Ceramide, a common sphingolipid, is composed of a sphingoid backbone (base) with amide linkages with fatty acids. It can be modified with monosaccharides (such as glucose) or phosphocholine to form compounds such as glucosylceramide (GlcCer) or sphingomyelin, respectively. Although these compounds are techni-
cally different from ceramides, commercially available ceramides are GlcCer or sphingomyelin to which glu-
cose or phosphocholine has been added (1). It is well
known that differences arise in the physiological effects of ceramide depending on its sphingoid backbone and constituent fatty acids (2, 3).

In humans, ceramide exists largely in the stratum corneum of the epidermis and is a main component of intercorneocyte lipids, particularly in the form of large amounts of GlcCer and sphingomyelin (4). Ceramide combines with water to form lamellar structures in the stratum corneum (5). At least 11 kinds of ceramide are known to exist in the horny layer of human skin (6). This lamellar structure contributes to skin moisture and acts as a barrier: when collapsed, desquamation of the stratum corneum occurs and barrier function declines (6, 7). The ceramide content of skin diminishes with age, and patients with atopic dermatitis have decreased ceramide content within the stratum corneum (8). Ceramide, free fatty acids, and cholesterol are three important classes of lipids for skin barrier function (9).

Interest in anti-aging beauty products is endless. The global market for anti-aging skin care products was approximately 88 billion JPY in 2002 and 2 trillion JPY in 2012, a 2.2-fold increase in 10 y (10). The intake of GlcCer has been reported to decrease transepidermal water loss (TEWL) in mice (11). In addition, moistur-
izing effects on human skin have been reported (12, 13). Ceramide was also reported to exert an inhibitory effect on melanin production (14); this inhibitory effect was observed in mouse melanoma cells (2) and dif-
f ered depending on the GlcCer source (bovine, yeast, or
flour. GlcCer derived from different foods has differences in its physiological effects, depend-
ing on the sphingoid backbone and constituent fatty acids. In this study, we investigated the moisturizing and skin conditioning effects of GlcCer derived from torula yeast (Candida utilis) in healthy human subjects. The participants were randomly distributed in a cross-
over, double-blind comparative manner. Seventeen volunteers were orally administered both 1.8 mg/d of GlcCer derived from torula yeast and a placebo for 4 wk. Before and after oral administration, transepidermal water loss (TEWL) was measured and the objective skin condition observation and a questionnaire on skin condition were conducted. The primary endpoint was TEWL; secondary endpoints included the objective and subjective skin condi-
tions. The change in TEWL over the study period on the forearm was $-0.97 \pm 0.48$ and $-1.26 \pm 0.46$ g/m$^2$·h in the placebo and GlcCer groups, respectively, with significantly lower ($p=0.01$) TEWL observed in the GlcCer group. Brown spots increased in the placebo group but significantly decreased in the GlcCer group ($p=0.04$). Although chapped skin worsened in the placebo group, it significantly improved in the GlcCer group ($p=0.04$). The use of torula yeast-derived GlcCer as a functional cosmeceutical food is a viable option to ameliorate skin conditions, including improvement in skin barrier function, reduction of brown spots, and fixation of chapped skin.

**Key Words** GlcCer, torula yeast, skin dryness, TEWL, skin condition

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**Summary** Glucosylceramide (GlcCer) is present in foods such as barley, corn, and wheat flour. GlcCer derived from different foods has differences in its physiological effects, depending on the sphingoid backbone and constituent fatty acids. In this study, we investigated the moisturizing and skin conditioning effects of GlcCer derived from torula yeast (Candida utilis) in healthy human subjects. The participants were randomly distributed in a crossover, double-blind comparative manner. Seventeen volunteers were orally administered both 1.8 mg/d of GlcCer derived from torula yeast and a placebo for 4 wk. Before and after oral administration, transepidermal water loss (TEWL) was measured and the objective skin condition observation and a questionnaire on skin condition were conducted. The primary endpoint was TEWL; secondary endpoints included the objective and subjective skin conditions. The change in TEWL over the study period on the forearm was $-0.97 \pm 0.48$ and $-1.26 \pm 0.46$ g/m$^2$·h in the placebo and GlcCer groups, respectively, with significantly lower ($p=0.01$) TEWL observed in the GlcCer group. Brown spots increased in the placebo group but significantly decreased in the GlcCer group ($p=0.04$). Although chapped skin worsened in the placebo group, it significantly improved in the GlcCer group ($p=0.04$). The use of torula yeast-derived GlcCer as a functional cosmeceutical food is a viable option to ameliorate skin conditions, including improvement in skin barrier function, reduction of brown spots, and fixation of chapped skin.
Chemicals Codex and the Code of Federal Regulations of the United States of America describe the safety of torula yeast (17, 18). Unlike common edible yeast, baker’s yeast (Saccharomyces cerevisiae), and brewer’s yeast (S. cerevisiae and S. pastorianus), which contain inositolphosphoceramide, torula yeast contains GlcCer and, therefore, is considered to be a better source of GlcCer (19). The continuous fermentation of torula yeast provides a stable supply of GlcCer, with the dry content reaching a relatively high value (0.1%). Furthermore, GlcCer is extracted from the yeast residue of yeast extract. This enables relatively low-cost production; indeed, its cost effectiveness is one of its advantages (20). We have focused on the moisturizing and anti-aging effects of GlcCer derived from torula yeast in healthy human subjects.

MATERIALS AND METHODS

Participants. The study began in November 2015 and ended in February 2016. The subjects requested to participate included both men and women, older than 20 y old, who experienced subjectively assessed skin chapping and dryness in winter. After informed consent was obtained from subjects, 17 volunteers (10 men and 7 women; mean age, 43.6 ± 10.3 y old) were enrolled. The following exclusion criteria were applied: participants with skin diseases, such as atopic dermatitis; undergoing drug treatment as outpatients; pregnant or planning to become pregnant; or breastfeeding. This study was granted approval from the Kyoto Prefectural University Ethics Committee (2015, no. 95).

Torula yeast-derived glucosylceramide. GlcCer derived from torula yeast was provided by KOHJIN Life Sciences Co., Ltd. (Tokyo, Japan). The purity of GlcCer was greater than 90%. In total, 1.8 mg of GlcCer was weighed into each Matsuya cellulose white capsule no. 3 (Matsuya Corporation, Osaka, Japan) and the remaining space was filled with dextrin. For the placebo, the capsules were filled with dextrin. The composition of the capsules is shown in Table 1.

Study design. The participants were randomly distributed in a crossover, double-blind comparative manner (Fig. 1). The baseline was measured at the first and third measurement. A 4-wk washout period separated the conditions; all participants completed both testing conditions. The participants ingested a capsule (1.8 mg torula yeast-derived GlcCer or the placebo) once daily. An email reminder to take their capsules was sent to the subjects every day. On each measurement date, TEWL, an objective observation of skin condition, a questionnaire on skin condition, and blood sample collection (general biochemistry) were conducted under the supervision of a physician. From 1 mo prior to the testing period to the end of the study, changes in cosmetics or the use of functional foods with moisturizing effects were prohibited.

Measurement methods. During measurements, the room temperature and humidity were maintained at 22 ± 2˚C and 50 ± 10%, respectively. The participants were requested to rest in a sitting posture, dressed in T-shirts, without jackets, for 30 min prior to measurements to unify the conditions of water loss. TEWL was determined by using a Tewameter TM300 (Courage+Khazaka electronic GmbH, Cologne, Germany) and measured in accordance with the manufacturer’s guidelines (21, 22). The average values were calculated from the most consistent values obtained over a 20 s measurement. The measurements sites were 10 cm from the right elbow toward the wrist (forearm) and at 3 cm from the earlobe (cheek) along a line extending from the right earlobe to the right corner of the mouth.

The objective skin conditions were measured by VISIA® Evolution (Canfield Scientific, Fairfield, NJ) (23–25) on a six-point scale (0 = none to 5 = very severe), with a lower score indicative of a favorable skin condition. The categories for this scale included fine wrinkles,

| Composition | Placebo | GlcCer |
|-------------|---------|--------|
| Capsule (mg) | 50.0 | 50.0 |
| Dextrin (mg) | 130.0 | 128.2 |
| Torula yeast-derived glucosylceramide (mg) | — | 1.8 |

GlcCer: glucosylceramide.
coarse wrinkles, mottled hyperpigmentation, lentigines, irregular depigmentation, pore size, visual roughness, erythema/telangiectasia, and overall solar damage. In our study, the following were evaluated: general hyperpigmentation for spots; deep pigmentation for ultraviolet spots; superficial melanin for brown spots; superficial hemoglobin for red area; porphyrin, metabolite of *Propionibacterium acnes* that indicates the risk of acne; color uniformity for texture; and length of wrinkle lines. Measurements of the right side of the face were taken with VISIA, as conducting measurements on the side of the face is easier than conducting measurements on the front of the face.

The questionnaire used to subjectively assess skin conditions asked participants about firmness, luster, dryness, itchiness, chapped skin, tautness, blemishes, dullness, ease of cosmetic application (for women only), and overall skin health. The responses were rated based on a free description.

**Statistical analysis.** All values were presented as the mean. SPSS Statistics 22 (IBM, Tokyo) was used to compute the data analysis, with values of *p* < 0.05 considered to represent statistical significance. The paired *t*-test was used to compare the data collected before and after within each group (placebo and GlcCer) and the differences between the groups. The analysis of TEWL and the subjective assessment of skin conditions were conducted only in participants with subjectively chapped skin and dryness in the winter. All participants underwent objective assessment of skin conditions. The data are shown as the mean ± standard error.

**RESULTS**

The comparison of the baseline data on TEWL in the forearm and cheek showed a slight, but insignificant difference between the placebo and GlcCer groups (*p* = 0.06) (Table 2). TEWL in the forearm was significantly decreased after ingestion of GlcCer compared with before ingestion (*p* = 0.02) (Table 2, Fig. 2). The change in TEWL over the study period on the forearm was 0.97 ± 0.48 and −1.26 ± 0.46 g/m²·h in the placebo and GlcCer groups, respectively, with significantly lower (*p* = 0.01) TEWL observed in the GlcCer group (Table 2).

**Objectively assessed skin conditions**

The comparison of the baseline data on the objective skin condition between the placebo and GlcCer groups revealed that there were significant differences pertaining to brown spots (*p* = 0.05; Table 2). For brown spots, the changes in score were 0.01 ± 0.01 and −0.01 ± 0.01 points in the placebo and GlcCer groups, respectively. Brown spots increased in the placebo group but decreased in the GlcCer group (*p* = 0.04, Table 2). No significant differences were found for the other indices when compared between the groups or within each group before and after the intervention (Table 2).

### Table 2. Transepidermal water loss rates (g/m²·h) and objective skin condition before and after the ingestion of placebo or GlcCer.

|                  | Placebo | GlcCer | Before | After | Difference | p value1 | p value2 | p value3 |
|------------------|---------|--------|--------|-------|------------|----------|----------|----------|
| **Transdermal water loss rates (g/m²·h)** |         |        |        |       |            |          |          |          |
| Forearm          | 12.44±0.55 | 9.44±0.45 | 0.97±0.48 | 0.04±0.2 | 0.03±0.01 | 0.04±0.09 | 0.04±0.03 | 0.00±0.01 |
| Cheek            | 13.64±1.15 | 10.49±0.69 | 2.22±0.69 | 0.22±0.09 | 0.01±0.01 | 0.02±0.03 | 0.02±0.00 | 0.00±0.00 |
| **Objective skin conditions** |         |        |        |       |            |          |          |          |
| Brown spots      | 0.01±0.01 | 0.01±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 |
| Porphyrin        | 0.01±0.01 | 0.01±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 |
| Red areas        | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Spots            | 0.01±0.01 | 0.01±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 |
| Texture          | 0.01±0.01 | 0.01±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 | 0.00±0.01 |
| UV spots         | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Wrinkles         | 0.03±0.01 | 0.03±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 |

1 Paired *t*-test comparing the placebo and GlcCer groups at the baseline.
2 Paired *t*-test comparing the placebo and GlcCer groups after ingestion. 3 Paired *t*-test comparing the placebo and GlcCer groups after ingestion.
Subjectively assessed skin conditions

The comparison of the baseline data on the subjective skin condition between the placebo and GlcCer groups, revealed that there were no significant differences in any evaluation index (Table 3). For chapped skin, the changes in score were \(-1.35 \pm 0.63\) and \(0.55 \pm 0.53\) points in the placebo and GlcCer groups, respectively. Although chapped skin deteriorated in the placebo group, it significantly improved in the GlcCer group (\(p = 0.04\), Table 3). For the other indices, no significant differences were observed for the other indices when compared between the groups or within each group before and after the intervention. There were no subjective physical symptoms of which participants complained.

DISCUSSION

In this study, the daily intake of 1.8 mg of torula yeast-derived GlcCer for 4 wk led to the following: (1) decreased TEWL from the forearm, indicating moisturizing effects; (2) improved scores for brown spots, with recognized inhibitory effects of melanin; and (3) improvement in the symptoms of chapped skin.

The most abundant GlcCer species derived from torula yeast were composed of hydroxylated 16- or 18-carbon fatty acids, with GlcCer (d19:2/C18:0h) accounting for approximately 80% of GlcCer, which was different from plant- or animal-derived ceramides. The ingestion of various kinds of ceramides may confer beneficial effects.

In a study in which participants ingested konjac extract (1.8 mg per day as GlcCer) or a placebo, TEWL was significantly reduced from the cheek after 8 wk and from the back after 12 wk compared with the placebo. In contrast, no significant difference was noted in TEWL from the forearm (12). An investigation of dry skin demonstrated that, compared with the placebo group, the ingestion of free ceramide-containing acetic acid bacteria (ceramide equivalent amounts: 400 and 800 \(\mu g\) per day) led to higher TEWL from the cheek after 4 wk. However, after 6 wk, the TEWL from the ceramide-treated group was significantly lower than that at baseline (13). In another study, the daily intake of beet extract (GlcCer equivalent amounts: 0.6 and 1.8 mg per day) did not exert significant effects on the TEWL after 4 and 8 wk from either the arm or the cheek. Furthermore, there were no differences in melanin content or erythema (27). The average baseline values of TEWL from the cheek for the ceramide-treated groups were 14.1, 26.3, 21.4, and 22.2 g/m² h for yeast (this study), konjac, acetic acid bacteria, and beets, respectively. The average baseline values of TEWL from the forearm were 9.3, 7.6, 8.3, and 7.2 g/m² h for yeast (this study), konjac, acetic acid bacteria, and beets, respectively (12, 13, 27). Compared with previous studies, we observed lower TEWL rates on the cheek and higher rates on the forearm. These results suggested that the effect of GlcCer on TEWL rates was not apparent on the cheek, but was on the forearm. In this study, the test ingestion period was short, only 4 wk, and this may also be the cause of the insignificant effects observed for the TEWL dynamics of the cheek.

We observed a significant improvement in brown spots in the GlcCer group compared with the placebo group, although the baseline differences between the two groups may have affected this result. Brown spots indicate the presence of melanin on the surface layer. In prior in vitro studies, the inhibitory effect of melanin on GlcCer was noted: the suppressive mechanisms of melanin production were reported to result from the inhibitory effects of tyrosinase on mRNA (28) or of the microphthalmia-associated transcription factor (14).

To date, there have been few studies that have assessed the effect of GlcCer ingestion on skin lightening in humans; to the best of our knowledge, this is one of the first reports. Furthermore, regarding the objective evaluation of wrinkles, although not statistically significant, wrinkles tended to be more suppressed in the GlcCer group than in the placebo group (\(p = 0.10\)). Wrinkles result from the age-related reduction in dermal collagen and stratum corneum ceramide (29). The topi-
Table 3. Subjective skin condition before and after the ingestion of placebo or GlcCer.

| Subjective skin conditions | Placebo | GlcCer | p value¹  | Before | After | Difference | p value²  | Before | After | Differece | p value³ |
|---------------------------|---------|--------|-----------|--------|-------|------------|-----------|--------|-------|-----------|---------|
| Firmness                  | 4.65±0.47 | 4.62±0.41 | 0.39      | 4.23±0.43 | 4.22±0.38 | 0.48       | 4.33±0.48 | 4.43±0.42 | 0.33      | 0.49      | 0.36      |
| Luster                    | 3.96±0.38 | 3.76±0.32 | 0.30      | 3.65±0.37 | 3.34±0.32 | 0.63       | 3.84±0.38 | 4.03±0.36 | 0.57      | 0.27      | 0.47      |
| Dryness                   | 4.95±0.38 | 4.84±0.32 | 0.63      | 4.58±0.37 | 4.82±0.32 | 0.48       | 4.78±0.38 | 4.94±0.32 | 0.64      | 0.29      | 0.43      |
| Itchiness                 | 4.69±0.38 | 4.39±0.32 | 0.63      | 4.35±0.37 | 4.75±0.32 | 0.48       | 4.84±0.38 | 4.95±0.32 | 0.64      | 0.36      | 0.53      |
| Chapped skin              | 7.44±0.38 | 6.76±0.32 | 0.63      | 6.08±0.37 | 6.25±0.32 | 0.48       | 6.95±0.38 | 7.12±0.32 | 0.64      | 0.53      | 0.70      |
| Blanched skin, dryness    | 4.69±0.38 | 4.93±0.32 | 0.63      | 4.35±0.37 | 4.64±0.32 | 0.48       | 4.84±0.38 | 5.06±0.32 | 0.64      | 0.36      | 0.53      |
| Easy of cosmetic application | 4.69±0.38 | 4.93±0.32 | 0.63      | 4.35±0.37 | 4.64±0.32 | 0.48       | 4.84±0.38 | 5.06±0.32 | 0.64      | 0.36      | 0.53      |
| Overall skin health       | 5.14±0.38 | 5.35±0.32 | 0.63      | 4.84±0.38 | 5.05±0.32 | 0.64       | 5.06±0.38 | 5.23±0.32 | 0.64      | 0.36      | 0.53      |

Mean±standard error.

1) Paired t-test comparing before and after ingestion in the groups.
2) Paired t-test comparing the placebo and GlcCer groups at the baseline.
3) Paired t-test comparing difference before and after ingestion in the placebo and GlcCer groups.

* p<0.05.

Kal application of ursolic acid and niacinamide has been reported to increase skin ceramide content and elicit an anti-wrinkle effect (30, 31). However, the oral administration of GlcCer may also be expected to produce an anti-wrinkle effect.

In this study, significant improvements in subjectively assessed chapped skin conditions were observed in the GlcCer group compared with the placebo group. The itching score decreased −0.78 points before and after administration, although this difference was not significant. Previous work on a konjac extract containing GlcCer showed that itching symptoms in the forearm were significantly improved after the use of GlcCer (12). In our study, the baseline subjective symptoms of itching scored highly; at 7.58 points; it is possible that such a high initial score may have masked any amelioration of itching symptoms after the ingestion of GlcCer. In contrast, as the subjective symptoms of chapped skin were moderate at 5.15 points, it was considered that GlcCer significantly improved the symptoms. Thus, the present study demonstrated that the ingestion of torula yeast-derived GlcCer inhibited TEWL and melanin production and improved chapped skin. It is meaningful that the beautifying effects of GlcCer intake were demonstrated in humans. Thus, the use of torula yeast-derived GlcCer as a functional cosmetic food is a viable option for the treatment of skin conditions.

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