin and the stratum corneum, which provides a barrier and water retention functions to control the release of moisture from the body [1]. The structure of the stratum corneum comprises regularly aligned epidermal keratinocyte cells with lipid filled interstitial spaces. Although ceramide is the main interstitial lipid component [11], its contents decrease with aging [12, 13]. Accordingly, numerous studies of ceramide report efficacy and safety following addition to food and/or cosmetics, and application to skin restores water content and permeability functions [14-17].

In this study, we determined changes in ceramide contents following application of peach-derived glucosylceramide to three-dimensional human cultured skin. We confirmed that oral administration of peach-derived glucosylceramide facilitates water retention, and showed that long term administration of excessive glucosylceramide doses is safe in humans.

Treatments with peach-derived glucosylceramide dose dependently increased ceramide-2 (NS), -3 (NP), -5 (AS), and -6 (AP) levels in a three-dimensional human cultured skin model. Thus, transdermal application of the peach-derived glucosylceramide likely improves the water retention

and barrier functions of skin. A previous study also showed that treatment of three-dimensional human cultured skin with rice-derived ceramide led to substantial increases in ceramide-5 (AS) levels, suggesting increased glucosylceramidase activity [10]. Although glycosphingolipid compositions of peach-derived glucosylceramide differ from those of rice-derived glucosylceramide, a similar mechanism of action is likely.

In the further experiments, oral administration of peach-derived glucosylceramide dose dependently decreased TEWL and increased water contents of the stratum corneum in human subjects. Furthermore, improvements in skin textures were observed, and these were substantial in subjects that had dry rough skin before taking the present peach derived ceramide tablets, indicating improved water retention and permeability barrier functions. In a previous study, rats were fed a corn-derived glucosylceramide, and following absorption through the digestive tract, glycosphingolipid, was used as a precursor to reconstitute skin ceramide levels [4, 18]. Hence, oral or transdermal administration of plant-derived ceramides, including those from rice, corn, and konjac, improved the water retention in skin [3, 14-16]. This study also confirmed the water retention effects of peach derived glucosylceramide, which has similar effects to those of other plant derived ceramides.

Peaches produce large amounts of ceramide [8] compared with other plants, and could be used as a stable and cost effective source of ceramide for cosmetics and supplements. Furthermore, the present open label trial in healthy 20-65 year-old Japanese subjects demonstrated the safety of long-term administration of peach ceramide. Our subjects consumed glucosylceramide capsules containing 4.5-9 times the recommended daily dose of 0.6-1.2 mg/day [3, 14, 19] for 12 weeks, and significant subjective or objective symptoms were observed. Moreover, although significant changes in physical examinations, hematological tests, and blood biochemical parameters were identified, these were all within normal physiological ranges.

Finally, mild reactions during glucosylceramide treatments were transient and were not specific to peach ceramide. Hence, these events were considered unrelated to the study material.

In conclusion, continuous daily administration of peach ceramide at high doses is safe and has beneficial effects on skin water retention and barrier function.

CONCLUSION

When peach-derived glucosylceramide was applied to three-dimensional human cultured skin model, a dose-dependent increase in the human ceramide in the skin was observed. As a result of consecutive ingestion of 0.6 mg/day and 1.2 mg/day of glucosylceramide by 26 adults (average age, 47.9 years; 13 males and 13 females) for 20 consecutive days, improvement in skin water retention, suppression, and improvement in the texture of the skin *etc.* were observed. In addition, as a result of consecutive ingestion of 5.4 mg/day of glucosylceramide, which is equivalent to 4.5-9 times the recommended intake, by 25 adults (average age, 46.0 years; 14 males and 11 females), special physiological changes were not observed, and glucosylceramide was confirmed to be safe.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted by Okayasu Shoten Co., Ltd. The investigator explained the contents of the study, using the informed consent form to the subjects before conducting the study. Each subject who fully understood the contents of the study voluntarily participated in the study.

HUMAN AND ANIMAL RIGHTS

No animal is used in this research.

The reported experiments were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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