Original Article

Double-Blind Study on Effects of Glucosyl Ceramide in Beet Extract on Skin Elasticity and Fibronectin Production in Human Dermal Fibroblasts

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Abstract

Objective: To determine the effect of beet extract on skin elasticity in female volunteers with dry skin and in human dermal fibroblasts.

Method: To assess the effects of oral administration of glucosyl ceramide contained in beet extract (beet ceramide), we conducted an 8-week double-blind comparison study with 35 females (mean age: 40.9±4.2 years) with mild subjective symptoms of dry skin and declining keratin moisture levels. The compound was administered as glucosyl ceramide at 0, 0.6, or 1.8 mg/day to 3 groups (n=11, 12, 12, respectively).

Results: Scores improved significantly for the following subjective skin symptoms: “concerned about dull skin,” “concerned about spots or freckles,” “sticky, oily skin,” “coarse and desiccated skin,” “not elastic, not glossy,” “concerned about rough skin,” “bags under eyes.” In addition, perspiration levels improved. The skin elasticity test (Cutometer) indicated that the elasticity index (R2 and R7) improved in a dose-dependent manner. However, we were unable to confirm the effects of ceramide on increasing skin moisture as reported in previous studies. In experiments involving human dermal fibroblasts, addition of beet ceramide promoted fibronectin synthesis and mRNA expression but had no effect on fibroblast proliferation or collagen synthesis.

Conclusion: Results from clinical trials and experiments suggested that oral ingestion of beet ceramide may stimulate intracellular signals and exert favorable effects on the extracellular matrix, including the induction of fibronectin synthesis. In addition, we confirmed the safety of administering beet ceramide to humans.

KEY WORDS: ceramide, skin elasticity, skin moisture, fibronectin, beet

Introduction

Lamellae, which form between cells in the outermost layer of skin, play an important role as a perspiration barrier in humans ¹⁾. In the human stratum corneum, 50% of the lipid lamella is composed of ceramide (N-acylsphingosine), one of the sphingolipids that make up the skin, and ceramide content decreases gradually with aging. As such, ceramide is considered to be a potentially important factor in maintaining young-looking, healthy skin.

We previously examined the effects of cosmetics ²⁾, supplements ³⁾, and health foods ⁴⁾ on skin in the field of Anti-Aging Medicine by utilizing common, objective parameters such as skin image analysis devices. According to the Incorporated Administrative Agency at the National Institute of Health and Nutrition, “Ceramide is said to have a ‘moisturizing effect’ as well as a ‘smoothing effect,’ but no reliable data regarding the effectiveness and safety of oral ingestion of ceramide can be found ⁵⁾.” However, the mechanism by which extrinsic ceramide is absorbed and

transported to the skin and ceramide’s essential role in physiology and effect on cell signals is still unclear.

Beets, from which sugar can be made, contain fibers which have relatively high amounts of glucosyl ceramide. Glucosyl ceramide extract from beets (beet ceramide) can be obtained through ethanol extraction. To examine the effects of beet ceramide on skin and quality of life, we conducted randomized controlled tests on the effect of oral administration of beet ceramide. In addition, since extracellular matrix proteins and natural moisturizing factors, both of which influence skin elasticity, are synthesized in dermal fibroblast cells, we examined the effect of beet ceramide on human fibroblast proliferation and synthesis of extracellular matrix proteins, such as collagen and fibronectin. Here, we report new information acquired through clinical and fundamental research.

Methods

Subjects

A total of 50 Japanese female volunteers (aged 35 to 50 years) reporting dry skin were screened based on level of skin moisture of the medial surface of their arms as measured by Corneometer (CM825; Courage + Khazaka Electronic, GmbH, Kern, Germany). Of these 50 initial volunteers, 35 with relatively low levels of skin moisture were selected to participate in the present study (mean age: 40.9±4.2 years, body mass index [BMI]: 21.1±3.4 kg/m²).

Participants were randomly divided into three groups: control (n=11), low-dose test group (LD group; n=12), and high-dose test group (HD group; n=12). One volunteer in the control group dropped out (drop out rate: 2.8% [1/36]) after suspecting pregnancy. In the LD group, one person made a mistake in answering the questionnaire, and her data for the evaluation of subjective symptoms was removed.

Test Product

The test product, beet ceramide, was provided by Nippon Beet Sugar Manufacturing Co. Ltd. (Minato-ku, Tokyo) in 200-mg capsules including gelatin, glycerin, and caramel color (Table 1). Beet ceramide is a botanical lipid contained in the beetroot domestically grown in Hokkaido as a raw material of sugar. Both the test product and placebo were odorless, tasteless, brown 11mm soft capsules. There were no differences in appearance or smell between the test product and placebo.

Water-soluble materials such as sugar are chiefly extracted from the beetroot using warm water. The cell walls of the residues (beet fibers) contain approximately 2% as lipids. In the present

study, this lipid was extracted and condensed using ethanol and then was filtrated using charcoal or diatomaceous earth to give the lipid segment. To this segment, cyclodextrin was added at a ratio of approximately 1:1, ultimately producing lipid powder known as “beet ceramide.” This powder contains 3.0%-3.5% glucosyl ceramide, which mainly consists of a sphingoid base with dihydroxy unsaturated bonds trans-located at the fourth and eighth positions (d18:2^{4t,8t}) and a 2-hydroxy fatty acid with 16 carbon atoms (16h:0) (Fig. 1)⁶⁾.

In Japan, beet fiber has been sold for 15 years or more as a food material, functioning as a source of dietary fiber, and neither serious adverse events nor side effects have yet been reported. Further, the Food and Drug Administration (FDA) in the United States is currently reviewing the application for beet fiber as a Generally Recognized As Safe (GRAS) material. At the Japan Health Food & Nutrition Association, a technical committee concerning specially designated health foods evaluated beet fibers and determined that a minimum of 5 g of beet fiber is needed to be ingested daily to achieve intestinal effect; the maximum amount determined not to induce a laxative effect was 20 g/day. Twenty to sixty milligrams of beet ceramide correspond to approximately 2-6 g as raw beet fiber. No issue has been raised with regard to its safety as a food item. Safety of beet ceramide has been confirmed by third party specialized agencies (New Drug Development Research Center Inc., Hokkaido, Japan) via a reverse mutation test, single dose toxicity test, and repeated dose toxicity test over 28 days.

Study Design

The present study was a double-blinded, dose-comparison study consisting of three groups: control group (11 cases), LD group (12 cases), and HD group (12 cases). Subjects in the control, LD, and HD groups were orally administered a capsule of placebo, low-dose test material, or high-dose test material, respectively, twice daily for eight weeks. Intake in the control, LD, and HD groups was 0, 20, or 60 mg/day as beet ceramide, and 0.0, 0.6, or 1.8 mg/day as glucosyl ceramide, respectively. Body and skin were examined three times during the observation period.

Participants ingested two soft capsules a day for eight weeks (one each after breakfast and dinner). Subjects were instructed to take only the test product, described below, if breakfast or dinner was skipped. Percentage intake was 98.6% in the control group, 98.7% in the LD group, and 98.0% in the HD group (average ingestion rate: 98.4%).

Table 1 Contents of test products in soft capsules

Group	Control	Low dose test group (LD group)	High dose test group (HD group)
amount (mg/capsule)	placebo	test product	test product
Beet ceramide	0	10	30
Cornstarch	30	20	0
Yellow beeswax	14	14	14
Fatty acid esters of glycerin	14	14	14
Safflower oil	142	142	142
Total	200	200	200

Fig. 1. Main structure of beet-derived glucosyl ceramide (6).

During the study, participants were instructed to maintain their normal lifestyles, including alcohol intake, and in particular to avoid excessive food intake, excessive exercise, and lack of sleep. Participants recorded the presence (and severity) or absence of adverse events, intake of the test product, lifestyle habits, and dietary/exercise habits in a diary.

The study was conducted from January 15 to March 13, 2010. Written consent was obtained from each participant after full explanation of the purpose and methods of the study, participants' rights, and that no penalty was associated with dropout.

Evaluation Methods

For the evaluation of subjective symptoms, items were divided into two categories: "physical symptoms" and "mental symptoms." The Anti-Aging Quality of Life (AAQoL) Common Questionnaire was used to rate individual symptoms on a five-point scale ranging from 1 to 5 points, as described previously⁷⁾.

Facial skin was evaluated on the right cheek with regard to moisture level, melanin levels, and presence of erythema, as in previous reports⁸⁾. Level of skin moisture was measured using a Corneometer (CM825; Courage + Khazaka Electronic, GmbH)^{9,10)}, which functions on the principle that electric capacitance changes according to the skin's moisture level. Transepidermal water loss (TEWL) was measured using a Tewameter (TM300; Courage + Khazaka Electronic, GmbH)¹¹⁾, which measures water vapor pressure at two points 1-2 mm from the skin surface. The volume of water transpiring from the epidermis was then calculated and expressed as volume of water (g/h·m²) lost per hour per square meter of skin. Measurements were taken 20 minutes after face-washing and becoming accustomed to the indoor environment. The temperatures and humidity levels during the measurements are shown in Table 2.

The level of melanin in the skin and degree of erythema were measured using a Mexameter (MX18; Courage + Khazaka Electronic, GmbH), which irradiates the skin with specific wavelength light and measures the reflected light using a diode to calculate the melanin and erythema indices^{12,13)}.

Skin elasticity was evaluated using a Cutometer (MPA580; Courage + Khazaka Electronic, GmbH)¹⁴⁻¹⁶⁾. Briefly, a probe was placed on the skin surface, and an area of skin was drawn up into the probe using negative pressure; the length of skin drawn into the probe was then measured using a glass prism. The physical principles underlying the results obtained from the Cutometer are shown in Fig. 2, and Table 3 shows the various indices that can be derived and their interpretations. The R2 index is the ratio of skin

Table 2 Temperatures and humidity levels

	Date	Weather	Average outdoor temperature/humidity		Average room temperature/humidity	
Week 0	1/15	Sunny	4.2°C	34.0%	24.5°C	43.0%
Week 4	2/12	Rain	2.9°C	68.0%	24.0°C	49.5%
Week 8	3/12	Sunny	13.2°C	47.5%	24.5°C	47.5%

Table 3 Definitions of skin elasticity indices by Cutometer

Index	Expression	Point of measurement
R0	Uf1	Length of the skin when elongated
R1	Uf1-Ua1	Length of the skin after elongation and constriction
R2	Ua1/Uf1	Recovery ratio of the skin length
R3	Uf2	Length of the skin when elongated at second time
R4	Uf2-Ua2	Length of the skin after elongation and constriction at second time
R5	Ur1/Ue1	Ratio of elasticity part when elongated and during constriction
R6	Uv1/Ue1	Ratio of viscosity and elasticity part when elongated
R7	Ur1/Uf1	Ratio of elasticity part during constriction
R8	Ua1	Length of skin constriction after first elongation
R9	Uf2-Uf1	Difference of skin length between first and second elongation
F0	Area A	Skin distortion during elongation
F1	Area B	Skin distortion during constriction

Fig. 2. Principles underlying the measurement of skin elasticity by Cutometer.

length recovery after elongation and constriction (U_{a1}/U_{f1}), indicating improvement in elasticity as the ratio approaches 1.00; an ideal elastic material has an R2 value of 1.00, and normal skin has values in the range of 0.3–0.5. The R7 index is a ratio of skin elasticity during constriction (U_{r1}/U_{f1}); the most elastic skin has an R7 value close to 1.00. The R2 and R7 indices are the most reliable ones derived from a Cutometer, and a previous study found that these indices decrease with aging, with the curve shifted down and forward in patients with diabetes mellitus¹⁷⁾. Skin elasticity was measured four times to reduce error, with the highest and lowest values removed before averaging calculations.

We evaluated accumulated amounts of advanced glycation endproducts (AGEs) in the skin as an index of glycation using an AGE Reader™ (DiagnOptics, Groningen, Netherlands). This measurement is a non-invasive evaluation method that excites AGEs accumulated in the skin tissue by exposure to ultraviolet radiation, using the observed characteristics to generate specific fluorescence (auto-fluorescence; AF)^{18,19)}. Examination of skin biopsies from diabetic and dialysis patients has confirmed that skin AF is correlated with accumulation of typical AGEs in the skin, such as pentosidine and *N*^ε-(carboxymethyl)lysine (CML). In addition, AF is high in type 2 diabetes patients and increases in healthy persons with age²⁰⁾. While AF measurements are generally taken at the forearm and upper arm, skin AF was impossible to measure at the forearms of a substantial number of subjects in our previous studies due to the presence of sun tan (data not shown). In this study, subjects were asked to rest their elbow on the AGE Reader™. Measurements were taken at the outer upper right arm, 10 cm away from the edge of the elbow. After wiping the measuring area using a cotton swab and alcohol, AF intensity was measured three times at the same point, and the results were expressed as the mean of three values.

Reflectance, which affects measured values of skin auto-fluorescence, was determined here, using spectra obtained from skin measurement, as subtraction between white standard skin and dark skin. An external reflection standard was measured using each apparatus above to determine correction factors for each wavelength. Reliability of measurement results is low when the reflectance value is low.

Experimental Method

Culture of Human Dermal Fibroblasts

Normal human dermal fibroblasts were obtained from AGC Techno Glass (Funabashi-city, Chiba). Cells were cultured and maintained in fibroblast basal medium (AGC Techno Glass) containing 10% fetal bovine serum and antibiotics at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

Measurement of Proliferation Rate of Human Dermal Fibroblasts

Appropriate concentrations of beet ceramide (adjusted to a final concentration of 0.1% with dimethyl sulfoxide [DMSO]) were added to the fibroblast basal medium in which human dermal fibroblasts were suspended. To observe the vehicle effect, 0.1% of DMSO was added to 0 μg/mL of beet ceramide. Suspended cells were plated onto 96-well tissue culture plates at 1×10^3 cells per well and incubated for 72 hours in a CO₂ incubator. After incubation, cell number in each well was estimated via colorimetric method using a Cell Counting Kit (Dojindo Laboratories, Kamimashiki-gun, Kumamoto).

Observation of Fibronectin and Type I Collagen Expression Via Fluorescent Immunostaining

Human dermal fibroblast cells were spread onto a 3.5-cm flat glass-bottom dish, and incubated for 24 hours in a CO₂ incubator with beet ceramide added to the medium at various concentrations. The cells can be stained directly with fluorescent antibodies with the glass-bottom dish used in this experiment. The cells were then fixed using 4% formaldehyde followed by treatment with 0.2% Triton X-100/PBS (-). After blocking in 10% goat serum, the specimens were treated with either mouse anti-human fibronectin monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or mouse anti-human type I collagen monoclonal antibody (Chemicon International, Billerica, MA, USA) as primary antibodies, followed by FITC-labeled goat anti-mouse IgG antibody (Santa Cruz Biotechnology) as a secondary antibody. After washing, fluorescence was observed using an inverted fluorescence microscope. Because anti-collagen type I antibodies can detect collagen type I in the cytosol²¹⁾, they were used for the relative comparison of collagen type I production.

Measurement of Fibronectin mRNA Gene Expression Using Real-Time Polymerase Chain Reaction (PCR)

Human dermal fibroblast cells were spread onto a 6-cm tissue culture dish and incubated for 24 hours in a CO₂ incubator with beet ceramide added to the medium at various concentrations. After collecting cells, total RNA was extracted and purified using Isogen (Nippon Gene, Chiyoda-ku, Tokyo), and cDNA was then adjusted using a high capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA, USA). The adjusted cDNA was used as a substrate and treated with human fibronectin gene (TaqMan Gene Expression Assay; Applied Biosystems). Fibronectin mRNA was assayed semi-quantitatively using real-time PCR. Glyceraldehyde-3-phosphate dehydrogenase was used as a reference gene.

Ethical Considerations and Declaration of No Commercial Relationship

The study was performed at a third party institution in compliance with the ethical principles of the Declaration of Helsinki and the Personal Information Protection Law, and with reference to the “Ordinance regarding the Good Clinical Practice (GCP)” (MHW Ordinance No. 28, dated March 27, 1997). At the medical institution where the study was performed, an ethical committee for clinical studies held meetings to review the ethical justification and validity of the study. This study was approved by the committee and conducted according to the approved protocol. The activities of the laboratory are annually declared to “the Committee of Conflicts of Interests” of Doshisha University. The authors have no commercial relationship with companies related to the field of the study.

Statistical Analysis

Results are expressed as average $\pm$ standard deviation (SD) in the clinical study (Table 4-8) or as average $\pm$ standard error of the mean (SEM) in the experimental study (Fig. 3 and 6). Statistical analyses were performed using the statistical analysis application Dr. SPSSII (SPSS Japan Inc., Shibuya-ku, Tokyo). Either a paired *t*-test or Dunnett’s test was used to compare measurements from Week 0 and data after treatment. For inter-group analysis, between-group differences before and after the study were compared using the Mann-Whitney U test. Correlation analysis was assessed using Spearman’s method, and significance was set at $p < 0.05$.

Table 4 Physical and mental symptoms, lifestyle-related behaviors

		Control (n=11)						LD group (n=12)						Inter-group analysis (vs. Control) <i>p</i> value	HD group (n=12)						Inter-group analysis (vs. Control) <i>p</i> value
		Week 0		Week 4		Weeks 8		Week 0		Week 4		Weeks 8			Week 0		Week 4		Weeks 8		
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		
Physical symptoms																					
Tired eyes		2.5 ± 0.7		2.5 ± 0.9		2.5 ± 0.8		3.2 ± 1.0		2.8 ± 1.0		2.8 ± 1.1		0.057	2.8 ± 1.2		2.8 ± 1.0		2.7 ± 1.2		0.936
Blurry eyes		2.0 ± 0.6		2.2 ± 0.9		1.8 ± 1.1		2.4 ± 1.2		2.1 ± 1.0		2.4 ± 1.0		0.888	2.5 ± 1.2		2.5 ± 1.0		2.3 ± 1.1		0.593
Eye pain		1.7 ± 0.6		1.8 ± 0.8		1.7 ± 0.6		1.6 ± 0.8		1.9 ± 1.0		1.8 ± 1.0		0.665	1.8 ± 1.1		1.7 ± 0.9		1.7 ± 0.8		0.748
Stiff shoulders		3.5 ± 1.0		3.5 ± 0.9		3.5 ± 1.0		3.5 ± 1.2		3.5 ± 1.2		3.2 ± 1.3		0.629	3.3 ± 0.9		3.3 ± 1.1		3.1 ± 1.0		0.898
Muscular pain/stiffness		2.6 ± 0.5		2.5 ± 0.8		2.5 ± 1.0		2.6 ± 1.2		2.4 ± 1.1		2.8 ± 1.3		0.391	2.8 ± 1.2		3.0 ± 1.3		2.9 ± 1.2		0.449
Palpitations		1.5 ± 0.5		1.5 ± 0.8		1.4 ± 0.7		1.7 ± 0.5		1.7 ± 0.5		1.8 ± 0.8		0.525	1.8 ± 1.0		2.1 ± 1.0		1.9 ± 0.8		0.511
Shortness of breath		1.5 ± 0.5		1.4 ± 0.5		1.4 ± 0.5		1.8 ± 0.6		1.6 ± 0.7		1.8 ± 0.8		0.805	1.6 ± 0.7		1.7 ± 0.8		2.1 ± 0.8**		0.131
Tendency to gain weight		2.9 ± 1.1		2.6 ± 1.2		2.5 ± 1.1		3.0 ± 1.1		3.0 ± 1.0		2.9 ± 1.2		0.443	2.8 ± 1.1		2.8 ± 0.9		3.0 ± 0.7		0.092
Weight loss; thin		1.4 ± 0.5		1.5 ± 0.5		1.4 ± 0.5		1.8 ± 0.8		1.8 ± 0.9		2.0 ± 1.0		0.562	1.4 ± 1.2		1.9 ± 1.2		1.7 ± 0.8		0.201
Lethargy		2.3 ± 0.5		2.4 ± 0.8		2.5 ± 0.7		2.3 ± 0.9		2.3 ± 1.1		2.4 ± 0.9		0.727	2.3 ± 0.8		2.8 ± 0.8*		2.3 ± 0.8		0.677
Lack of sense of wellness		2.4 ± 0.8		2.1 ± 0.5		1.8 ± 0.6*		1.8 ± 0.7		1.7 ± 0.9		1.9 ± 1.0		0.343	2.3 ± 0.6		2.3 ± 0.9		2.1 ± 0.8		0.343
Thirst		2.4 ± 0.7		2.2 ± 0.9		2.1 ± 0.8		2.1 ± 1.1		2.1 ± 1.2		2.2 ± 1.4		0.433	1.8 ± 0.9		2.0 ± 1.0		2.3 ± 1.0*		0.053
Skin problems		2.5 ± 0.8		2.1 ± 0.3		2.2 ± 0.6*		2.5 ± 0.5		2.5 ± 1.0		2.4 ± 1.0		0.354	2.9 ± 0.9		2.4 ± 0.5		2.8 ± 0.6		0.630
Anorexia		1.5 ± 0.5		1.9 ± 1.1		1.4 ± 0.5		1.5 ± 0.5		1.7 ± 0.8		1.8 ± 1.0		0.726	1.4 ± 0.5		1.7 ± 0.7		1.6 ± 0.7		0.363
Early satiety		2.1 ± 0.5		1.6 ± 0.5		1.7 ± 0.6		1.7 ± 0.8		1.8 ± 1.1		1.4 ± 0.5		0.195	2.1 ± 1.0		2.1 ± 1.0		1.9 ± 0.7		0.444
Epigastralgia		2.1 ± 0.7		1.9 ± 0.8		2.0 ± 0.8		1.8 ± 1.0		1.8 ± 1.0		1.6 ± 0.9		0.677	1.9 ± 0.9		2.1 ± 0.9		2.1 ± 1.1		0.238
Liable to catch colds		2.2 ± 0.6		2.3 ± 0.6		2.1 ± 0.5		1.8 ± 0.8		2.1 ± 1.0*		1.8 ± 0.9		0.293	2.1 ± 0.8		2.1 ± 1.1		2.0 ± 0.7		0.974
Coughing and sputum		2.0 ± 0.8		1.9 ± 0.7		2.0 ± 0.8		1.7 ± 0.7		1.9 ± 1.2		1.8 ± 1.0		0.191	1.9 ± 1.2		2.2 ± 1.0		2.3 ± 0.9		0.110
Diarrhea		1.8 ± 0.8		1.9 ± 0.8		2.0 ± 0.8		1.8 ± 0.8		1.7 ± 0.7		1.8 ± 0.8		0.768	1.8 ± 0.8		1.8 ± 0.8		1.9 ± 0.8		0.919
Constipation		2.7 ± 0.9		2.5 ± 0.8		2.5 ± 0.8		2.6 ± 1.4		2.5 ± 1.2		2.5 ± 1.2		0.649	2.7 ± 0.8		2.8 ± 1.1		2.7 ± 1.1		0.549
Hair loss		2.8 ± 0.9		2.4 ± 0.7		2.1 ± 0.9*		1.8 ± 0.9		2.0 ± 0.9		1.9 ± 0.9		0.010	2.6 ± 1.2		2.5 ± 1.0		2.5 ± 1.2		0.057
Gray hair		3.2 ± 0.9		3.0 ± 0.6		2.9 ± 1.2		2.8 ± 1.1		2.8 ± 1.1		2.8 ± 1.2		0.433	2.4 ± 1.1		2.4 ± 1.2		2.6 ± 0.9		0.184
Headache		1.7 ± 0.8		1.7 ± 0.8		1.9 ± 0.7		1.8 ± 0.9		2.0 ± 1.0		1.6 ± 0.8		0.423	2.1 ± 0.9		1.8 ± 0.7		2.1 ± 0.8		0.899
Dizziness		1.7 ± 0.6		1.8 ± 1.0		1.8 ± 0.6		1.7 ± 0.7		1.7 ± 0.8		1.7 ± 0.8		0.609	1.8 ± 0.9		1.8 ± 0.7		2.0 ± 0.7*		0.875
Tinnitus		1.7 ± 0.9		1.6 ± 0.8		1.6 ± 0.7		1.4 ± 0.5		1.5 ± 0.7		1.7 ± 0.9		0.226	1.8 ± 0.8		1.8 ± 0.9		2.2 ± 0.9		0.224
Lumbago		2.7 ± 0.8		2.2 ± 0.9*		2.3 ± 1.0		2.5 ± 1.4		2.4 ± 1.3		2.6 ± 1.3		0.174	2.8 ± 1.2		2.9 ± 1.3		2.3 ± 1.2		0.191
Arthralgia		1.6 ± 0.7		1.7 ± 0.6		1.8 ± 0.6		1.8 ± 1.2		2.1 ± 1.2		1.9 ± 1.0		0.850	1.8 ± 0.6		1.7 ± 0.8		2.0 ± 0.9		0.752
Edematous		2.8 ± 0.6		2.6 ± 0.9		2.6 ± 1.0		2.3 ± 0.8		2.3 ± 1.1		2.0 ± 0.9		0.752	2.4 ± 0.8		2.0 ± 0.6		2.5 ± 0.8		0.922
Easily breaking into a sweat		2.5 ± 0.9		2.3 ± 0.9		2.2 ± 1.1		2.8 ± 1.3		2.6 ± 1.3		2.3 ± 1.2*		0.561	1.8 ± 0.8		2.0 ± 0.9		2.2 ± 0.7		0.045
Frequent urination		2.1 ± 0.8		2.4 ± 0.9		2.4 ± 1.0		2.3 ± 1.4		2.5 ± 1.3		2.3 ± 1.3		0.788	2.3 ± 1.2		2.4 ± 1.2		2.5 ± 1.1		0.713
Hot flashes		2.0 ± 1.2		1.8 ± 0.8		1.8 ± 0.8		1.9 ± 1.1		1.8 ± 0.8		1.9 ± 0.9		0.678	1.8 ± 0.9		1.8 ± 0.7		1.9 ± 0.8		0.404
Cold skin		3.4 ± 1.1		3.3 ± 1.1		3.1 ± 1.3		3.3 ± 1.1		2.8 ± 1.5		2.9 ± 1.4		0.490	3.1 ± 1.3		3.1 ± 1.5		3.0 ± 1.4		0.557
Mental symptoms																					
Irritability		2.2 ± 0.4		2.4 ± 0.7		2.1 ± 0.5		2.0 ± 1.2		2.3 ± 1.0		2.2 ± 1.0		0.582	2.6 ± 0.7		2.7 ± 1.0		2.4 ± 0.8		0.653
Easily angered		2.1 ± 0.5		2.2 ± 0.4		1.9 ± 0.3		1.9 ± 1.2		2.3 ± 1.2*		2.1 ± 0.9		0.301	2.3 ± 0.7		2.3 ± 0.9		2.1 ± 0.7		0.739
Loss of motivation		2.1 ± 0.5		2.2 ± 0.4		2.0 ± 0.4		1.8 ± 0.9		1.8 ± 0.8		1.8 ± 1.0		0.833	2.2 ± 0.8		2.3 ± 0.9		2.0 ± 0.7		0.888
No feeling of happiness		1.8 ± 0.6		2.0 ± 0.4		1.9 ± 0.3		1.8 ± 0.9		1.8 ± 0.8		1.6 ± 0.7		0.403	2.1 ± 0.5		1.9 ± 0.5		1.8 ± 0.5		0.155
Nothing to look forward to in life		1.8 ± 0.6		2.0 ± 0.4		2.0 ± 0.4		1.8 ± 0.9		1.8 ± 0.8		1.8 ± 0.6		0.575	1.8 ± 0.8		1.8 ± 0.6		1.8 ± 0.6		0.491
Daily life is not enjoyable		1.8 ± 0.6		2.1 ± 0.5		1.9 ± 0.3		1.8 ± 0.9		1.8 ± 0.8		1.7 ± 0.8		0.447	1.6 ± 0.5		1.8 ± 0.5		1.6 ± 0.5		0.623
Lose confidence		2.0 ± 0.6		2.2 ± 0.6		2.0 ± 0.4		2.1 ± 0.8		1.8 ± 0.8		1.9 ± 0.8		0.251	1.9 ± 0.5		1.8 ± 0.6		1.7 ± 0.7		0.233
Reluctance to talk with others		1.7 ± 0.5		1.8 ± 0.4		1.9 ± 0.5		1.7 ± 0.8		1.9 ± 0.8		1.8 ± 0.8		0.681	1.7 ± 0.5		1.8 ± 0.6		1.8 ± 0.7		0.860
Depressed		1.8 ± 0.8		2.0 ± 0.6		1.7 ± 0.6		1.8 ± 1.0		1.7 ± 0.7		1.7 ± 0.8		0.433	1.6 ± 0.5		1.8 ± 0.6		1.7 ± 0.7		0.916
Feeling of uselessness		1.8 ± 0.6		2.0 ± 0.6		1.8 ± 0.4		1.7 ± 0.9		1.9 ± 0.8		1.4 ± 0.5		0.789	1.9 ± 0.7		1.9 ± 0.7		1.8 ± 0.8		0.452
Shallow sleep		2.2 ± 0.6		2.2 ± 0.6		1.8 ± 0.8		2.3 ± 1.3		2.5 ± 1.1		1.8 ± 0.9		0.547	1.8 ± 0.8		1.9 ± 0.7		1.7 ± 0.7		0.505
Difficulty in falling asleep		1.7 ± 0.6		1.6 ± 0.7		1.8 ± 0.8		2.4 ± 1.4		2.2 ± 1.2		1.9 ± 1.1*		0.108	1.7 ± 1.0		1.7 ± 0.7		1.6 ± 0.7		1.000
Pessimism		2.5 ± 0.5		2.1 ± 0.5*		1.9 ± 0.8**		1.8 ± 0.9		1.9 ± 0.9		1.9 ± 0.8		0.013	2.0 ± 0.7		1.9 ± 0.7		1.7 ± 0.7*		0.204
Lapse of memory		2.5 ± 0.7		2.3 ± 0.6		2.5 ± 0.5		2.9 ± 0.9		2.8 ± 0.7		2.9 ± 0.9		0.896	2.6 ± 1.0		2.6 ± 0.9		2.3 ± 1.1		0.949
Inability to concentrate		2.3 ± 0.6		2.3 ± 0.6		2.2 ± 0.6		2.1 ± 0.7		2.2 ± 0.7		1.9 ± 0.7		1.000	2.6 ± 0.9		2.4 ± 0.9		2.1 ± 1.0		0.300
Inability to solve problems		2.2 ± 0.6		2.2 ± 0.6		1.9 ± 0.5		1.8 ± 0.7		1.6 ± 0.7		1.8 ± 0.8		0.948	2.1 ± 0.7		2.1 ± 0.7		1.7 ± 0.7		0.918
Inability to make judgments readily		2.0 ± 0.6		2.0 ± 0.6		1.9 ± 0.5		2.1 ± 0.8		1.9 ± 0.8		2.1 ± 0.8		0.806	2.0 ± 0.7		2.1 ± 0.7		1.8 ± 0.6		0.733
Inability to sleep because of worries		1.8 ± 0.8		2.1 ± 0.5		1.6 ± 0.8		1.9 ± 1.0		1.7 ± 0.8		1.8 ± 0.8		0.437	1.9 ± 0.7		1.7 ± 0.5		1.5 ± 0.5*		0.101
A sense of tension		2.5 ± 0.8		2.4 ± 0.7		2.2 ± 0.6		2.1 ± 0.9		1.9 ± 0.7		2.0 ± 0.7		0.676	2.6 ± 1.0		2.4 ± 1.0		2.2 ± 0.9		0.896
Feeling of anxiety for no special reason		2.0 ± 0.4		2.1 ± 0.5		1.9 ± 0.7		1.7 ± 0.7		1.7 ± 0.7		1.6 ± 0.7		0.533	1.9 ± 0.5		1.9 ± 0.8		1.7 ± 0.7		0.838
Vague feeling of fear		1.7 ± 0.5		1.8 ± 0.6		1.6 ± 0.5		1.3 ± 0.5		1.4 ± 0.5		1.4 ± 0.5		0.377	1.8 ± 0.6		1.7 ± 0.7		1.6 ± 0.7		0.623
Lifestyle-related behaviors																					
Smoking	Cigarettes/ day	5.5 ± 6.5		4.8 ± 6.4		5.5 ± 6.5		0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0		1.000	0.8 ± 2.9		0.				

Table 5 *Anthropometry*

		Control (n=11)						LD group (n=12)				Inter-group analysis (vs. Control) <i>p</i> value	HD group (n=12)				Inter-group analysis (vs. Control) <i>p</i> value				
		Week 0		Week 4		Weeks 8		Week 0		Week 4			Weeks 8		Week 0			Week 4		Weeks 8	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		mean	SD	mean	SD		mean	SD	mean	SD
Height	cm	158.2 ± 6.6						152.5 ± 8.2							158.2 ± 7.7						
Weight	kg	51.2 ± 6.0		51.2 ± 5.8		50.9 ± 5.8		50.6 ± 12.4		50.5 ± 12.1		50.4 ± 12.2		0.666	52.5 ± 6.8		52.0 ± 7.0		52.4 ± 7.0		0.926
Percentage body fat	%	28.4 ± 4.6		28.4 ± 4.1		27.7 ± 4.3*		29.7 ± 7.2		29.6 ± 6.8		29.0 ± 7.1		0.853	30.3 ± 5.3		30.3 ± 5.2		29.6 ± 5.3		0.975
Amount of fat	kg	14.7 ± 3.5		14.7 ± 3.4		14.2 ± 3.3 **		15.7 ± 8.3		15.5 ± 7.9		15.2 ± 8.0		0.689	16.1 ± 4.3		16.0 ± 4.3		15.7 ± 4.2		1.000
Lean body mass	kg	36.5 ± 3.6		36.5 ± 3.3		36.6 ± 3.5		34.9 ± 5.4		35.0 ± 5.5		35.2 ± 5.6		1.000	36.4 ± 3.8		36.0 ± 3.9		36.7 ± 3.8		0.926
Water content	kg	26.7 ± 2.6		26.7 ± 2.4		26.8 ± 2.6		25.6 ± 3.9		25.6 ± 4.1		25.8 ± 4.1		0.902	26.6 ± 2.8		26.4 ± 2.8		26.9 ± 2.8		0.951
Basal metabolic rate	kcal	1029.6 ± 93.7		1029.5 ± 87.4		1031.7 ± 90.9		992 ± 149		992 ± 151		997 ± 155		0.951	1029 ± 103		1019 ± 106		1036 ± 105		0.926
BMI		20.5 ± 2.7		20.5 ± 2.7		20.4 ± 2.6		21.7 ± 4.5		21.6 ± 4.4		21.6 ± 4.4		0.514	21.0 ± 2.8		20.8 ± 2.8		21.0 ± 2.6		0.926
Systolic blood pressure	mmHg	119.5 ± 18.2		115.9 ± 16.2		108.2 ± 13.7**		119.8 ± 19.5		115.8 ± 14.6		114.2 ± 14.4		0.218	118.0 ± 12.0		113.9 ± 16.8		110.5 ± 10.4*		0.712
Diastolic blood pressure	mmHg	72.0 ± 12.9		72.4 ± 12.9		68.3 ± 12.8		72.0 ± 13.4		72.1 ± 13.4		70.0 ± 10.4		0.902	73.9 ± 7.8		73.2 ± 12.1		70.2 ± 9.7		0.735
Heart Rate	bpm	74.6 ± 11.6		71.4 ± 9.5		73.1 ± 7.2		72.0 ± 7.9		72.9 ± 8.0		71.9 ± 9.8		0.268	77.9 ± 6.8		82.8 ± 16.9		75.4 ± 10.9		0.268

Average±SD, **p*<0.05, ***p*<0.01 vs. Week 0 by paired-t test. Inter-group analysis by Mann-Whitney U test.**Table 6** *Blood biochemistry*

		Control (n=11)						LD group (n=12)						Inter-group analysis (vs. Control) <i>p</i> value	HD group (n=12)						Inter-group analysis (vs. Control) <i>p</i> value
		Week 0		Week 4		Weeks 8		Week 0		Week 4		Weeks 8			Week 0		Week 4		Weeks 8		
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		mean	SD	mean	SD	mean	SD	
Leukocyte count	/μl	5691 ± 1297		5909 ± 1532		5900 ± 1448		5509 ± 1472		5536 ± 908		5236 ± 1267	0.538	5083 ± 1357		5550 ± 1643		5242 ± 1277		0.805	
Erythrocyte count	×10 ⁴ /μl	430.1 ± 29.3		437.0 ± 30.1		429.0 ± 22.2		439.4 ± 28.2		436.4 ± 25.7		431.8 ± 25.1	0.340	440.3 ± 27.7		451.6 ± 36.5*		443.2 ± 35.7		0.498	
Hemoglobin	g/dl	13.0 ± 0.6		13.1 ± 0.8		12.9 ± 0.6		13.0 ± 0.8		12.9 ± 0.7		12.8 ± 0.8	0.441	12.8 ± 1.5		13.0 ± 1.5		12.8 ± 1.2		0.388	
Hematocrit	%	40.8 ± 1.9		41.7 ± 2.4		40.3 ± 1.7		40.9 ± 2.4		40.5 ± 1.4		39.4 ± 2.2*	0.140	40.7 ± 3.5		41.6 ± 3.2		40.2 ± 2.8		0.805	
Platelet count	×10 ⁴ /μl	27.8 ± 7.2		28.7 ± 7.4		27.5 ± 6.3		27.6 ± 7.6		27.1 ± 6.2		25.6 ± 5.1	0.176	27.5 ± 7.0		26.6 ± 7.1		26.5 ± 5.9		0.242	
MCV	fl	94.9 ± 3.7		95.5 ± 3.2		94.1 ± 2.8		93.5 ± 6.5		93.0 ± 6.6		91.5 ± 6.3	0.277	92.8 ± 10.2		92.7 ± 9.6		91.3 ± 9.6*		0.203	
MCH	pg	30.3 ± 1.1		30.1 ± 1.1		30.0 ± 1.0		29.7 ± 2.2		29.6 ± 2.2		29.7 ± 2.1	0.205	29.2 ± 3.9		29.0 ± 3.8		29.2 ± 3.6		0.388	
MCHC	%	31.9 ± 0.6		31.5 ± 0.6		31.9 ± 0.9		31.8 ± 0.9		31.7 ± 0.8		32.4 ± 0.8*	0.064	31.4 ± 1.5		31.3 ± 1.5		31.9 ± 1.1		0.242	
Neutrophils	%	56.7 ± 9.3		61.0 ± 8.6		62.3 ± 6.8		57.5 ± 9.6		59.9 ± 7.8		60.3 ± 5.8	0.424	56.9 ± 7.4		59.8 ± 12.5		59.2 ± 8.1		0.667	
Lymphocytes	%	34.8 ± 9.3		30.6 ± 8.2		29.1 ± 6.1		33.8 ± 8.4		31.6 ± 6.8		31.1 ± 5.6	0.356	33.6 ± 4.7		31.3 ± 10.6		30.6 ± 6.2		0.538	
Monocytes	%	5.2 ± 0.9		4.9 ± 1.1		5.1 ± 1.5		5.7 ± 1.4		5.9 ± 1.1		4.9 ± 1.0*	0.878	5.4 ± 1.2		5.2 ± 1.3		5.0 ± 1.0		1.000	
Eosinophils	%	2.7 ± 1.6		2.9 ± 2.1		2.8 ± 1.9		2.4 ± 1.7		1.9 ± 1.2		3.0 ± 1.9	0.805	3.5 ± 3.2		3.3 ± 2.8		4.5 ± 3.7		0.387	
Basophils	%	0.7 ± 0.4		0.6 ± 0.4		0.7 ± 0.3		0.7 ± 0.4		0.6 ± 0.4		0.7 ± 0.4	0.926	0.6 ± 0.4		0.6 ± 0.4		0.6 ± 0.3		0.925	
Total protein	g/dl	7.5 ± 0.5		7.5 ± 0.4		7.4 ± 0.4		7.6 ± 0.5		7.5 ± 0.5		7.4 ± 0.3*	0.163	7.7 ± 0.3		7.7 ± 0.6		7.6 ± 0.3		0.643	
Albumin	g/dl	4.6 ± 0.2		4.6 ± 0.3		4.5 ± 0.2*		4.6 ± 0.2		4.5 ± 0.1		4.5 ± 0.2	0.805	4.6 ± 0.2		4.6 ± 0.2		4.6 ± 0.2		0.353	
A/G ratio	-	1.6 ± 0.1		1.6 ± 0.2		1.6 ± 0.2		1.6 ± 0.2		1.5 ± 0.2		1.6 ± 0.2	0.457	1.5 ± 0.2		1.5 ± 0.3		1.6 ± 0.3		0.142	
AST	IU/l	16.1 ± 1.9		15.9 ± 1.9		15.4 ± 1.6		17.9 ± 3.1		17.4 ± 2.9		16.8 ± 3.1	0.597	22.3 ± 5.9		20.6 ± 5.3		20.8 ± 5.1		0.302	
ALT	IU/l	12.3 ± 2.9		12.3 ± 3.1		11.1 ± 3.0*		13.7 ± 3.8		13.4 ± 3.7		13.1 ± 3.6	0.926	17.6 ± 6.0		16.6 ± 4.8		17.3 ± 7.4		0.643	
LDH	IU/l	156.3 ± 20.8		154.7 ± 11.6		147.5 ± 14.8*		175.6 ± 17.2		163.5 ± 27.5		153.3 ± 23.0**	0.176	182.5 ± 31.5		177.3 ± 20.4		177.8 ± 29.8		0.666	
Total bilirubin	mg/dl	0.8 ± 0.3		0.8 ± 0.2		0.7 ± 0.3		0.7 ± 0.2		0.7 ± 0.2		0.7 ± 0.2	0.619	0.8 ± 0.2		0.7 ± 0.2		0.7 ± 0.2*		0.493	
ALP	IU/l	189.0 ± 63.7		184.0 ± 58.0		186.4 ± 54.2		159.5 ± 41.1		146.5 ± 34.7*		147.0 ± 32.4	0.460	190.5 ± 59.9		189.2 ± 54.1		200.8 ± 63.4		0.268	
γ-GTP	IU/l	18.5 ± 9.4		19.4 ± 9.4		18.9 ± 7.9		13.5 ± 2.8		14.3 ± 2.9		14.0 ± 3.2	0.902	22.6 ± 14.7		22.5 ± 12.7		21.9 ± 13.2		0.975	
CPK	IU/l	62.4 ± 16.8		62.5 ± 15.6		62.3 ± 14.5		73.1 ± 17.3		79.9 ± 24.0		73.3 ± 21.1	0.666	82.9 ± 21.7		80.5 ± 28.6		101.1 ± 64.6		0.951	
Urea nitrogen	mg/dl	11.2 ± 2.8		10.1 ± 3.0		11.4 ± 4.0		11.5 ± 3.0		11.8 ± 3.7		11.9 ± 3.6	0.735	11.1 ± 2.4		11.0 ± 1.8		10.8 ± 2.9		0.689	
Creatinine	mg/dl	0.6 ± 0.1		0.6 ± 0.1		0.6 ± 0.1		0.5 ± 0.1		0.6 ± 0.1		0.6 ± 0.1	0.735	0.6 ± 0.1		0.6 ± 0.1		0.6 ± 0.1		0.517	
Uric acid	mg/dl	3.9 ± 1.2		3.8 ± 1.0		4.0 ± 0.9		3.5 ± 1.1		3.4 ± 1.3		3.6 ± 1.4	0.666	4.0 ± 0.8		3.8 ± 0.8		4.0 ± 1.0		0.405	
Sodium	mEq/l	140.5 ± 1.4		139.8 ± 1.6		139.3 ± 2.2*		140.5 ± 1.8		139.6 ± 2.2*		140.2 ± 1.9	0.401	140.8 ± 1.9		139.3 ± 1.4**		140.2 ± 1.5		0.975	
Chlorine	mEq/l	102.8 ± 1.9		102.3 ± 2.0		103.7 ± 1.5		102.1 ± 1.7		102.2 ± 2.1		104.5 ± 1.8**	0.172	102.3 ± 1.8		101.9 ± 2.1		103.3 ± 2.1		0.975	
Potassium	mEq/l	4.0 ± 0.2		4.1 ± 0.3		4.1 ± 0.2		4.0 ± 0.3		4.0 ± 0.3		3.9 ± 0.2	0.322	4.0 ± 0.2		4.2 ± 0.3		4.0 ± 0.2		0.852	
Calcium	mg/dl	9.8 ± 0.3		9.7 ± 0.6		9.7 ± 0.3		9.7 ± 0.2		9.6 ± 0.2		9.7 ± 0.3	0.355	9.7 ± 0.2		9.8 ± 0.3		9.7 ± 0.2		0.497	
Serum iron	μg/dl	110.0 ± 26.4		92.7 ± 31.6		88.2 ± 28.5*		79.7 ± 29.9		85.5 ± 32.8		87.9 ± 46.1	0.242	102.6 ± 32.9		97.4 ± 53.9		99.7 ± 51.4		0.424	
Total cholesterol	mg/dl	206.1 ± 29.7		208.5 ± 31.2		200.2 ± 28.7		206.9 ± 33.1		202.4 ± 28.7		203.7 ± 25.8	1.000	203.6 ± 38.0		199.6 ± 31.8		198.6 ± 32.9		0.829	
LDL cholesterol	mg/dl	112.3 ± 25.1		111.2 ± 27.4		108.2 ± 24.2		117.4 ± 35.9		109.7 ± 30.1*		113.4 ± 28.1	0.805	115.2 ± 36.2		112.7 ± 38.6		114.3 ± 36.9		0.538	
HDL cholesterol	mg/dl	73.1 ± 12.8		76.4 ± 12.4		76.8 ± 12.0		72.5 ± 10.5		73.5 ± 11.7		74.3 ± 12.3	0.324	64.2 ± 17.9		62.4 ± 16.2		64.8 ± 15.8		0.079	
Triglyceride	mg/dl	73.0 ± 21.9		78.9 ± 31.8		63.6 ± 20.4		66.5 ± 26.7		65.4 ± 34.5		73.5 ± 42.8	0.854	108.9 ± 137.3		81.1 ± 35.8		89.3 ± 74.5		0.879	
Glucose	mg/dl	86.2 ± 5.9		86.2 ± 5.8		86.3 ± 7.6		83.2 ± 8.8		83.2 ± 5.5		82.8 ± 6.6	0.644	90.8 ± 15.6		93.8 ± 11.8		87.6 ± 8.6		0.644	
HbA1c	%	4.8 ± 0.2		4.7 ± 0.2**		4.8 ± 0.2		4.8 ± 0.2		4.7 ± 0.2*		4.8 ± 0.2	0.826	4.9 ± 0.4		4.9 ± 0.4		5.0 ± 0.4		0.103	
Arterial sclerosis index	-	1.9 ± 0.5		1.8 ± 0.6		1.6 ± 0.5**		1.9 ± 0.6		1.8 ± 0.6		1.8 ± 0.6	0.106	2.4 ± 1.2		2.4 ± 1.1		2.3 ± 1.1		0.156	
Insulin	μU/ml	4.4 ± 1.7		4.6 ± 1.5		5.3 ± 2.0		5.4 ± 4.2		5.0 ± 2.0		6.9 ± 4.3	1.000	6.7 ± 4.1		8.3 ± 6.3		6.0 ± 2.6		0.878	

Table 7 Skin symptoms

	Control (n=11)				LD group (n=11)			HD group (n=12)				
	Week 0		Week 4+8		Week 0	Week 4+8	Inter-group analysis (vs. Control) p value	Week 0		Week 4+8		Inter-group analysis (vs. Control) p value
	mean	SD	mean	SD				mean	SD	mean	SD	
Concerned about pores	2.8 ± 0.8		2.5 ± 0.7*		3.2 ± 0.8	2.8 ± 0.8**	0.642	3.2 ± 0.9		2.8 ± 0.8		0.803
Dry skin	3.9 ± 0.8		3.4 ± 0.8**		3.9 ± 1.0	3.3 ± 0.9*	0.837	3.8 ± 1.0		3.4 ± 0.8		0.295
Concerned about dull skin	3.1 ± 0.7		2.9 ± 0.6		3.1 ± 0.8	3.0 ± 1.1	0.784	3.5 ± 1.2		3.0 ± 1.1*		0.292
Frequent pimples	2.4 ± 0.8		2.1 ± 0.6		2.2 ± 1.2	2.2 ± 1.1	0.263	2.9 ± 1.2		2.8 ± 1.1		0.536
Itchy skin	2.7 ± 0.5		2.3 ± 0.5*		3.0 ± 0.6	2.2 ± 0.7*	0.480	2.9 ± 1.4		2.5 ± 0.9		0.900
Concerned about spots or freckles	3.0 ± 0.6		2.8 ± 0.6		3.1 ± 1.0	3.2 ± 0.6	0.632	3.8 ± 0.8		3.3 ± 1.0*		0.287
Make-up runs easily	2.8 ± 0.4		2.5 ± 0.5*		3.0 ± 0.6	2.7 ± 0.6	0.756	3.1 ± 0.8		2.8 ± 0.7		0.706
Poor complexion	2.7 ± 0.6		2.4 ± 0.5		2.7 ± 0.9	2.5 ± 0.8	0.697	2.8 ± 1.0		2.7 ± 1.1		0.330
Sticky oily skin	2.0 ± 0.7		2.0 ± 0.6		2.4 ± 1.0	2.1 ± 0.6	0.189	2.4 ± 0.7		2.0 ± 0.7*		0.072
Coarse and desiccated skin	2.6 ± 0.7		2.2 ± 0.4		3.0 ± 0.7	2.4 ± 0.5**	0.508	3.3 ± 1.0		2.6 ± 0.8**		0.239
Slackened skin	3.3 ± 0.6		2.7 ± 0.8**		3.1 ± 0.9	3.0 ± 0.9	0.038	3.3 ± 1.3		3.0 ± 0.8		0.488
Not elastic, not glossy	2.9 ± 0.5		2.7 ± 0.6		3.3 ± 0.5	3.0 ± 0.7	0.973	3.4 ± 1.1		3.0 ± 0.9*		0.428
Concerned about crows feet	2.6 ± 0.9		2.8 ± 0.8		3.5 ± 1.1	3.1 ± 1.1*	0.012	3.5 ± 1.0		3.3 ± 0.8		0.141
Concerned about rough skin	2.8 ± 0.6		2.2 ± 0.5**		3.1 ± 1.0	2.7 ± 0.9	0.687	3.2 ± 0.9		2.7 ± 1.0**		0.407
Bags under eyes	2.4 ± 0.8		2.4 ± 1.0		2.1 ± 0.7	2.2 ± 0.8	0.939	2.6 ± 1.0		2.3 ± 0.8*		0.085
Thinning eyebrows	1.7 ± 0.5		1.9 ± 0.5		1.9 ± 0.8	2.0 ± 0.8	0.639	2.1 ± 0.7		2.0 ± 0.7		0.114
Concerned about slackened skin and chin	2.7 ± 0.5		2.6 ± 0.6		2.8 ± 0.7	2.6 ± 1.0	1.000	2.8 ± 0.9		2.5 ± 0.7		0.779
Thinning hair	2.5 ± 0.5		2.4 ± 0.6		2.7 ± 1.2	2.6 ± 1.2	0.914	3.0 ± 1.5		2.6 ± 1.2*		0.246
Slow growing nails	1.7 ± 0.5		1.9 ± 0.3		2.1 ± 0.5	2.4 ± 0.8	0.605	1.9 ± 0.8		1.9 ± 0.6		0.795
Dull, fragile nails	2.6 ± 1.1		2.5 ± 1.2		2.4 ± 0.9	2.6 ± 1.0	0.342	3.3 ± 1.3		3.0 ± 1.1*		0.423

SD, standard deviation

Average±SD, * $p<0.05$, ** $p<0.01$ vs. Week 0 by Wilcoxon signed rank test. Inter-group analysis by Mann-Whitney U test.

Table 8 Skin analysis

		Control (n=11)						LD group (n=12)						Inter-group analysis (vs. Control) p value	HD group (n=12)						Inter-group analysis (vs. Control) p value
		Week 0		Week 4		Weeks 8		Week 0		Week 4		Weeks 8			Week 0		Week 4		Weeks 8		
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		mean	SD	mean	SD	mean	SD	
Moisture level	Cheak	47.1 ± 9.6		52.9 ± 6.5		53.1 ± 5.9		47.4 ± 5.9		49.2 ± 9.1		49.7 ± 7.3		0.295	43.2 ± 8.5		43.6 ± 9.9		42.7 ± 8.7		0.110
	Arm	30.3 ± 6.0		33.1 ± 5.5		34.9 ± 6.2**		32.5 ± 5.5		32.2 ± 5.3		34.4 ± 9.8		0.196	31.9 ± 5.0		32.8 ± 6.8		34.2 ± 4.9		0.218
pH		6.4 ± 0.2		6.5 ± 0.1*		6.5 ± 0.2		6.53 ± 0.14		6.46 ± 0.32		6.51 ± 0.28		0.056	6.41 ± 0.29		6.50 ± 0.15		6.45 ± 0.27		0.622
TEWL	Cheak	20.2 ± 4.8		17.7 ± 3.2		19.7 ± 3.4		22.6 ± 5.2		21.3 ± 5.7		20.2 ± 5.3		0.538	22.2 ± 7.2		18.7 ± 4.7		19.5 ± 3.8		0.340
	Arm	8.1 ± 2.0		6.8 ± 1.7**		7.9 ± 2.3		8.9 ± 3.1		8.4 ± 2.5		9.5 ± 3.4		0.580	7.2 ± 1.9		6.6 ± 2.1		8.3 ± 2.7		0.242
Skin image analysis																					
	Melanin	125.4 ± 36.0		117.8 ± 37.0		109.2 ± 38.7**		129.3 ± 23.9		126.4 ± 29.9		118.6 ± 23.9**		0.388	109.8 ± 24.5		108.2 ± 25.9		110.3 ± 23.5		0.052
	Erythema	354.2 ± 56.7		342.8 ± 52.8		345.4 ± 58.0		325.8 ± 64.1		306.3 ± 71.5		319.2 ± 45.6		0.902	351.6 ± 50.5		328.9 ± 41.5		330.1 ± 34.1		0.131
Skin Elasticity																					
	R0	0.33 ± 0.03		0.33 ± 0.06		0.31 ± 0.04		0.35 ± 0.05		0.34 ± 0.07		0.31 ± 0.05**		0.255	0.34 ± 0.06		0.32 ± 0.06		0.31 ± 0.06**		0.601
	R1	0.16 ± 0.03		0.15 ± 0.04		0.14 ± 0.03*		0.16 ± 0.03		0.16 ± 0.03		0.13 ± 0.03*		0.712	0.16 ± 0.02		0.14 ± 0.03*		0.13 ± 0.03**		0.166
	R2	0.51 ± 0.07		0.54 ± 0.07		0.54 ± 0.07*		0.55 ± 0.07		0.54 ± 0.05		0.58 ± 0.07		0.085	0.51 ± 0.05		0.56 ± 0.06*		0.58 ± 0.05**		0.023
	R3	0.35 ± 0.03		0.35 ± 0.06		0.33 ± 0.05		0.37 ± 0.05		0.36 ± 0.07		0.33 ± 0.05**		0.268	0.35 ± 0.06		0.34 ± 0.06		0.33 ± 0.06**		0.667
	R4	0.18 ± 0.03		0.17 ± 0.04		0.16 ± 0.03*		0.18 ± 0.03		0.17 ± 0.03		0.15 ± 0.03*		0.878	0.18 ± 0.02		0.16 ± 0.03*		0.15 ± 0.03**		0.056
	R5	0.33 ± 0.08		0.33 ± 0.05		0.33 ± 0.05		0.34 ± 0.05		0.33 ± 0.03		0.35 ± 0.05		0.758	0.30 ± 0.05		0.34 ± 0.04*		0.34 ± 0.07		0.074
	R6	0.41 ± 0.06		0.41 ± 0.09		0.43 ± 0.09		0.38 ± 0.05		0.43 ± 0.11		0.42 ± 0.09		0.176	0.42 ± 0.08		0.41 ± 0.10		0.40 ± 0.11		0.667
	R7	0.23 ± 0.05		0.24 ± 0.04		0.23 ± 0.05		0.24 ± 0.04		0.23 ± 0.03		0.25 ± 0.03		0.242	0.21 ± 0.03		0.24 ± 0.02**		0.24 ± 0.03*		0.012
	R8	0.17 ± 0.03		0.17 ± 0.04		0.17 ± 0.04		0.19 ± 0.04		0.18 ± 0.04*		0.18 ± 0.04		0.176	0.17 ± 0.04		0.18 ± 0.04		0.18 ± 0.03		0.460
	R9	0.02 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		0.829	0.02 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		0.497
	F0	0.06 ± 0.01		0.06 ± 0.00		0.05 ± 0.00		0.06 ± 0.01		0.06 ± 0.01		0.06 ± 0.01		0.806	0.06 ± 0.01		0.05 ± 0.01		0.05 ± 0.01		0.951
	F1	0.05 ± 0.01		0.04 ± 0.01		0.04 ± 0.01		0.04 ± 0.01		0.05 ± 0.01		0.05 ± 0.01		0.268	0.04 ± 0.01		0.04 ± 0.01		0.05 ± 0.01		0.538
AGEs measurement																					
	Auto fluorescence	2.13 ± 0.41		2.08 ± 0.41		2.04 ± 0.40		2.16 ± 0.35		1.93 ± 0.36*		2.11 ± 0.24		0.460	1.98 ± 0.35		1.88 ± 0.36		1.92 ± 0.27		0.806
	Reflectance value	0.14 ± 0.03		0.15 ± 0.03		0.16 ± 0.02*		0.15 ± 0.04		0.17 ± 0.04**		0.16 ± 0.04		0.622	0.16 ± 0.04		0.15 ± 0.04		0.17 ± 0.04		0.758

TEWL, transepidermal water loss; AGEs: advanced glycation end-products; SD, standard deviation

Average±SD, * $p<0.05$, ** $p<0.01$ vs. Week 0 by paired- t test. Inter-group analysis by Mann-Whitney U test.

Fig. 3. Increase in human dermal fibroblast growth.

Relative cell numbers were compared by the colormetric method using tetrazolium salt 72 h after cultivation of human dermal fibroblasts in various concentrations of beet ceramide (0-300 µg/ml). The y-axis represents absorbance at 415 nm. (n=6, average±standard error of the mean [SEM])

Fig. 4. Fibronectin production in human dermal fibroblasts

Immunofluorescent staining was performed with anti-fibronectin antibodies 24 h after cultivation of human dermal fibroblasts in various concentrations of beet ceramide. Left column: phase-contrast microscopy image. Right column: fluorescence microscope image.

Fig. 5. Collagen type I production in human dermal fibroblasts.

24 hours after the cultivation of human dermal fibroblasts with (100 μ g/ml) and without the addition of beet ceramide, immunofluorescent staining was performed with anti-collagen type I antibodies. Left column: phase-contrast microscopy image. Right column: fluorescence microscopy image.

Fig. 6. Fibronectin mRNA expression in human dermal fibroblast.

Fibronectin mRNA expression was semi-quantitatively measured using real time PCR 24 h after cultivation of human dermal fibroblasts in beet ceramide (0-100 μ g/ml). The y-axis represents relative amount of fibronectin mRNA adjusted with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression, a standard for mRNA gene expression. (n=3, average $\pm$ standard error of the mean [SEM])

Results

Evaluation of Subjective Symptoms

Subjective symptom scores assessed using the AAQoL questionnaire are shown in [Table 4](#). Scores for the three groups (control, LD [glucosyl ceramide at 0.6 mg/day], HD [glucosyl ceramide at 1.8 mg/day]) after the intake of beet ceramide (averages after 4 and 8 weeks) were compared using a Mann-Whitney *U* test. Of the 32 physical symptom items, only “easily break into sweat” differed statistically significantly compared to the control ($p=0.045$); the score decreased from the relatively high pre-value (2.8 ± 1.3) in the LD group and increased from the relatively low pre-value (1.8 ± 0.8) in the HD group, suggesting that inter-group differences are due to background difference between the LD and HD groups.

Of the 21 emotional symptom items, none differed significantly from the control values. Although the LD group showed significant differences from the control with regard to “hair loss” ($p=0.010$) and “pessimism” ($p=0.013$), we deemed this result to be due to the large improvement observed in the control group.

Physical Examination

The results of the participants’ physical examination are presented in [Table 5](#). No significant differences in body composition, blood pressure, or pulse were noted between the control and experimental groups.

Biochemical Blood Testing

Results of biochemical blood tests are presented in [Table 6](#). No significant differences in results for tested items were noted between the control and experimental groups.

Skin Analysis

Results for skin symptom analysis are presented in [Table 7](#). With regard to skin symptoms for which significant improvement was reported after the experiment, improvement was noted for six symptoms in both the control and LD group and nine in the HD group. The most overall improvement in skin was reported among members of the HD group. Items for which improvement was reported in the HD group were, “Concerned about dull skin,” “Concerned about spots or freckles,” “Sticky oily skin,” “Coarse and desiccated skin,” “Not elastic, not glossy,” “Bags under eyes,” “Thinning hair,” and “Dull, fragile nails.”

No skin symptom items showed a significant difference in score change between HD group and Control. In the LD group, a significant difference in score change (vs. Control) was seen for “Slackened skin” ($p=0.038$) and “Concerned about crow’s feet” ($p=0.012$), but the change in “Slackened skin” was attributed to the high level of improvement seen in the control group.

Results of skin analysis are presented in [Table 8](#). No significant differences in skin moisture or transepidermal water loss (TEWL) in the cheek or arm were observed between the control and experimental groups. Although we analyzed a number of subclasses in our assessment of moisture and TEWL before oral intake, we noted no significant observations. Results of correlation analysis between moisture level and TEWL prior to beet ceramide intake were $r=-0.072$ and $p=0.680$ in the cheek and $r=0.223$ and $p=0.197$ in the arm. Results for correlation analysis of changes in moisture levels and TEWL were $r=-0.017$ and $p=0.921$ in the cheek and $r=0.309$ and $p=0.071$ in the arm. No significant correlation was observed.

No significant difference was noted in the level of melanin or severity of erythema in the center of the right cheek between the control and experimental groups. Results of the skin elasticity test (measured at the center of the right cheek) showed a significant difference in elasticity indices R2 ($p=0.023$) and R7 ($p=0.012$) between the control and LD groups. Given their dose-dependent nature, these changes were associated with ceramide intake.

A significant correlation was confirmed between improvement in elasticity index R2 and score improvement for “Thinning eyebrows” ($r=-0.361$, $p=0.036$). In addition, a significant correlation was also observed between improvement in elasticity index R7 and score improvement for, “Concerned about dull skin” ($r=-0.469$, $p=0.005$), “Concerned about spots of freckles” ($r=-0.343$, $p=0.047$), and “Thinning eyebrows” ($r=-0.360$, $p=0.036$).

No significant differences were noted in the advanced glycation end-product fluorescence AF values (measured on the skin of the outer side of the upper arm) between the control and experimental groups.

Test Product Safety

No adverse events due to intake of the test product were reported during or after the examination period, suggesting the safety of the test product.

Results from Experiments with Human Dermal Fibroblasts

Results from experiments investigating the influence of beet ceramide on human fibroblast proliferation are presented in [Fig. 3](#). No influence on cell proliferation was observed at beet ceramide concentrations at or below 100 $\mu\text{g/ml}$, with inhibition only evident from 300 $\mu\text{g/ml}$. With regard to apoptosis, no cell apoptosis was noted at beet ceramide concentrations with 100 $\mu\text{g/ml}$ (data not shown).

The influence of beet ceramide on fibronectin production in human fibroblasts was examined using fluorescent immunostaining ([Fig. 4](#)). Results showed that fibronectin production increased after culturing cells for 24 h in beet ceramide. Further, the level of increase was found to be dependent on the concentration of beet ceramide used for culturing.

Analysis results regarding the influence of beet ceramide on production of human fibroblast collagen type I are presented in [Fig. 5](#). Unlike fibronectin production, collagen type I production levels did not change significantly with the addition of beet ceramide.

The influence of the beet ceramide on fibronectin mRNA expression in human fibroblast was also examined ([Fig. 6](#)). Results showed that expression increased in a dose-dependent manner after culturing fibroblasts for 24 h with beet ceramide in the medium.

Discussion

We conducted a double-blind comparison study to assess the effects of eight-week oral administration of glucosyl ceramide contained in beet extract (beet ceramide) on skin condition. Results showed that oral ingestion of beet ceramide did indeed improve subjective symptoms, and a skin elasticity test showed that the elasticity index improved in a dose-dependent manner. In experiments involving human dermal fibroblast cells, beet ceramide promoted fibronectin synthesis as well as fibronectin mRNA expression. Given these results, we postulate that orally ingested beet ceramide stimulates cell signals, thereby inducing favorable effects on the extracellular matrix. However, further studies are needed to determine the digestion and absorption dynamics of beet ceramide, as well as its transference to and effects on human skin.

Changes in the Skin Due to Aging and Ceramide

Various structural changes occur in the skin due to aging²²⁾. In the epidermis, for example, such changes include decline in ability to carry out cell division in keratinocytes, decrease in the number of spinous cells, and decrease in lipid levels between keratinocytes (primarily ceramide). Prominent changes in the dermis include decrease in collagen fibers and thinning of fiber bundles; in addition, numbers of elastic fibers decrease, and normal elasticity is lost. As general consequences of aging, the extracellular matrix (mainly hyaluronic acid) diminishes, skin moisture decreases, and reductions are noted in perspiration levels, sebum secretion, and number of hair follicles, along with thinning and whitening of hair.

Dry skin is often seen among the elderly, with the degree of dryness increasing with age²³⁾. Subjective symptoms such as itchiness, burning sensations, tingling pains, and stiffness are also occasionally experienced. Morphologically, the dermis and subcutaneous tissue become atrophic and thin in aged skin. Physiologically, sebum secretion and sweat gland function decrease, while TEWL increases²⁴⁾. Biochemically, ceramide and amino acid levels decrease²⁵⁾. With regard to ceramide fractions, the ratio of ceramide 1 decreases while that of ceramides 4 and 5 increases in the elderly compared to the younger population. Levels of sphingomyelinase and propositin, important components in the ceramide synthesis pathway, also decrease with age²³⁾.

Data Analysis

In the present study, we noted improvements in skin symptoms and skin elasticity on oral administration of beet ceramide. The fact that the number of improved skin symptom items increased significantly in a dose-dependent manner and that many improvements were observed among members of the high-dose group suggests the efficacy of the product evaluated here.

Improved items included “Coarse and desiccated skin” and “Skin not elastic or glossy.” We were unable to explain the reason for the improvement in “Corase and desiccated skin,” for improvements in skin moisture were not confirmed. Functions of skin appendages (sweat glands, sebaceous glands, etc.) and sweat secretion decline with aging²⁴⁾, and it is possible that sweat gland function improved during the study. Further studies are required in this area. Improvements in “Skin not elastic or glossy” scores can be shown using a skin elasticity test. Strong correlation between the degree of improvement in elasticity indices R2/R7 and the degree of improvement in skin symptoms support the possibility that improvements in skin symptoms are directly related to improvements in skin elasticity.

Skin elasticity is affected by a number of factors, such as the extracellular matrix and collagen fibers, and is lost with age^{23,24)}. Given that the amount of ceramide contained in the test product is less than both the daily intake of ceramide and the total amount in the body, administration of exogenous ceramide is unlikely to affect ceramide content in the skin. Therefore, ceramide is extremely likely to instead affect intracellular signaling.

Results from experiments with human skin fibroblasts showed that addition of beet ceramide to the culture medium induced an increase in fibronectin production and mRNA levels. However, given that no increase in levels of collagen I production was observed, the effect of beet ceramide is believed to be selective. These results indicate that beet ceramide does indeed exert favorable effects on the skin’s extracellular matrix, sebum line, and elasticity.

Fibronectin, an extracellular matrix protein produced by fibroblasts and resides in the matrix between collagen fibers and elastic fibers in the dermis, can bind to collagen, heparin, and other cells, and plays a critical role in elasticity, morphology, and wound healing in the dermis. Because beet ceramide promotes fibronectin production in fibroblasts, it is thought to aid in maintaining proper dermal structure, thereby maintaining skin elasticity.

Pharmacokinetics of Orally Ingested Ceramide in the Body

Results from previous studies revealed that a specific type of ceramide is transported to the skin, liver, and brain after oral ingestion, where it is then metabolically converted and distributed throughout the body, migrating from the dermis to the epidermis in mice²⁶⁾ and rats²⁷⁾. Approximately 5% of dosed ceramide is excreted in urine, and approximately 90% in feces²⁷⁾. Respective ratios of orally ingested ceramide transported to the skin in mice and rats are 4.5% (continuous administration for 12 days)²⁶⁾ and 1.67% (96 h after a single administration)²⁷⁾.

Typical daily ceramide intake from food in humans is approximately 50-290 mg²⁸⁾. With plant-derived orally ingested glucosyl ceramide, glucoside bonds break off and become ceramide, which is then broken down into sphingoid bases and fatty acids. Sphingoid bases are absorbed by the epithelial cells of the small intestine via selective absorption involving p-sugar protein, a drug excretion transporter²⁹⁾. However, while plant-derived sphingoid bases such as sphingadienine (d18:2^{4t,8t}, d18:2^{4t,8c}, etc.) are absorbed by epithelial cells, they are absorbed and excreted into the intestines by p-glycoproteins. As such, absorption of plant-derived sphingoid bases has been reported to be lower than that of animal-derived ones such as sphingosine³⁰⁾.

In the present study, only 1.8 mg of glucosyl ceramide was administered to the high-dose group, an amount which accounts for approximately 4% of daily intake. Only a small portion of ingested ceramide is actually absorbed by the body, and even if 5% of the total amount absorbed were transported to the skin, that amount is expected to be less than 1 µg. Further, the skin of an adult human weighs 3-4 kg³¹⁾, and 2 mg of ceramide is less than 0.0001% of this weight. As such, it is therefore unrealistic to hypothesize that, “2 mg of authentic glucosyl ceramide orally ingested is absorbed by the intestinal tract and thereby increases the level of ceramide in the epidermis.” We alternatively hypothesize that glucoside bases which appear during metabolism acts as second messengers in the cell and control intracellular signals, thereby leading to physiological changes.

Observations on Oral Ingestion of Authentic Glucosyl Ceramide

Findings on oral administration of authentic glucosyl ceramide (such as ceramide from rice, corn, and konjac) have been previously reported and are briefly described below.

In a double blind study involving 33 individuals with dry skin, oral administration of rice ceramide (1.2 mg/day, administered as glucosyl ceramide for 6 weeks) induced a significant increase skin moisture, improvement in skin smoothness as determined by epidermal image analysis (Visioscan, Courage & Khazaka Electric GmbH), and improvement in skin spuma and texture^{32,33}. In another double blind study involving 23 healthy individuals, oral administration of corn ceramide (3 groups: placebo and glucosyl ceramide at 0.6 and 1.2 mg/day; administered for 3 weeks) induced an increase in moisture levels in the stratum corneum (Skicon-200, IBS, Hamamatsu-city, Shizuoka) and inhibited perspiration in the epidermis (using Tewameter)³⁴.

In a double blind study involving 100 individuals with high TEWL in the cheek, oral administration of konjac-derived ceramide (1.8 mg/day as glucosyl ceramide for 12 weeks) improved TEWL in the cheek as well as moisture levels in the stratum corneum³⁵. Further, in a double blind study involving 16 healthy men and women, 3-week oral administration of konjac-derived ceramide (1.2 mg/day as glucosyl ceramide for 8 weeks) induced improvements in subjective symptoms related to skin moisture and significantly reduced TEWL in the left arm³⁶. Similarly, in a recent double blind study involving 60 individuals with dry skin, oral administration of acetic acid bacteria (*Acetobacter malorum*)-derived ceramide (3 groups: placebo and ceramide at 0.4 and 0.8 mg/day, administered for 8 weeks) induced improvements in TEWL in the cheek as well as moisture levels in the stratum corneum³⁷.

However, results from the present study differed from those obtained in the above-mentioned previous studies with rice, corn, and konjac ceramide; specifically, beet ceramide did not induce any significant improvements in TEWL. Further, while previous experiments involving cultured normal human dermal fibroblasts found that konjac ceramide dose-dependently induced an increase in collagen type I production³⁶, we noted no such effect with beet ceramide (100 µg/ml).

Differences between the types of sphingoid bases and fatty acid molecules that make up the various ceramide compounds may account for the disparity observed when culturing in konjac ceramide and beet ceramide. With regard to sphingoid bases, rice, corn, and konjac ceramide consist primarily of 8-cis-unsaturated bonds, such as d18:2^{4t,8c} and d18:1^{8c}, while beet ceramide consists primarily of 8-trans-unsaturated bonds, such as d18:2^{4t,8t}, d18:1^{8t}, and t18:1^{8t}^{6,38}. In addition, fatty acids in rice, corn, and konjac typically have 16-24 carbon atoms (mainly 18 and 20), while beet ceramide is rich in palmitinic acids (16 carbon atoms). Acetic acid bacteria-derived ceramide differs from authentic glucosyl ceramide altogether, being primarily composed of saturated dihydroxyl base sphinganine (dihydrosphingosine) (d18:0)³⁹.

Fibroblasts play an essential role in maintaining healthy skin by producing extracellular matrices comprised of collagens type I, III, VI, and VII; fibronectin; glycosaminoglycan; tissue inhibitor of metalloproteinase (TIMP), which inhibits the breakdown of the extracellular matrix; and matrix metalloproteinase (MMP), which degrades the extracellular matrix^{40,41}. In addition, fibroblasts also produce cytokines, such as TGF-β. While decreased reduction in fibroblast activity can delay wound healing and ulcer formation, excessive activation is related to aggravation of fibrosis and manifestation of scleroderma.

Fibronectin exists as a dimer, consisting of two polypeptide

chains (A and B), each with a molecular weight of 230 kDa and linked by a pair of C-terminal disulfide bonds. The N-terminal domains of the dimers bond and assemble to form long fibers. Fibronectin encloses and extends outside of cells to form a network, contributing to cell adhesion, growth, migration, and differentiation. Fibronectin levels in human plasma are 0.12±0.02 mg/ml for ages 0-29 years, 0.16±0.04 mg/ml for ages 30-59 years, and 0.26±0.08 mg/ml for ages 60-100 years. As is evident, plasma levels of fibronectin increase slowly until the 50s, and then increases significantly thereafter⁴². However, given that fibronectin levels in skin decrease with age, beet ceramide's promotive effect on fibronectin production and enhancement of fibronectin mRNA expression is noteworthy. Tretinoin tocoferil, which promotes wound healing, has been reported to have a promotive effect on fibronectin production⁴⁰. Given that fibronectin is responsible for maintaining structure and strength in skin, fibronectin is expected to be involved in improving skin elasticity (Cutometer indices R2 and R7), as noted in this study.

The glucosyl ceramide contained in the beet ceramide used in this study differs from authentic glucosyl ceramide in several respects, such as types of sphingoid bases and fatty acids, as previously described. Further, although beet ceramide is comprised of approximately 3% glucosyl ceramide, beets also contain many unidentified lipid components, such as plant sterols, which may also contribute to alterations in skin condition. Further research is needed to clarify our understanding of these other components in beet ceramide.

Safety

Regarding the safety of administering authentic glucosyl ceramide to humans, 12-week oral administration of konjac potato flour extract (glucosyl ceramide, 5.4 mg/day) produced no adverse events in scientific or clinical examinations⁴³. In the present study as well, no adverse events were reported as subjective symptoms or in scientific or clinical examinations. Further, significantly greater improvements were noted for "Easily breaking into a sweat" scores in the high-dose group than in the control group, indicating that elevated perspiration was caused by improvements in sweat gland function. In addition, "Hair loss" and "Pessimism" scores were significantly increased in the low-dose group compared with the control group. However, given that this finding was not observed in the high-dose group, nor did it display dose-dependency, this observed increase was determined to be due to the strong placebo effect experienced by participants in the control group. Taking these findings into account, we therefore conclude that ingestion of beet ceramide is safe.

Conclusion

To assess the effects of 8-week oral administration of glucosyl ceramide contained in beet extract (beet ceramide; administered as glucosyl ceramide to 3 groups at 0, 0.6, and 1.8 mg/day), we conducted a double-blind comparison study involving 35 females (mean age: 40.9±4.2 years). Results showed that oral ingestion of beet ceramide improved subjective skin symptoms, and skin elasticity tests showed that elasticity index improved in a dose-dependent manner. In addition, beet ceramide promoted fibronectin synthesis and mRNA expression in experiments involving human dermal fibroblast cells. Given these results, we postulate that orally ingested beet ceramide stimulates cell signals, thereby exerting favorable effects on the extracellular matrix. We have confirmed the safety of beet ceramide in humans. However, further studies are needed to determine the digestion and absorption dynamics of beet ceramide, as well as its transferability to and effects on human skin.

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