Glycative Stress Research

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*Original article*

# A placebo-controlled, randomized, single-blind, parallel-group comparative study to evaluate the anti-glycation effect of a functional soymilk beverage supplemented with rice bran/rice bran oil

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## Abstract

***Objective*:** We evaluated the effect on glycation stress of a consecutive 12-week ingestion of a soymilk beverage supplemented with rice bran and rice bran oil (test diet) in a placebo-controlled, randomized, single-blind, parallel-group comparison design. Specifically, changes in soluble receptor for advanced glycation end product (sRAGE) and endogenous secretary RAGE (esRAGE), among others, were evaluated.

***Methods*:** : Women with high glycation stress, defined as an autofluorescence (AF) intensity of ≥ 2.0 on an AGE Reader, with a mean age of 47.24 ± 7.24, were randomized to receive either a control soymilk beverage (placebo; 11 subjects) or the test diet (12 subjects, daily intake of rice bran and rice bran oil of 8.2 g and 2.7 g, respectively). After the 12-week ingestion of placebo or the test diet, subjects were assessed for skin elasticity, as measured with Cutometer, skin AF intensity, glycation stress-related parameters in blood, including carboxymethyl-lysine (CML), sRAGE and esRAGE, and skin color as measured with a color-difference meter.

***Results*:** The following parameters were significantly improved in the test group compared to the control group: decreased CML and improved skin elasticity index R6 in an overall analysis; and a slower increase in plasma glucose level and a slower decrease in skin elasticity index R7 in a subclass analysis in the subjects with a BMI of ≥ 25. Although no significant intergroup difference was observed, the following parameters were significantly improved only in the test group: decreased HbA1c, increased sRAGE, increased esRAGE, and improved melanin index for skin color difference in the upper arm. No adverse event occurred during or after the study period.

***Conclusion*:** The ingestion of the test diet resulted in significant improvement in glucose metabolism, CML content in blood and skin elasticity, as well as a significant increase in esRAGE, a decoy receptor for AGEs. These results suggest that the test diet reduces glycation stress.

***KEY WORDS*:** Rice bran, rice bran oil, advanced glycation end products, glycation stress

## Introduction

A variety of byproducts can be obtained from rice, Japan's staple food, such as rice bran and rice bran oil. Rice bran consists of rice pericarp, seed coat, perisperm, aleurone layer, and other byproducts of the pearling (polishing) of brown rice into white rice, and is known to be rich in oil, protein, minerals and B vitamins (especially B1) [1-7]. Rice bran oil is a vegetable oil extracted from rice bran; it is characterized by a high ratio of oleic acid to total fatty acids, a high concentration of vitamin E and high resistance to oxidation on heating [8].

A previous *in vitro* study evaluated the effect of High-Bref™ (Sunbran Co., Ltd., Yamagata, Japan), a functional material made of powdered rice bran, on the inhibition of

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advanced glycation end products (AGEs) produced by a glucose-human serum albumin (HSA) reaction. Both the hot water and ethanol extracts of High-Bref™ inhibited the production of fluorescent AGEs in a dose-dependent manner, suggesting that this material has an anti-glycation activity [8]. The objective of the present study in humans was to evaluate the effect of a soymilk beverage supplemented with rice bran and rice bran oil as the test diet, in terms of glycation stress reduction.

## Methods

### Subjects

Healthy Japanese women between 35 and 60 years of age who 1) have been found to have high blood glucose or HbA1c levels, 2) have diabetic parents or close relatives, and/or 3) have a waist circumference of 90 cm or more were preferably recruited. Eligible subjects were selected according to the criteria below. A total of 67 subjects who provided written informed consent to participating in the study underwent screening assessments, including skin AGE deposition and blood HbA1c level, and 25 subjects with a skin AGEs deposition of ≥ 2.0, which is the average for 50-year-old women (= 50 years of glycation age) and the highest HbA1c levels were eventually included in the study. Those with a glycation age younger than the chronological age, *i.e.* with a skin AGE deposition lower than the average for the age of the subject, were excluded. The principal investigator determined the eligibility of each candidate subject using the following inclusion/exclusion criteria. Specifically, subjects were excluded if they: 1) were smokers, 2) had an average sleep time of less than 5 hours/night, 3) were regularly consuming an anti-glycation diet and/or using an anti-glycation cosmetic, 4) were regularly consuming an anti-hyperglycemic diet, 5) were regularly consuming a soymilk beverage, rice bran or rice bran oil, or health food containing any of these as the main ingredient, 6) had food allergy or were allergic to specific food materials (especially soybean allergy), or 7) were considered ineligible for other reasons as assessed by the principal investigator.

### Study design

This placebo-controlled, randomized, single-blind, parallel-group comparative study was designed to include 25 eligible subjects who started taking the test diet as the full analysis set (FAS), which was further divided into the control (12 subjects) and test (13 subject) groups. Subjects allocated to the control and test groups consumed a control diet (placebo) and the test diet for 12 weeks, respectively. During this period, clinical evaluations were performed at four time points (weeks 0, 4, 8 and 12) and blood/urine tests and skin function test performed at weeks 0, 8 and 12.

Each subject consumed a bottle (195 g) of the test or control diet once daily before the evening meal. Subjects were instructed to consume the investigational diet even when not having a meal. In the event of a missed dose, the subject drank the investigational diet as soon as possible within the day. The mean compliance rate was 99.8% in the control group, 98.5% in the test group, and 99.1% in the entire study population.

This study was conducted between September 2013 and December 2013 at Senrigaoka Kyouritu Clinic (Settsu, Osaka, Japan). Subjects were given a sufficient explanation of the purpose and details of the study and participants' rights and provided written informed consent. They were also told that early withdrawal from the study would not disadvantage them at all.

### Test diet

A beverage containing rice bran and rice bran oil was used as the test diet. The test and control (placebo) diets were provided by Sunstar Inc. (Takatsuki, Osaka). The daily intakes of each ingredient are shown in ***Table 1***. The differential compositions of the test and control diets are shown in ***Table 2***. The daily intake of each ingredient was as follows: 8.2 g of rice bran and 2.7 g of rice bran oil in the test group, and 2.0 g of rice flour and 5.5 g of corn oil in the control group.

The safety of the test diet was confirmed as follows. All ingredients of the test diet have long been consumed and recognized as safe food ingredients. Also the ingredients being tested are contained at concentrations lower than the safe concentrations demonstrated by safety testing. Rice flour and corn oil, which constituted the control diet in place of the ingredients being tested, are also widely distributed in food and have never been reported to cause any serious adverse events. The test diet is produced by a food manufacturing process which is strictly controlled to ensure product quality from the receiving of raw materials through packaging. The following batch release criteria are being applied to assure the safety of the test diet as a food product. For product identification, the test diet had to be a nearly homogeneous, milky-brown liquid while the control diet had to be a nearly homogeneous, milky colored liquid. Both products had to be negative for coliform bacteria, with viable bacteria and fungal/yeast counts of 0 cell/ml. A pilot study was conducted to test

***Table 1. The estimated daily intake of ingredients/nutrients of the test and control diets***

| | Test diet (195 g) | Control diet (placebo) (195 g) |
|---|---|---|
| Calorie (kcal) | 109 | 110 |
| Protein (g) | 3.4 | 2.1 |
| Fat (g) | 4.8 | 6.5 |
| Carbohydrates (g) | 9.5 | 9.6 |
| Dietary fiber (g) | 6.4 | 1.1 |
| Solid soybean | 3.5 | 3.5 |

***Table 2. Compositions of the test and control diets***

| Test diet | Control diet |
|---|---|
| Soymilk | Soymilk |
| Rice bran | Rice flour |
| Rice bran oil | Corn oil |
| Indigestible dextrin | — |
| Honey | Honey |
| Isomaltooligosaccharide | Isomaltooligosaccharide |
| Maltitol | Maltitol |
| Sucralose | Sucralose |
| Emulsifier | Emulsifier |
| Stabilizer (polysaccharide thickener) | Stabilizer (polysaccharide thickener) |

The control diet is formulated with rice flour and corn oil, rather than rice bran, rice bran oil and indigestible dextrin, as contained in the test diet.

administration of the test diet at a dosage of 16 g/day for 12 weeks to 13 volunteers and confirmed no adverse event related to the test diet (Sunstar in-house data). In another pilot study, a total of 8 volunteers were given the test diet at a dosage of 20 g/day for 8 weeks and no abnormal changes were observed in liver function or routine laboratory parameters (Sunstar in-house data).

## *Test procedure*

**Anti-Aging QOL Common Questionnaire (AAQol)**

Subjective symptoms were divided into physical and mental symptoms and evaluated on a 5-point scale using the Anti-Aging QOL Common Questionnaire (AAQol), as described previously [8,9].

**Physical examination**

The following physical parameters were recorded: height (cm), body weight (kg), body composition (body fat percentage, fat mass, fat-free mass, muscle mass, and basal metabolic rate), systolic and diastolic blood pressure (mmHg) and pulse rate (beats/min). Body composition was analyzed using Well Scan 500 (Canon Lifecare Solutions Inc., Chuo-ku, Osaka).

**Blood biochemistry**

The following routine laboratory parameters were evaluated: hematological parameters, including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential WBC count, and platelets; biochemical parameters, including total protein, albumin, total bilirubin, AST (GOT), ALT (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-GTP, blood urea nitrogen (BUN), creatine phosphokinase (CPK), creatinine (CRE), uric acid (UA), sodium (Na), chloride (Cl), potassium (K), calcium (Ca), iron (Fe), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglyceride (TG), fasting plasma glucose (FPG), HbA1c [JDS] [NGSP], insulin (immunoreactive insulin: IRI) and glycated albumin; and AGEs and their intermediates, including carboxymethyl-lysine (CML), pentosidine, and 3-deoxyglucosone (3DG). The serum concentration of soluble receptor for AGE (sRAGE) was measured with Human RAGE Quantikine ELISA Kit DRG00 (R&D Systems Inc., Minneapolis, MN, USA) and that of endogenous secreted RAGE (esRAGE) was measured with esRAGE Human ELISA Kit K1009-1 (B-BridgeInternational Inc., Cupertino, CA, USA). Except for 3DG, which was measured at SRL, Inc. (Tokyo, Japan), all laboratory parameters were measured at LSI Medience Corporation (Tokyo, Japan).

**Skin AGE fluorometry**

Skin AGE deposition was analyzed as a measure of glycation using AGE Reader™ (DiagnOptics, Groningen, Netherlands) [10,11]. This non-invasive test detects unique autofluorescence (AF) emitted from AGEs accumulated in skin tissue as excited by ultraviolet irradiation. Skin AF emission has been shown to be well correlated with the skin accumulation of typical AGEs, such as fluorescent pentosidine and non-fluorescent CML, in studies using skin biopsies from diabetic and dialysis patients. As described previously [12], a subject was instructed to rest his/her cheeks in his/her hands, and AF intensity was measured at a site about 10 cm from the elbow on the medial aspect of the right upper arm. The average of three measurements at the same site was reported.

**CML content in stratum corneum**

Stratum corneum was sampled using the tape stripping technique and used for protein extraction and the subsequent measurement of CML and protein contents [13]. CML was quantified using $N^{\varepsilon}$-(carboxymethyl) lysine ELISA Kit CY8066 (CircuLex Inc, Nagano, Japan). A sample of stratum corneum was collected from the medial aspect of the right upper arm (about 10 cm from the olecranon toward the shoulder).

**Skin elasticity test**

Skin elasticity, as previously described [14-16], was measured using Cutometer (Dual MPA580, Courage + Khazaka, Cologne, Germany) [17-19]. Using a single suction technique, five measurements were obtained at and around the measurement site. The average of three measurements, excluding the maximum and minimum values using the value of elasticity index R2 as the reference value, was reported. This test was performed in the left cheek (at the center between the bottom of the earlobe and the lip end) and the medial aspect of the right upper arm (at about 10 cm from the olecranon toward the shoulder) on a subject placed in a supine position.

**Skin color difference analysis**

Skin color difference was analyzed using a spectrophotometer (CM-600d, Konica Minolta Inc., Tokyo, Japan), as described previously [20]. Five measurements were obtained at the center of the measurement site. The average of three measurements, excluding the maximum and minimum values using L* value as the reference value, was reported. This test was performed in the left cheek (at the center between the bottom of the earlobe and the lip end) and at the medial aspect of the right upper arm (at about 10 cm from the olecranon toward the shoulder).

All of these skin parameters were measured after washing the measurement site or face and a 20-minute acclimation in a room with temperature and humidity controlled at 21°C and 50%, respectively.

**Statistical analysis**

Results were reported as the mean ± standard deviation. For AAQol assessment, Dunnett's test was used to compare data obtained before diet intake with those obtained 4, 8 and 12 weeks after diet intake, and the unpaired t-test was used to compare absolute and percent changes from baseline (before diet intake) between groups. For measured data, Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake, and the unpaired t-test was used to compare absolute and percent changes from baseline (before diet intake) between groups. All statistical analyses were performed using SPSS Statistics 20.0 software (IBM Japan, Tokyo, Japan). Differences were considered significant for a two-tailed significance level of 0.05.

**Ethical considerations**

This study was conducted at Senrigaoka Kyouritu Clinic, Settsu, Osaka, in compliance with the ethical principles based on the Declaration of Helsinki, the Private Information Protection Law and the Ministerial Ordinance on Good Clinical Practice (GCP) for Drugs (Ministry of Health and Welfare Ordinance No. 28 of March 27, 1997). The protocol of this study was reviewed for the ethical aspects and appropriateness of the study and approved by the institutional review board at Tokyo Synergy Clinic (Chuo-ku, Tokyo,

Japan). The study was conducted according to the approved protocol. This study is registered with UMIN (#000017141).

## Results

### Analysis sets

The FAS consisted of 25 subjects (mean age 47.2 ± 7.2 years) who were enrolled in the study and consumed the investigational diet. One subject in the control group was prematurely withdrawn from the study and the remaining 24 subjects completed the study. Another subject in the control group who was taking more than one supplement was excluded from the analysis, and the remaining 23 subjects were included in the per-protocol set (PPS) and used for efficacy analysis.

### AAQol

The results of AAQol assessment are summarized in ***Tables 3*** and ***4***.

The parameters that were significantly improved after diet intake only in the test group were physical symptom "easily breaking into a sweat" at week 12 ( $p = 0.038$) and skin symptom "make-up does not apply smoothly" at week 8 ( $p = 0.037$). No significant intergroup difference in scores was found for these parameters.

The only parameter that was significantly improved in the test group compared to the control group was lifestyle behavior "exercise", in particular the absolute change in score from baseline to week 4 ( $p = 0.029$), absolute and percent changes from baseline to week 8 ( $p = 0.007$ and 0.014, respectively), and absolute and percent changes from baseline to week 12 ( $p = 0.036$ and 0.032, respectively).

### Insulin resistance

The results of analysis of insulin resistance-related parameters and subclass analysis are shown in ***Tables 5*** and ***6***, respectively.

An overall analysis with the PPS (n = 23) showed that significant improvements were observed in HbA1c [JDS] at week 8 ($p = 0.015$) and HbA1c [NGSP] at week 8 ($p = 0.009$) after diet intake in the test group, with no significant difference between groups.

In a subclass analysis of the subjects with a body mass index (BMI) of ≥ 25 (*i.e.* the cut-off for mild obesity) (n = 19), significant improvements were observed in HbA1c [JDS] at week 8 ($p = 0.025$) and HbA1c [NGSP] at week 8 ($p = 0.015$) after diet intake in the test group. Increased FPG level was noted at week 8 in both the test and control groups, with a significantly greater increase in the control group compared to the test group ($p = 0.023$ and 0.023 for the absolute and percent changes at week 8, respectively).

### AGE-related parameters

The results of analysis of AGE-related parameters and subclass analysis are shown in ***Tables 7*** and ***8***, respectively.

In the PPS (n = 23) analysis, 1 subject in the control group was excluded due to a negative value of parameter [sRAGE - esRAGE]. Compared to the baseline values, significant improvements were observed in skin AGE deposition, glycation age (calculated from skin AGE deposition) and the difference between glycation age and chronological age at week 8 ($p = 0.004$ for all parameters) in the test group and at week 8 ($p = 0.014$ for all parameters), week 12 ($p = 0.001$ for all parameters) in the control group. The parameters that were significantly improved after diet intake only in the test group were sRAGE at week 12 ($p = 0.001$), esRAGE at week 12 ($p = 0.003$), and [sRAGE - esRAGE] at week 8 ($p = 0.040$) and week 12 ($p = 0.002$). The parameters with significant differences between the test and control groups were the difference between glycation age and chronological age at week 8 ($p = 0.042$ for intergroup comparison of measured values) (***Fig. 1***) and CML at week 12 ($p = 0.047$ for intergroup comparison of measured values) (***Fig. 2a***).

In a subclass analysis of the subjects with a BMI of ≥ 25 (*i.e.* the cut-off for mild obesity) (n = 19), where 1 subject in the control group was excluded due to a negative value of parameter [sRAGE - esRAGE], significant improvements were observed compared to the baseline values in skin AGE deposition, glycation age (calculated from skin AGE deposition) and the difference between glycation age and chronological age at week 8 ($p = 0.002$ and 0.037, respectively) and week 12 ($p = 0.049$ and 0.003, respectively) in both groups. The parameters that were significantly improved after diet intake only in the test group were sRAGE at week 12 ($p = 0.001$), esRAGE at week 12 ($p = 0.003$), and [sRAGE - esRAGE] at week 8 ($p = 0.030$) and week 12 ($p = 0.001$). A significant difference between the test and control groups was observed in CML at week 12 ($p = 0.019$ for intergroup comparison of measured values) (***Fig. 2b***).

### Skin assessment

The results of analysis of skin-related parameters and subclass analysis for the subjects with a BMI of ≥ 25 (*i.e.* the cut-off for mild obesity) (n = 19) are shown in ***Tables 9*** and ***10***, respectively.

The only parameter that was significantly improved after diet intake only in the test group was melanin index, as determined by the measurement of skin color difference in the upper arm, at week 8 ($p = 0.016$). The parameters that were significantly worsened after diet intake only in the control group were skin elasticity indexes R2 ($p = 0.003$ at week 8 and $p = 0.003$ at week 12) and R7 ($p = 0.004$ at week 8) measured in the cheek. The only parameter that showed a significant improvement in the test group compared to the control group was skin elasticity index R6 measured in the cheek. Although a significant intergroup difference was seen in cheek R6 at week 0, an intergroup comparison of the absolute change in this parameter showed a significant improvement at week 8 in the test group ($p = 0.028$, ***Fig. 3a***). In a subclass analysis of the subjects with a BMI of ≥ 25 (*i.e.* the cut-off for mild obesity) (n = 19), the only parameter that was significantly improved after diet intake only in the test group was melanin index for skin color difference in the upper arm at week 8 ($p = 0.020$). The parameters that were significantly worsened after diet intake only in the control group were skin elasticity indexes R2 ($p = 0.027$ at week 8 and $p = 0.018$ at week 12) and R7 ($p = 0.041$ at week 8) measured in the cheek. In both groups, a decrease in elasticity index R7 in the upper arm was observed at week 12. In the control group, the decrease in R7 was significant at weeks 8 ($p = 0.021$) and 12 ($p < 0.001$), with a significantly greater decrease compared to the test group ($p = 0.013$ and 0.016 for the absolute and percent changes at week 12, respectively) (***Figs. 3b and 3c***).

***Table 3. Results of AAQol assessment (physical and mental symptoms)***

| Parameter | | Unit | Group | n | 0w | 4w | | 8w | | 12w | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | Mean SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Age | Age | Year | Test group | 12 | 49.25 ± 7.57 | – – | – | – – | – | – – | – | | | | | | | | | | |
| | | | Control | 11 | 45.64 ± 7.05 | – – | – | – – | – | – – | – | | | | | | | | | | |
| Physical symptoms | Tired eyes | | Test group | 12 | 2.67 ± 0.49 | 2.83 ± 1.11 | 0.836 | 3.00 ± 0.85 | 0.395 | 2.83 ± 1.11 | 0.836 | 0.519 | 0.393 | 0.970 | 0.809 | 0.718 | 0.623 | 0.865 | 0.688 | 0.657 | 0.889 |
| | | | Control | 11 | 2.73 ± 1.10 | 2.64 ± 1.21 | 0.951 | 2.82 ± 1.08 | 0.951 | 2.91 ± 1.45 | 0.731 | | | | | | | | | | |
| | Blurry eyes | | Test group | 12 | 2.08 ± 0.79 | 2.50 ± 0.90 | 0.252 | 2.50 ± 0.67 | 0.252 | 2.58 ± 0.90 | 0.138 | 0.078 | 0.217 | 0.901 | 0.063 | 0.178 | 0.406 | 0.612 | 0.053 | 0.097 | 0.638 |
| | | | Control | 11 | 1.91 ± 0.83 | 1.73 ± 0.90 | 0.772 | 1.91 ± 0.94 | 1.000 | 2.36 ± 1.29 | 0.143 | | | | | | | | | | |
| | Eye pain | | Test group | 12 | 1.33 ± 0.49 | 1.83 ± 0.83 | **0.028** | 1.67 ± 0.65 | 0.188 | 1.58 ± 0.67 | 0.397 | 0.592 | 0.570 | 0.211 | 0.630 | 0.123 | 0.201 | 0.802 | 0.597 | 0.602 | 0.379 |
| | | | Control | 11 | 1.27 ± 0.65 | 1.64 ± 0.92 | 0.346 | 1.45 ± 1.21 | 0.809 | 1.91 ± 1.04 | **0.043** | | | | | | | | | | |
| | Stiff shoulders | | Test group | 12 | 3.17 ± 0.83 | 3.58 ± 0.90 | 0.076 | 3.58 ± 1.00 | 0.076 | 3.67 ± 1.07 | **0.027** | **0.003** | **0.036** | **0.011** | **0.003** | **0.023** | **0.008** | 0.853 | 0.118 | 0.415 | 0.222 |
| | | | Control | 11 | 3.27 ± 1.68 | 2.73 ± 1.49 | 0.053 | 3.09 ± 1.70 | 0.753 | 2.91 ± 1.76 | 0.260 | | | | | | | | | | |
| | Muscular pain/ stiffness | | Test group | 12 | 2.33 ± 0.89 | 2.92 ± 1.00 | 0.082 | 2.75 ± 1.06 | 0.275 | 2.75 ± 1.14 | 0.275 | **0.021** | **0.030** | 0.130 | **0.040** | **0.021** | 0.253 | 0.699 | 0.159 | 0.296 | 0.337 |
| | | | Control | 11 | 2.55 ± 1.57 | 2.18 ± 1.40 | 0.414 | 2.18 ± 1.47 | 0.414 | 2.27 ± 1.19 | 0.630 | | | | | | | | | | |
| | Palpitations | | Test group | 12 | 1.42 ± 0.67 | 1.58 ± 0.67 | 0.559 | 1.50 ± 0.80 | 0.903 | 1.75 ± 0.87 | 0.087 | 0.701 | 0.684 | 0.263 | 0.318 | 0.801 | 0.342 | 0.895 | 0.904 | 0.886 | 0.540 |
| | | | Control | 11 | 1.45 ± 0.69 | 1.55 ± 0.82 | 0.834 | 1.45 ± 0.69 | 1.000 | 1.55 ± 0.69 | 0.834 | | | | | | | | | | |
| | Shortness of breath | | Test group | 12 | 1.58 ± 0.79 | 1.58 ± 0.67 | 1.000 | 1.50 ± 0.67 | 0.963 | 1.83 ± 0.94 | 0.524 | 0.657 | 0.754 | 0.932 | 0.714 | 0.599 | 0.925 | 0.484 | 0.683 | 0.633 | 0.572 |
| | | | Control | 11 | 1.36 ± 0.67 | 1.45 ± 0.82 | 0.798 | 1.36 ± 0.67 | 1.000 | 1.64 ± 0.67 | 0.083 | | | | | | | | | | |
| | Tendency to gain weight | | Test group | 12 | 4.00 ± 1.04 | 3.92 ± 1.00 | 0.973 | 4.08 ± 0.79 | 0.973 | 4.17 ± 1.03 | 0.831 | 0.381 | 0.815 | 0.394 | 0.451 | 0.729 | 0.586 | 0.849 | 0.546 | 0.677 | 0.335 |
| | | | Control | 11 | 3.91 ± 1.22 | 4.18 ± 1.08 | 0.499 | 3.91 ± 1.14 | 1.000 | 3.73 ± 1.10 | 0.766 | | | | | | | | | | |
| | Weight loss; thin | | Test group | 12 | 1.08 ± 0.29 | 1.25 ± 0.45 | 0.432 | 1.08 ± 0.29 | 1.000 | 1.08 ± 0.29 | 1.000 | 0.610 | 0.565 | 0.565 | 0.610 | 0.715 | 0.715 | 0.952 | 0.708 | 0.506 | 0.506 |
| | | | Control | 11 | 1.09 ± 0.30 | 1.18 ± 0.40 | 0.698 | 1.18 ± 0.40 | 0.698 | 1.18 ± 0.40 | 0.698 | | | | | | | | | | |
| | Lethargy | | Test group | 12 | 1.83 ± 0.83 | 2.33 ± 1.07 | 0.068 | 2.00 ± 0.60 | 0.780 | 2.08 ± 0.90 | 0.521 | 0.074 | **0.029** | 0.503 | **0.033** | **0.031** | 0.680 | 0.495 | 0.486 | 0.359 | 0.985 |
| | | | Control | 11 | 2.09 ± 0.94 | 2.00 ± 1.18 | 0.960 | 1.73 ± 0.79 | 0.285 | 2.09 ± 1.04 | 1.000 | | | | | | | | | | |
| | No feeling of good health | | Test group | 12 | 1.75 ± 0.75 | 2.17 ± 0.72 | 0.130 | 1.83 ± 0.58 | 0.958 | 2.00 ± 0.74 | 0.492 | 0.097 | 0.731 | 0.861 | 0.055 | 0.648 | 0.618 | 0.708 | 0.111 | 0.459 | 0.619 |
| | | | Control | 11 | 1.64 ± 0.67 | 1.64 ± 0.81 | 1.000 | 1.64 ± 0.67 | 1.000 | 1.82 ± 0.98 | 0.719 | | | | | | | | | | |
| | Thirst | | Test group | 12 | 1.92 ± 1.00 | 1.75 ± 0.87 | 0.862 | 1.75 ± 0.87 | 0.862 | 2.00 ± 0.74 | 0.978 | 0.796 | 0.840 | 0.546 | 0.346 | 0.723 | 0.336 | 0.655 | 0.868 | 0.528 | 0.829 |
| | | | Control | 11 | 2.09 ± 0.83 | 1.82 ± 1.08 | 0.637 | 2.00 ± 1.00 | 0.976 | 1.91 ± 1.22 | 0.848 | | | | | | | | | | |
| | Skin problems | | Test group | 12 | 1.50 ± 0.52 | 2.08 ± 0.67 | **0.009** | 1.58 ± 0.51 | 0.942 | 2.08 ± 0.51 | **0.009** | **0.001** | 0.157 | 0.066 | **0.001** | 0.290 | **0.015** | **0.029** | 0.454 | 0.217 | 0.639 |
| | | | Control | 11 | 2.27 ± 1.01 | 1.82 ± 0.98 | 0.159 | 2.00 ± 1.00 | 0.532 | 2.27 ± 1.27 | 1.000 | | | | | | | | | | |
| | Anorexia | | Test group | 12 | 1.25 ± 0.45 | 1.67 ± 0.78 | 0.086 | 1.33 ± 0.49 | 0.945 | 1.50 ± 0.67 | 0.416 | **0.031** | 0.437 | **0.045** | **0.048** | 0.475 | 0.058 | 0.907 | 0.075 | 0.432 | 0.190 |
| | | | Control | 11 | 1.27 ± 0.47 | 1.18 ± 0.40 | 0.818 | 1.18 ± 0.40 | 0.818 | 1.18 ± 0.40 | 0.818 | | | | | | | | | | |
| | Early satiety | | Test group | 12 | 1.50 ± 0.67 | 1.83 ± 0.72 | 0.311 | 1.92 ± 1.00 | 0.160 | 2.17 ± 1.03 | **0.013** | 0.903 | 0.090 | 0.051 | 0.764 | 0.149 | 0.134 | 0.183 | 0.338 | **0.017** | **0.015** |
| | | | Control | 11 | 1.18 ± 0.40 | 1.55 ± 0.69 | 0.194 | 1.09 ± 0.30 | 0.943 | 1.27 ± 0.47 | 0.943 | | | | | | | | | | |

| Parameter | | Unit | Group | n | 0w | 4w | | 8w | | 12w | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | Mean SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Physical symptoms | Epigastralgia | | Test group | 12 | 1.58 ± 0.67 | 1.58 ± 0.79 | 1.000 | 1.58 ± 1.00 | 1.000 | 1.75 ± 0.87 | 0.717 | 1.000 | 0.708 | 0.801 | 0.726 | 0.527 | 0.583 | 0.271 | 0.263 | 0.546 | 0.312 |
| | | | Control | 11 | 1.27 ± 0.65 | 1.27 ± 0.47 | 1.000 | 1.36 ± 0.67 | 0.924 | 1.36 ± 0.92 | 0.924 | | | | | | | | | | |
| | Liable to catch colds | | Test group | 12 | 1.58 ± 0.67 | 1.92 ± 0.79 | 0.438 | 1.83 ± 1.03 | 0.651 | 2.08 ± 1.08 | 0.149 | 0.855 | 0.568 | 0.624 | 0.962 | 0.363 | 0.337 | 0.095 | 0.152 | 0.618 | 0.710 |
| | | | Control | 11 | 1.18 ± 0.40 | 1.45 ± 0.69 | 0.738 | 1.64 ± 0.81 | 0.376 | 1.91 ± 1.14 | 0.083 | | | | | | | | | | |
| | Coughing and sputum | | Test group | 12 | 1.33 ± 0.65 | 1.92 ± 0.90 | 0.143 | 2.25 ± 0.97 | **0.011** | 2.33 ± 0.89 | **0.006** | 0.390 | 0.075 | 0.260 | 0.199 | 0.082 | 0.272 | 0.498 | 0.814 | 0.196 | 0.502 |
| | | | Control | 11 | 1.55 ± 0.82 | 1.82 ± 1.08 | 0.631 | 1.73 ± 0.90 | 0.845 | 2.00 ± 1.41 | 0.247 | | | | | | | | | | |
| | Diarrhea | | Test group | 12 | 1.42 ± 0.51 | 1.83 ± 0.58 | 0.248 | 1.67 ± 0.89 | 0.633 | 1.50 ± 0.67 | 0.976 | 0.173 | 0.944 | 0.502 | 0.050 | 0.880 | 0.263 | 0.271 | 0.744 | 0.407 | 0.708 |
| | | | Control | 11 | 1.73 ± 0.79 | 1.73 ± 0.90 | 1.000 | 2.00 ± 1.00 | 0.401 | 1.64 ± 1.03 | 0.942 | | | | | | | | | | |
| | Constipation | | Test group | 12 | 1.75 ± 0.97 | 2.08 ± 1.16 | 0.363 | 1.50 ± 0.67 | 0.585 | 1.83 ± 1.03 | 0.970 | 0.610 | 0.090 | 0.979 | 0.384 | 0.197 | 0.590 | 0.598 | 0.856 | 0.119 | 0.645 |
| | | | Control | 11 | 2.00 ± 1.26 | 2.18 ± 1.40 | 0.689 | 2.27 ± 1.49 | 0.393 | 2.09 ± 1.58 | 0.941 | | | | | | | | | | |
| | Hair loss | | Test group | 12 | 2.25 ± 0.97 | 2.33 ± 0.89 | 0.970 | 2.42 ± 0.79 | 0.818 | 2.33 ± 0.78 | 0.970 | 0.288 | 0.214 | 0.226 | 0.348 | 0.262 | 0.256 | 0.567 | 0.672 | 0.546 | 0.472 |
| | | | Control | 11 | 2.00 ± 1.10 | 2.55 ± 1.44 | 0.276 | 2.64 ± 0.92 | 0.173 | 2.73 ± 1.68 | 0.103 | | | | | | | | | | |
| | Gray hair | | Test group | 12 | 2.75 ± 0.97 | 3.08 ± 1.38 | 0.270 | 3.00 ± 1.13 | 0.492 | 2.92 ± 1.31 | 0.762 | 0.443 | 0.708 | 0.757 | 0.616 | 0.923 | 0.576 | 0.481 | 0.860 | 0.378 | 0.443 |
| | | | Control | 11 | 3.09 ± 1.30 | 3.18 ± 1.25 | 0.965 | 3.45 ± 1.29 | 0.318 | 3.36 ± 1.43 | 0.541 | | | | | | | | | | |
| | Headache | | Test group | 12 | 1.75 ± 0.62 | 2.17 ± 0.94 | **0.046** | 2.17 ± 0.72 | **0.046** | 2.33 ± 0.89 | **0.004** | **0.049** | 0.130 | **0.021** | 0.057 | 0.165 | **0.018** | 0.248 | 0.495 | 0.434 | 0.200 |
| | | | Control | 11 | 2.18 ± 1.08 | 1.91 ± 0.83 | 0.708 | 1.91 ± 0.83 | 0.708 | 1.82 ± 0.98 | 0.510 | | | | | | | | | | |
| | Dizziness | | Test group | 12 | 1.50 ± 0.67 | 1.67 ± 0.89 | 0.515 | 1.58 ± 0.79 | 0.887 | 1.50 ± 0.80 | 1.000 | 0.701 | 0.506 | 0.307 | 0.510 | 0.298 | 0.242 | 0.633 | 0.559 | 0.904 | 0.894 |
| | | | Control | 11 | 1.36 ± 0.67 | 1.45 ± 0.82 | 0.867 | 1.55 ± 0.69 | 0.466 | 1.55 ± 0.82 | 0.466 | | | | | | | | | | |
| | Tinnitus | | Test group | 12 | 1.25 ± 0.45 | 1.17 ± 0.39 | 0.619 | 1.17 ± 0.39 | 0.619 | 1.08 ± 0.29 | 0.124 | 0.172 | 0.952 | 0.093 | 0.187 | 0.830 | 0.098 | 0.357 | 0.142 | 0.307 | 0.113 |
| | | | Control | 11 | 1.55 ± 0.93 | 1.64 ± 0.92 | 0.747 | 1.45 ± 0.82 | 0.747 | 1.64 ± 1.12 | 0.747 | | | | | | | | | | |
| | Hearing difficulty | | Test group | 12 | 1.67 ± 0.65 | 2.00 ± 0.74 | 0.265 | 1.50 ± 0.67 | 0.758 | 1.67 ± 0.98 | 1.000 | 0.105 | 0.610 | 0.741 | 0.066 | 0.610 | 0.981 | **0.044** | **0.004** | 0.076 | 0.274 |
| | | | Control | 11 | 1.18 ± 0.40 | 1.18 ± 0.40 | 1.000 | 1.09 ± 0.30 | 0.721 | 1.27 ± 0.65 | 0.721 | | | | | | | | | | |
| | Lumbago | | Test group | 12 | 2.25 ± 0.97 | 2.25 ± 1.14 | 1.000 | 2.25 ± 1.14 | 1.000 | 2.50 ± 1.38 | 0.541 | 0.359 | 0.741 | 0.592 | 0.407 | 0.899 | 0.393 | 0.481 | 0.899 | 0.411 | 0.809 |
| | | | Control | 11 | 1.91 ± 1.30 | 2.18 ± 1.40 | 0.613 | 1.82 ± 1.33 | 0.973 | 2.36 ± 1.29 | 0.229 | | | | | | | | | | |
| | Arthralgia | | Test group | 12 | 1.58 ± 0.79 | 1.92 ± 0.79 | 0.501 | 2.00 ± 0.74 | 0.327 | 2.08 ± 1.16 | 0.198 | 0.640 | 0.242 | 0.315 | 0.226 | 0.100 | 0.146 | 0.911 | 0.655 | 0.236 | 0.342 |
| | | | Control | 11 | 1.55 ± 0.82 | 1.73 ± 1.19 | 0.561 | 1.55 ± 1.04 | 1.000 | 1.64 ± 1.03 | 0.903 | | | | | | | | | | |
| | Edematous | | Test group | 12 | 2.00 ± 0.85 | 2.25 ± 0.97 | 0.467 | 2.00 ± 0.95 | 1.000 | 2.08 ± 1.00 | 0.954 | 0.106 | 0.478 | 0.780 | 0.297 | 0.842 | 0.538 | 0.602 | 0.731 | 0.843 | 0.447 |
| | | | Control | 11 | 2.27 ± 1.49 | 2.09 ± 1.22 | 0.778 | 2.09 ± 1.22 | 0.778 | 2.45 ± 1.29 | 0.778 | | | | | | | | | | |
| | Easily breaking into a sweat | | Test group | 12 | 2.50 ± 1.00 | 2.08 ± 1.08 | 0.268 | 2.08 ± 0.90 | 0.268 | 1.83 ± 0.83 | **0.038** | 0.576 | 0.891 | 0.168 | 0.984 | 0.554 | 0.432 | 0.435 | 0.246 | 0.377 | 0.087 |
| | | | Control | 11 | 2.91 ± 1.45 | 2.73 ± 1.49 | 0.836 | 2.55 ± 1.51 | 0.398 | 2.73 ± 1.49 | 0.836 | | | | | | | | | | |
| | Frequent urination | | Test group | 12 | 1.75 ± 0.75 | 1.92 ± 0.79 | 0.763 | 2.00 ± 0.74 | 0.495 | 1.83 ± 0.94 | 0.959 | 0.434 | 0.074 | 0.612 | 0.335 | 0.069 | 0.516 | 0.939 | 0.411 | **0.043** | 0.597 |
| | | | Control | 11 | 1.73 ± 0.65 | 1.64 ± 0.81 | 0.948 | 1.36 ± 0.67 | 0.215 | 1.64 ± 0.81 | 0.948 | | | | | | | | | | |

| Parameter | | Unit | Group | n | 0w | 4w | | 8w | | 12w | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | Mean SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Physical symptoms | Hot flash | | Test group | 12 | 1.50 ± 0.67 | 1.42 ± 0.67 | 0.935 | 1.50 ± 0.80 | 1.000 | 1.50 ± 0.80 | 1.000 | 0.728 | 0.527 | 1.000 | 0.572 | 0.844 | 0.686 | 0.901 | 0.852 | 0.708 | 0.916 |
| | | | Control | 11 | 1.55 ± 1.04 | 1.36 ± 0.67 | 0.767 | 1.36 ± 0.92 | 0.767 | 1.55 ± 1.21 | 1.000 | | | | | | | | | | |
| | Cold skin | | Test group | 12 | 2.17 ± 1.03 | 2.50 ± 1.09 | 0.497 | 2.33 ± 1.07 | 0.880 | 2.67 ± 0.89 | 0.194 | 0.127 | 0.345 | 0.363 | 0.121 | 0.244 | 0.191 | 0.585 | 0.421 | 0.957 | 0.955 |
| | | | Control | 11 | 2.45 ± 1.44 | 2.09 ± 1.30 | 0.162 | 2.36 ± 1.57 | 0.934 | 2.64 ± 1.57 | 0.664 | | | | | | | | | | |
| | Get tired easily | | Test group | 12 | 2.17 ± 0.72 | 2.50 ± 1.00 | 0.255 | 2.25 ± 0.62 | 0.956 | 2.42 ± 0.90 | 0.476 | **0.039** | 0.087 | 0.475 | **0.031** | 0.052 | 0.766 | 0.469 | 0.303 | 0.720 | 0.940 |
| | | | Control | 11 | 2.45 ± 1.13 | 2.00 ± 1.26 | 0.175 | 2.09 ± 1.38 | 0.329 | 2.45 ± 1.44 | 1.000 | | | | | | | | | | |
| Mental symptoms | Irritability | | Test group | 12 | 2.42 ± 0.51 | 2.25 ± 0.75 | 0.823 | 2.33 ± 0.78 | 0.971 | 2.50 ± 1.00 | 0.971 | 0.394 | 0.470 | 0.835 | 0.319 | 0.396 | 0.856 | 0.677 | 0.787 | 0.251 | 0.593 |
| | | | Control | 11 | 2.27 ± 1.01 | 2.36 ± 1.21 | 0.969 | 1.91 ± 0.94 | 0.350 | 2.27 ± 1.01 | 1.000 | | | | | | | | | | |
| | Easily angered | | Test group | 12 | 2.00 ± 0.74 | 2.08 ± 0.67 | 0.952 | 2.25 ± 0.75 | 0.451 | 2.33 ± 1.07 | 0.233 | 0.498 | 0.090 | **0.018** | 0.592 | 0.053 | 0.067 | 0.799 | 0.482 | 0.248 | 0.127 |
| | | | Control | 11 | 2.09 ± 0.94 | 2.36 ± 1.12 | 0.554 | 1.82 ± 0.98 | 0.554 | 1.64 ± 1.03 | 0.176 | | | | | | | | | | |
| | Loss of motivation | | Test group | 12 | 2.08 ± 0.90 | 2.33 ± 0.89 | 0.500 | 1.83 ± 0.83 | 0.500 | 2.33 ± 0.98 | 0.500 | 0.944 | 0.607 | 0.115 | 0.831 | 0.868 | 0.102 | 0.612 | 0.728 | 0.968 | 0.063 |
| | | | Control | 11 | 1.91 ± 0.70 | 2.18 ± 1.17 | 0.583 | 1.82 ± 0.98 | 0.970 | 1.55 ± 0.93 | 0.361 | | | | | | | | | | |
| | No feeling of happiness | | Test group | 12 | 1.75 ± 0.62 | 2.00 ± 0.74 | 0.356 | 1.67 ± 0.89 | 0.933 | 1.83 ± 0.72 | 0.933 | 0.944 | 0.981 | 0.548 | 0.808 | 0.736 | 0.967 | 0.939 | 1.000 | 0.928 | 0.506 |
| | | | Control | 11 | 1.73 ± 0.79 | 2.00 ± 1.10 | 0.584 | 1.64 ± 0.67 | 0.970 | 1.64 ± 0.67 | 0.970 | | | | | | | | | | |
| | Nothing to look forward to in life | | Test group | 12 | 1.92 ± 0.79 | 2.00 ± 0.60 | 0.959 | 1.67 ± 0.89 | 0.500 | 1.67 ± 0.65 | 0.500 | 0.404 | 0.776 | 0.708 | 0.322 | 0.989 | 0.340 | 0.620 | 0.056 | 0.332 | 0.914 |
| | | | Control | 11 | 1.73 ± 1.01 | 1.45 ± 0.69 | 0.610 | 1.36 ± 0.50 | 0.391 | 1.64 ± 0.67 | 0.973 | | | | | | | | | | |
| | Daily life is not enjoyable | | Test group | 12 | 1.58 ± 0.67 | 2.00 ± 0.60 | 0.091 | 1.75 ± 0.62 | 0.715 | 1.75 ± 0.75 | 0.715 | 0.201 | 0.291 | 0.239 | 0.220 | 0.245 | 0.293 | 0.507 | 0.359 | 0.462 | 0.505 |
| | | | Control | 11 | 1.82 ± 0.98 | 1.73 ± 0.79 | 0.971 | 1.55 ± 0.69 | 0.596 | 1.55 ± 0.69 | 0.596 | | | | | | | | | | |
| | Lose confidence | | Test group | 12 | 1.75 ± 0.62 | 2.08 ± 1.00 | 0.417 | 1.83 ± 0.72 | 0.976 | 1.92 ± 1.00 | 0.846 | 0.196 | 0.381 | 0.182 | 0.186 | 0.385 | 0.205 | 0.843 | 0.174 | 0.338 | 0.113 |
| | | | Control | 11 | 1.82 ± 0.98 | 1.55 ± 0.82 | 0.541 | 1.55 ± 0.69 | 0.541 | 1.36 ± 0.50 | 0.166 | | | | | | | | | | |
| | Reluctance to talk with others | | Test group | 12 | 1.75 ± 0.75 | 1.75 ± 0.87 | 1.000 | 1.58 ± 0.67 | 0.850 | 1.75 ± 0.97 | 1.000 | 0.565 | 0.508 | 0.784 | 0.325 | 0.924 | 0.813 | 0.462 | 0.249 | 0.895 | 0.749 |
| | | | Control | 11 | 1.55 ± 0.52 | 1.36 ± 0.67 | 0.445 | 1.55 ± 0.69 | 1.000 | 1.64 ± 0.67 | 0.858 | | | | | | | | | | |
| | Depressed | | Test group | 12 | 1.75 ± 0.75 | 1.83 ± 0.72 | 0.925 | 1.67 ± 0.65 | 0.925 | 1.67 ± 0.78 | 0.925 | 0.977 | 0.548 | 0.331 | 0.525 | 0.568 | 0.406 | 0.339 | 0.492 | 0.669 | 0.933 |
| | | | Control | 11 | 1.45 ± 0.69 | 1.55 ± 1.21 | 0.944 | 1.55 ± 0.69 | 0.944 | 1.64 ± 0.92 | 0.702 | | | | | | | | | | |
| | Feeling of uselessness | | Test group | 12 | 1.42 ± 0.51 | 1.67 ± 0.49 | 0.492 | 1.50 ± 0.67 | 0.958 | 1.83 ± 0.83 | 0.130 | **0.017** | 0.331 | **0.034** | **0.018** | 0.241 | **0.017** | 0.215 | 0.160 | 0.859 | 0.211 |
| | | | Control | 11 | 1.73 ± 0.65 | 1.36 ± 0.50 | 0.066 | 1.55 ± 0.52 | 0.513 | 1.45 ± 0.52 | 0.208 | | | | | | | | | | |
| | Shallow sleep | | Test group | 12 | 1.83 ± 1.03 | 2.08 ± 1.08 | 0.104 | 1.75 ± 0.97 | 0.821 | 1.83 ± 1.03 | 1.000 | 0.285 | 0.206 | 0.807 | 0.489 | 0.145 | 0.499 | 0.968 | 0.507 | 0.503 | 0.863 |
| | | | Control | 11 | 1.82 ± 0.75 | 1.82 ± 0.75 | 1.000 | 2.00 ± 0.77 | 0.802 | 1.91 ± 1.04 | 0.967 | | | | | | | | | | |
| | Difficulty in falling asleep | | Test group | 12 | 1.92 ± 1.00 | 2.17 ± 1.19 | 0.492 | 1.83 ± 1.03 | 0.958 | 2.00 ± 1.13 | 0.958 | 0.853 | 0.762 | 0.326 | 0.830 | 0.680 | 0.456 | 0.443 | 0.472 | 0.618 | 0.120 |
| | | | Control | 11 | 1.64 ± 0.67 | 1.82 ± 1.08 | 0.766 | 1.64 ± 0.81 | 1.000 | 1.36 ± 0.67 | 0.499 | | | | | | | | | | |
| | Pessimism | | Test group | 12 | 2.17 ± 0.83 | 2.08 ± 0.67 | 0.976 | 1.83 ± 0.58 | 0.428 | 2.17 ± 0.94 | 1.000 | 0.984 | 0.200 | 0.843 | 0.984 | 0.272 | 0.635 | 0.711 | 0.699 | 0.436 | 0.589 |
| | | | Control | 11 | 2.00 ± 1.26 | 1.91 ± 1.38 | 0.969 | 2.18 ± 1.33 | 0.812 | 1.91 ± 1.30 | 0.969 | | | | | | | | | | |

| Parameter | | Unit | Group | n | 0w | | 4w | | | 8w | | | 12w | | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean | SD | Mean | SD | p values | Mean | SD | p values | Mean | SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Mental symptoms | Lapse of memory | | Test group | 12 | 2.67 ± 0.78 | | 2.33 ± 0.78 | | 0.188 | 2.67 ± 0.65 | | 1.000 | 2.92 ± 0.79 | | 0.397 | 0.842 | 0.741 | 0.119 | 0.855 | 0.680 | 0.123 | 0.370 | 0.507 | 0.241 | **0.044** |
| | | | Control | 11 | 2.36 ± 0.81 | | 2.09 ± 0.94 | | 0.454 | 2.27 ± 0.90 | | 0.952 | 2.09 ± 1.04 | | 0.454 | | | | | | | | | | |
| | Inability to concentrate | | Test group | 12 | 2.25 ± 0.62 | | 2.25 ± 0.75 | | 1.000 | 2.33 ± 0.78 | | 0.958 | 2.50 ± 1.00 | | 0.491 | 1.000 | 0.780 | 0.528 | 0.582 | 0.473 | 0.933 | **0.034** | 0.074 | 0.150 | **0.025** |
| | | | Control | 11 | 1.64 ± 0.67 | | 1.64 ± 0.81 | | 1.000 | 1.82 ± 0.87 | | 0.807 | 1.64 ± 0.67 | | 1.000 | | | | | | | | | | |
| | Inability to solve problems | | Test group | 12 | 1.83 ± 0.72 | | 2.08 ± 0.79 | | 0.425 | 1.83 ± 0.58 | | 1.000 | 1.83 ± 0.83 | | 1.000 | 0.147 | 0.527 | 0.527 | 0.510 | 0.590 | 0.844 | 0.543 | **0.037** | 0.115 | 0.251 |
| | | | Control | 11 | 1.64 ± 0.81 | | 1.45 ± 0.52 | | 0.719 | 1.45 ± 0.52 | | 0.719 | 1.45 ± 0.69 | | 0.719 | | | | | | | | | | |
| | Inability to make judgments readily | | Test group | 12 | 1.92 ± 0.79 | | 1.92 ± 0.79 | | 1.000 | 1.92 ± 0.67 | | 1.000 | 1.75 ± 0.87 | | 0.696 | 0.307 | 0.527 | 0.966 | 0.209 | 0.359 | 0.877 | 0.539 | 0.117 | 0.204 | 0.540 |
| | | | Control | 11 | 1.73 ± 0.65 | | 1.45 ± 0.52 | | 0.409 | 1.55 ± 0.69 | | 0.702 | 1.55 ± 0.69 | | 0.702 | | | | | | | | | | |
| | Inability to sleep because of worries | | Test group | 12 | 1.83 ± 0.83 | | 1.83 ± 0.83 | | 1.000 | 1.58 ± 0.67 | | 0.397 | 1.83 ± 0.83 | | 1.000 | 0.465 | 0.948 | 0.811 | 0.524 | 0.651 | 0.935 | 0.968 | 0.414 | 0.895 | 0.868 |
| | | | Control | 11 | 1.82 ± 0.98 | | 1.55 ± 0.82 | | 0.610 | 1.55 ± 0.69 | | 0.610 | 1.91 ± 1.30 | | 0.973 | | | | | | | | | | |
| | A sense of tension | | Test group | 12 | 1.83 ± 0.58 | | 1.83 ± 0.72 | | 1.000 | 1.92 ± 0.79 | | 0.928 | 2.33 ± 0.65 | | **0.016** | 1.000 | 0.636 | 0.242 | 0.709 | 0.876 | 0.517 | 0.779 | 0.852 | 0.763 | 0.275 |
| | | | Control | 11 | 1.91 ± 0.70 | | 1.91 ± 1.14 | | 1.000 | 1.82 ± 0.75 | | 0.981 | 2.00 ± 0.77 | | 0.981 | | | | | | | | | | |
| | Feeling of anxiety for no special reason | | Test group | 12 | 1.58 ± 0.67 | | 1.92 ± 0.79 | | 0.211 | 1.67 ± 0.65 | | 0.947 | 1.92 ± 0.90 | | 0.211 | 0.345 | 0.731 | 0.220 | 0.599 | 0.818 | 0.279 | 0.654 | 0.246 | 0.401 | 0.113 |
| | | | Control | 11 | 1.45 ± 0.69 | | 1.55 ± 0.69 | | 0.944 | 1.45 ± 0.52 | | 1.000 | 1.36 ± 0.67 | | 0.944 | | | | | | | | | | |
| | Vague feeling of fear | | Test group | 12 | 1.58 ± 0.67 | | 1.50 ± 0.67 | | 0.903 | 1.42 ± 0.51 | | 0.559 | 1.42 ± 0.51 | | 0.559 | 0.979 | 0.957 | 0.562 | 0.928 | 0.865 | 0.588 | 0.683 | 0.688 | 0.492 | 0.904 |
| | | | Control | 11 | 1.45 ± 0.82 | | 1.36 ± 0.92 | | 0.958 | 1.27 ± 0.47 | | 0.763 | 1.45 ± 0.93 | | 1.000 | | | | | | | | | | |
| | Stressful | | Test group | 12 | 2.58 ± 0.79 | | 2.83 ± 0.83 | | 0.425 | 2.58 ± 0.79 | | 1.000 | 2.75 ± 1.06 | | 0.715 | 0.339 | 0.479 | 0.970 | 0.253 | 0.230 | 0.550 | 0.214 | 0.651 | 0.590 | 0.337 |
| | | | Control | 11 | 2.09 ± 1.04 | | 2.64 ± 1.21 | | 0.140 | 2.36 ± 1.12 | | 0.637 | 2.27 ± 1.27 | | 0.848 | | | | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 4, 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in scores from baseline (before diet intake) between groups.

***Table 4. Results of AAQol assessment (lifestyle behavior and skin symptoms)***

| Parameter | | Unit | Group | n | 0w | 4w | | 8w | | 12w | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | Mean SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Lifestyle behavior | Smoking | (Cigaretts/day) | Test group | 12 | 0.00 ± 0.00 | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | | | | | | | | | | |
| | | | Control | 11 | 0.00 ± 0.00 | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | | | | | | | | | | |
| | Frequency of alcohol drinking | (times/week) | Test group | 12 | 1.19 ± 1.93 | 1.42 ± 1.96 | 0.518 | 1.21 ± 1.72 | 0.999 | 0.90 ± 1.61 | 0.330 | 0.237 | 0.647 | 0.758 | 0.403 | 0.723 | 0.303 | 0.479 | 0.641 | 0.522 | 0.339 |
| | | | Control | 11 | 1.86 ± 2.55 | 1.86 ± 2.55 | 1.000 | 1.77 ± 2.40 | 0.955 | 1.68 ± 2.22 | 0.747 | | | | | | | | | | |
| | Alcohol consumption | (ml/day) | Test group | 12 | 175.00 ± 235.97 | 247.92 ± 283.74 | 0.210 | 218.75 ± 248.89 | 0.594 | 220.83 ± 290.34 | 0.560 | 0.056 | 0.586 | 0.661 | 0.172 | 0.912 | 0.713 | 0.349 | 0.703 | 0.519 | 0.490 |
| | | | Control | 11 | 323.18 ± 454.60 | 305.00 ± 418.60 | 0.972 | 323.18 ± 486.48 | 1.000 | 336.36 ± 482.10 | 0.989 | | | | | | | | | | |
| | Converted into ethanol T | (g/day) | Test group | 12 | 3.21 ± 6.10 | 3.96 ± 6.04 | 0.488 | 3.42 ± 5.88 | 0.974 | 3.60 ± 6.98 | 0.865 | 0.077 | 0.732 | 0.815 | 0.347 | 0.839 | 0.937 | 0.230 | 0.309 | 0.282 | 0.253 |
| | | | Control | 11 | 9.10 ± 14.40 | 8.48 ± 12.95 | 0.968 | 8.48 ± 13.86 | 0.968 | 8.98 ± 14.12 | 1.000 | | | | | | | | | | |
| | Exercise | (dat/week) | Test group | 12 | 1.04 ± 1.48 | 1.88 ± 2.13 | **0.025** | 1.54 ± 1.64 | 0.247 | 1.25 ± 1.23 | 0.830 | **0.029** | **0.007** | **0.036** | 0.072 | **0.014** | **0.032** | 0.219 | 0.804 | 0.786 | 0.975 |
| | | | Control | 11 | 2.09 ± 2.42 | 1.64 ± 2.41 | 0.289 | 1.32 ± 2.24 | **0.032** | 1.23 ± 2.14 | **0.015** | | | | | | | | | | |
| | Sleeping hours | (hour/day) | Test group | 12 | 6.50 ± 0.64 | 6.63 ± 0.61 | 0.755 | 6.54 ± 0.66 | 0.986 | 6.63 ± 0.88 | 0.755 | 0.446 | 0.906 | 0.961 | 0.442 | 0.965 | 0.987 | 0.159 | 0.353 | 0.134 | 0.233 |
| | | | Control | 11 | 6.89 ± 0.63 | 6.86 ± 0.60 | 0.992 | 6.95 ± 0.61 | 0.842 | 7.02 ± 0.64 | 0.409 | | | | | | | | | | |
| | Water consumption | ( ℓ /day) | Test group | 12 | 1.41 ± 0.48 | 1.30 ± 0.53 | 0.488 | 1.36 ± 0.50 | 0.899 | 1.17 ± 0.49 | **0.029** | 0.662 | 0.583 | 0.450 | 0.461 | 0.663 | 0.273 | 0.997 | 0.812 | 0.689 | 0.583 |
| | | | Control | 11 | 1.41 ± 0.44 | 1.35 ± 0.46 | 0.825 | 1.28 ± 0.39 | 0.317 | 1.27 ± 0.41 | 0.267 | | | | | | | | | | |
| | VDT working hours | (hour/day) | Test group | 12 | 5.54 ± 2.87 | 5.33 ± 1.78 | 0.972 | 5.83 ± 2.62 | 0.930 | 4.92 ± 2.23 | 0.601 | 0.314 | 0.827 | 0.183 | 0.614 | 0.522 | 0.183 | 0.846 | 0.470 | 0.989 | 0.424 |
| | | | Control | 11 | 5.27 ± 3.68 | 6.09 ± 3.05 | 0.670 | 5.82 ± 2.76 | 0.866 | 6.00 ± 3.97 | 0.740 | | | | | | | | | | |
| Skin symptoms | Concerned about pores | | Test group | 12 | 3.25 ± 0.75 | 3.08 ± 1.00 | 0.821 | 2.83 ± 0.83 | 0.205 | 2.92 ± 1.08 | 0.367 | 0.572 | 0.911 | 0.928 | 0.707 | 0.962 | 0.754 | 0.123 | 0.288 | 0.146 | 0.187 |
| | | | Control | 11 | 3.91 ± 1.14 | 3.55 ± 1.04 | 0.203 | 3.45 ± 1.13 | 0.086 | 3.55 ± 1.13 | 0.203 | | | | | | | | | | |
| | Dry skin | | Test group | 12 | 2.42 ± 0.67 | 2.50 ± 0.52 | 0.963 | 2.50 ± 0.80 | 0.963 | 2.58 ± 0.67 | 0.784 | 0.762 | 0.793 | 0.968 | 0.646 | 0.578 | 0.496 | 0.917 | 0.910 | 0.738 | 0.875 |
| | | | Control | 11 | 2.45 ± 1.04 | 2.45 ± 1.21 | 1.000 | 2.64 ± 1.12 | 0.825 | 2.64 ± 0.92 | 0.825 | | | | | | | | | | |
| | Frequent pimples | | Test group | 12 | 2.33 ± 0.78 | 2.00 ± 0.43 | 0.219 | 2.00 ± 0.74 | 0.219 | 2.08 ± 0.67 | 0.434 | 0.825 | 0.554 | 0.462 | 0.623 | 0.387 | 0.382 | 0.602 | 0.569 | 0.236 | 0.982 |
| | | | Control | 11 | 2.55 ± 1.13 | 2.27 ± 1.49 | 0.634 | 2.45 ± 1.04 | 0.975 | 2.09 ± 0.94 | 0.249 | | | | | | | | | | |
| | Oily face | | Test group | 12 | 2.75 ± 0.45 | 2.58 ± 0.67 | 0.756 | 2.33 ± 0.78 | 0.125 | 2.25 ± 0.97 | 0.053 | 0.088 | 0.654 | 0.886 | 0.122 | 0.769 | 0.397 | 0.244 | 0.940 | 0.445 | 0.216 |
| | | | Control | 11 | 3.27 ± 1.35 | 2.55 ± 1.51 | **0.003** | 2.73 ± 1.49 | **0.028** | 2.82 ± 1.17 | 0.077 | | | | | | | | | | |
| | Itchy skin | | Test group | 12 | 1.75 ± 0.75 | 2.08 ± 0.90 | 0.183 | 1.92 ± 0.67 | 0.688 | 2.08 ± 0.67 | 0.183 | 0.228 | 0.172 | 0.399 | 0.360 | 0.201 | 0.520 | 0.528 | 0.835 | 0.804 | 0.985 |
| | | | Control | 11 | 2.00 ± 1.10 | 2.00 ± 1.00 | 1.000 | 1.82 ± 1.17 | 0.741 | 2.09 ± 1.22 | 0.953 | | | | | | | | | | |
| | Concerned about spots or freckles | | Test group | 12 | 3.58 ± 0.67 | 3.42 ± 1.08 | 0.860 | 3.25 ± 0.97 | 0.448 | 3.67 ± 0.78 | 0.978 | 0.316 | 0.880 | **0.021** | 0.206 | 0.950 | **0.015** | 0.419 | 0.189 | 0.634 | 0.051 |
| | | | Control | 11 | 3.27 ± 1.10 | 2.73 ± 1.35 | 0.087 | 3.00 ± 1.48 | 0.557 | 2.73 ± 1.35 | 0.087 | | | | | | | | | | |
| | Make-up runs easily | | Test group | 12 | 2.83 ± 1.03 | 3.00 ± 0.95 | 0.710 | 2.67 ± 0.78 | 0.710 | 2.67 ± 0.78 | 0.710 | **0.005** | 0.188 | **0.043** | **0.003** | 0.124 | **0.029** | 0.067 | 0.643 | 0.486 | 0.891 |
| | | | Control | 11 | 3.70 ± 1.06 | 2.73 ± 1.68 | **0.002** | 3.00 ± 1.41 | **0.025** | 2.73 ± 1.27 | 0.002 | | | | | | | | | | |
| | Poor complexion | | Test group | 12 | 2.17 ± 0.72 | 2.00 ± 0.85 | 0.693 | 2.00 ± 0.60 | 0.693 | 2.25 ± 0.87 | 0.942 | 0.562 | 0.268 | 0.763 | 0.478 | 0.238 | 0.555 | 0.268 | 0.619 | 1.000 | 0.567 |
| | | | Control | 11 | 1.82 ± 0.75 | 1.82 ± 0.87 | 1.000 | 2.00 ± 0.89 | 0.735 | 2.00 ± 1.18 | 0.735 | | | | | | | | | | |

| | Parameter | Unit | Group | n | 0w | 4w | | 8w | | 12w | | Inter-group analysis by change values | | | Inter-group analysis by % change | | | Inter-group analysis by measured values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | Mean SD | p values | 4w | 8w | 12w | 4w | 8w | 12w | 0w | 4w | 8w | 12w |
| Skin symptoms | Coarse skin | | Test group | 12 | 2.08 ± 0.67 | 2.08 ± 0.79 | 1.000 | 1.92 ± 0.67 | 0.536 | 2.08 ± 0.51 | 1.000 | 0.527 | 0.495 | 0.253 | 0.497 | 0.658 | 0.215 | 0.539 | 0.984 | 0.319 | 0.785 |
| | | | Control | 11 | 2.27 ± 0.79 | 2.09 ± 0.94 | 0.793 | 2.36 ± 1.29 | 0.965 | 2.00 ± 0.89 | 0.541 | | | | | | | | | | |
| | Not elastic, not glossy | | Test group | 12 | 3.00 ± 0.95 | 2.83 ± 0.83 | 0.860 | 2.50 ± 0.67 | 0.156 | 3.00 ± 0.95 | 1.000 | 0.968 | 0.901 | 0.490 | 0.861 | 0.698 | 0.467 | 0.463 | 0.472 | 0.406 | 0.859 |
| | | | Control | 11 | 3.36 ± 1.36 | 3.18 ± 1.40 | 0.837 | 2.91 ± 1.45 | 0.233 | 3.09 ± 1.45 | 0.617 | | | | | | | | | | |
| | Concerned about crows feet | | Test group | 12 | 2.83 ± 1.03 | 2.92 ± 1.24 | 0.968 | 2.58 ± 0.67 | 0.566 | 2.92 ± 1.08 | 0.968 | 0.780 | 0.792 | **0.045** | 0.684 | 0.579 | **0.038** | 0.756 | 0.667 | 0.669 | 0.490 |
| | | | Control | 11 | 3.00 ± 1.48 | 3.18 ± 1.66 | 0.829 | 2.82 ± 1.66 | 0.829 | 2.55 ± 1.44 | 0.218 | | | | | | | | | | |
| | Frequent exposure to UV | | Test group | 12 | 2.83 ± 0.72 | 2.67 ± 1.07 | 0.840 | 2.08 ± 0.67 | **0.013** | 2.25 ± 0.62 | 0.062 | 0.211 | 0.475 | 0.217 | 0.249 | 0.387 | 0.095 | 0.757 | 0.251 | 0.209 | 0.119 |
| | | | Control | 11 | 2.73 ± 0.90 | 2.18 ± 0.87 | 0.119 | 1.73 ± 0.65 | **0.002** | 1.73 ± 0.90 | **0.002** | | | | | | | | | | |
| | Concerned about rough skin | | Test group | 12 | 2.58 ± 1.00 | 2.33 ± 0.78 | 0.500 | 2.33 ± 0.89 | 0.500 | 2.50 ± 1.17 | 0.959 | 0.172 | 0.172 | 0.117 | 0.111 | 0.169 | 0.179 | 0.335 | 0.950 | 0.953 | 0.934 |
| | | | Control | 11 | 3.09 ± 1.45 | 2.36 ± 1.43 | **0.031** | 2.36 ± 1.50 | **0.031** | 2.55 ± 1.44 | 0.130 | | | | | | | | | | |
| | Bags under eyes | | Test group | 12 | 2.08 ± 0.51 | 2.58 ± 1.31 | 0.132 | 2.17 ± 0.83 | 0.975 | 2.42 ± 1.08 | 0.412 | **0.008** | **0.044** | **0.006** | **0.012** | **0.028** | **0.002** | 0.126 | 0.363 | 0.698 | 0.303 |
| | | | Control | 11 | 2.82 ± 1.40 | 2.09 ± 1.22 | 0.073 | 2.00 ± 1.18 | **0.039** | 1.91 ± 1.22 | **0.020** | | | | | | | | | | |
| | Dull skin | | Test group | 12 | 2.83 ± 1.03 | 2.75 ± 0.87 | 0.940 | 2.58 ± 0.67 | 0.387 | 2.92 ± 1.08 | 0.940 | **0.015** | 0.109 | **0.018** | **0.037** | 0.343 | 0.229 | 0.244 | 0.963 | 0.891 | 0.847 |
| | | | Control | 11 | 3.45 ± 1.44 | 2.73 ± 1.42 | **0.007** | 2.64 ± 1.12 | **0.002** | 2.82 ± 1.33 | **0.019** | | | | | | | | | | |
| | Dull, fragile nails | | Test group | 12 | 2.33 ± 1.07 | 2.67 ± 1.15 | 0.323 | 2.25 ± 1.14 | 0.966 | 2.42 ± 1.24 | 0.966 | 0.640 | 0.763 | 0.835 | 0.766 | 0.686 | 0.993 | 0.825 | 0.959 | 0.965 | 0.945 |
| | | | Control | 11 | 2.45 ± 1.51 | 2.64 ± 1.63 | 0.836 | 2.27 ± 1.35 | 0.836 | 2.45 ± 1.37 | 1.000 | | | | | | | | | | |
| | Skin clearness | | Test group | 12 | 2.50 ± 0.80 | 2.58 ± 0.51 | 0.963 | 2.67 ± 0.65 | 0.784 | 2.25 ± 0.75 | 0.527 | 0.802 | 0.649 | 0.678 | 0.792 | 0.694 | 0.408 | 0.575 | 0.699 | 0.825 | 0.312 |
| | | | Control | 11 | 2.73 ± 1.10 | 2.73 ± 1.10 | 1.000 | 2.73 ± 0.65 | 1.000 | 2.64 ± 1.03 | 0.977 | | | | | | | | | | |
| | Skin brightness | | Test group | 12 | 2.58 ± 0.79 | 2.75 ± 0.45 | 0.768 | 2.75 ± 0.62 | 0.768 | 2.75 ± 0.75 | 0.768 | 0.540 | 0.786 | 0.354 | 0.637 | 0.909 | 0.538 | 0.706 | 0.687 | 0.442 | 0.339 |
| | | | Control | 11 | 2.73 ± 1.01 | 2.64 ± 0.81 | 0.984 | 3.00 ± 0.89 | 0.732 | 2.45 ± 0.69 | 0.732 | | | | | | | | | | |
| | Not anemic face | | Test group | 12 | 2.83 ± 0.72 | 2.83 ± 0.72 | 1.000 | 2.75 ± 1.06 | 0.962 | 2.83 ± 0.58 | 1.000 | 0.490 | 0.852 | 0.120 | 0.488 | 0.815 | 0.101 | 0.268 | 0.817 | 0.583 | 0.380 |
| | | | Control | 11 | 3.18 ± 0.75 | 2.91 ± 0.83 | 0.756 | 3.00 ± 1.10 | 0.907 | 2.55 ± 0.93 | 0.164 | | | | | | | | | | |
| | Skin elasticity | | Test group | 12 | 2.58 ± 0.90 | 2.67 ± 0.49 | 0.965 | 2.83 ± 0.83 | 0.541 | 2.83 ± 0.58 | 0.541 | 0.620 | 0.861 | 0.362 | 0.510 | 0.807 | 0.388 | 0.935 | 0.507 | 0.773 | 0.214 |
| | | | Control | 11 | 2.55 ± 1.29 | 2.91 ± 1.14 | 0.683 | 2.73 ± 0.90 | 0.939 | 2.36 ± 1.12 | 0.939 | | | | | | | | | | |
| | Skin smoothness | | Test group | 12 | 2.33 ± 0.78 | 2.42 ± 0.67 | 0.964 | 2.58 ± 0.90 | 0.534 | 2.33 ± 0.89 | 1.000 | 0.873 | 0.965 | 0.672 | 0.346 | 0.544 | 0.551 | 0.946 | 0.771 | 0.901 | 0.685 |
| | | | Control | 11 | 2.36 ± 1.29 | 2.55 ± 1.29 | 0.956 | 2.64 ± 1.12 | 0.872 | 2.18 ± 0.87 | 0.956 | | | | | | | | | | |
| | Skin moisture | | Test group | 12 | 2.50 ± 0.90 | 2.58 ± 0.67 | 0.947 | 2.83 ± 0.58 | 0.210 | 2.75 ± 0.75 | 0.424 | 0.852 | 0.855 | 0.239 | 0.454 | 0.724 | 0.362 | 0.593 | 0.379 | 0.649 | 0.465 |
| | | | Control | 11 | 2.73 ± 1.10 | 2.91 ± 1.04 | 0.935 | 3.00 ± 1.10 | 0.821 | 2.45 ± 1.13 | 0.821 | | | | | | | | | | |
| | Good skin condition | | Test group | 12 | 2.58 ± 0.79 | 2.67 ± 0.78 | 0.976 | 2.83 ± 0.83 | 0.635 | 2.75 ± 0.75 | 0.847 | 0.760 | 0.964 | 0.669 | 0.367 | 0.651 | 0.825 | 0.922 | 0.728 | 0.972 | 0.465 |
| | | | Control | 11 | 2.55 ± 1.04 | 2.82 ± 1.25 | 0.884 | 2.82 ± 1.17 | 0.884 | 2.45 ± 1.13 | 0.995 | | | | | | | | | | |
| | Make-up does not apply smoothly | | Test group | 12 | 2.83 ± 1.03 | 2.67 ± 1.07 | 0.854 | 2.17 ± 0.72 | **0.037** | 2.50 ± 0.90 | 0.435 | 0.918 | 0.598 | 0.353 | 0.621 | 0.827 | 0.265 | 0.894 | 0.779 | 0.574 | 0.388 |
| | | | Control | 11 | 2.90 ± 1.29 | 2.82 ± 1.47 | 0.994 | 2.45 ± 1.51 | 0.560 | 2.09 ± 1.30 | 0.135 | | | | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 4, 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in scores from baseline (before diet intake) between groups.

***Table 5. Insulin resistance-related parameters***

| Parameter | Unit | Group | n | 0w | 8w | | 12w | | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean SD | Mean SD | p values | Mean SD | p values | | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Fasting plasma glucose | mg/mL | Test group | 12 | 89.92 ± 6.54 | 92.50 ± 8.59 | 0.107 | 92.25 ± 9.32 | 0.153 | | 0.147 | 0.549 | 0.140 | 0.608 | 0.718 | 0.634 | 0.526 |
| | | Control | 11 | 88.55 ± 11.03 | 94.82 ± 14.00 | **0.010** | 89.36 ± 12.09 | 0.888 | | | | | | | | |
| Insulin | μU/mL | Test group | 12 | 5.40 ± 3.21 | 6.19 ± 2.31 | 0.262 | 6.00 ± 3.49 | 0.443 | | 0.208 | 0.063 | **0.020** | **0.029** | 0.366 | 0.816 | 0.791 |
| | | Control | 11 | 6.67 ± 3.40 | 6.50 ± 3.86 | 0.951 | 5.62 ± 3.33 | 0.211 | | | | | | | | |
| HbA1c [JDS] | % | Test group | 12 | 5.18 ± 0.31 | 5.06 ± 0.29 | **0.015** | 5.21 ± 0.25 | 0.621 | | 0.116 | 0.733 | 0.132 | 0.830 | 0.914 | 0.492 | 0.816 |
| | | Control | 11 | 5.19 ± 0.39 | 5.16 ± 0.42 | 0.713 | 5.25 ± 0.48 | 0.298 | | | | | | | | |
| HbA1c [NGSP] | % | Test group | 12 | 5.56 ± 0.33 | 5.43 ± 0.33 | **0.009** | 5.59 ± 0.28 | 0.615 | | 0.124 | 0.633 | 0.136 | 0.701 | 0.973 | 0.574 | 0.833 |
| | | Control | 11 | 5.56 ± 0.42 | 5.53 ± 0.46 | 0.595 | 5.63 ± 0.50 | 0.240 | | | | | | | | |
| Glycoalbumin | % | Test group | 12 | 14.00 ± 1.05 | 13.83 ± 0.99 | 0.075 | 13.95 ± 0.95 | 0.744 | | 0.058 | 0.152 | 0.062 | 0.163 | 0.443 | 0.802 | 0.693 |
| | | Control | 11 | 13.69 ± 0.82 | 13.74 ± 0.82 | 0.784 | 13.81 ± 0.71 | 0.243 | | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation; FPG, fasting plasama glucose.

***Table 6. Insulin resistance-related parameters (subclass analysis)***

| Parameter | Unit | Group | n | 0w | 8w | | 12w | | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean SD | Mean SD | p values | Mean SD | p values | | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Fasting plasma glucose | mg/mL | Test group | 11 | 89.64 ± 6.79 | 91.55 ± 8.31 | 0.274 | 91.82 ± 9.65 | 0.196 | | **0.023** | 0.947 | **0.023** | 0.820 | 0.767 | 0.126 | 0.742 |
| | | Control | 8 | 91.00 ± 11.28 | 99.38 ± 12.97 | **0.011** | 93.38 ± 10.51 | 0.566 | | | | | | | | |
| Insulin | μU/mL | Test group | 11 | 5.60 ± 3.28 | 6.27 ± 2.41 | 0.403 | 5.99 ± 3.67 | 0.718 | | 0.342 | 0.151 | 0.064 | 0.125 | 0.230 | 0.455 | 0.774 |
| | | Control | 8 | 7.58 ± 3.59 | 7.40 ± 4.03 | 0.972 | 6.48 ± 3.43 | 0.384 | | | | | | | | |
| HbA1c [JDS] | % | Test group | 11 | 5.17 ± 0.33 | 5.05 ± 0.31 | **0.025** | 5.21 ± 0.27 | 0.622 | | 0.233 | 0.604 | 0.262 | 0.692 | 0.352 | 0.188 | 0.272 |
| | | Control | 8 | 5.33 ± 0.36 | 5.29 ± 0.44 | 0.669 | 5.40 ± 0.47 | 0.249 | | | | | | | | |
| HbA1c [NGSP] | % | Test group | 11 | 5.55 ± 0.35 | 5.43 ± 0.34 | **0.015** | 5.59 ± 0.29 | 0.615 | | 0.177 | 0.500 | 0.195 | 0.558 | 0.362 | 0.192 | 0.246 |
| | | Control | 8 | 5.71 ± 0.38 | 5.68 ± 0.45 | 0.681 | 5.80 ± 0.47 | 0.177 | | | | | | | | |
| Glycoalbumin | % | Test group | 11 | 13.95 ± 1.08 | 13.81 ± 1.04 | 0.165 | 13.94 ± 1.00 | 0.990 | | 0.167 | 0.231 | 0.170 | 0.231 | 0.554 | 0.812 | 0.770 |
| | | Control | 8 | 13.66 ± 0.89 | 13.70 ± 0.87 | 0.904 | 13.81 ± 0.73 | 0.263 | | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation; FPG, fasting plasama glucose.

### *Table 7. AGE-related parameters*

| Parameter | Unit | Group | n | 0w | 8w | | 12w | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean SD | Mean SD | p values | Mean SD | p values | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Skin AGE fluorescence | | Test group | 12 | 2.27 ± 0.22 | 2.05 ± 0.35 | **0.004** | 2.12 ± 0.31 | 0.055 | 0.422 | 0.406 | 0.261 | 0.505 | 0.258 | 0.121 | 0.630 |
| | | Control | 11 | 2.40 ± 0.31 | 2.25 ± 0.24 | **0.014** | 2.18 ± 0.27 | **0.001** | | | | | | | |
| Glycation age | year | Test group | 12 | 66.07 ± 12.77 | 53.45 ± 19.73 | **0.004** | 57.76 ± 17.75 | 0.055 | 0.422 | 0.406 | 0.164 | 0.596 | 0.258 | 0.121 | 0.630 |
| | | Control | 11 | 73.45 ± 17.49 | 65.01 ± 13.76 | **0.014** | 61.15 ± 15.22 | **0.001** | | | | | | | |
| Glycation age – chronological age | year | Test group | 12 | 16.82 ± 9.74 | 4.20 ± 17.97 | **0.004** | 8.51 ± 15.57 | 0.055 | 0.422 | 0.406 | 0.208 | 0.185 | 0.074 | **0.042** | 0.316 |
| | | Control | 11 | 27.82 ± 17.58 | 19.37 ± 15.37 | **0.014** | 15.51 ± 17.10 | **0.001** | | | | | | | |
| Pentosidine (serum) | pmol/mL | Test group | 12 | 81.05 ± 34.88 | 93.36 ± 24.13 | 0.233 | 123.04 ± 32.85 | **<0.001** | 0.420 | 0.714 | 0.286 | 0.555 | 0.833 | 0.546 | 0.919 |
| | | Control | 11 | 78.22 ± 28.19 | 100.24 ± 29.49 | **0.018** | 124.43 ± 31.36 | **<0.001** | | | | | | | |
| 3-deoxyglucosone (serum) | ng/mL | Test group | 12 | 19.01 ± 13.87 | 26.98 ± 25.16 | 0.497 | 28.86 ± 11.74 | 0.358 | 0.378 | 0.769 | 0.281 | 1.000 | 0.948 | 0.326 | 0.758 |
| | | Control | 11 | 19.34 ± 9.51 | 18.31 ± 15.19 | 0.984 | 26.83 ± 19.00 | 0.467 | | | | | | | |
| CML (serum) | μg/mL | Test group | 12 | 1.17 ± 0.31 | 1.56 ± 0.39 | **0.001** | 1.55 ± 0.31 | **0.001** | 0.525 | 0.424 | 0.896 | 0.986 | 0.190 | 0.113 | **0.047** |
| | | Control | 11 | 1.38 ± 0.44 | 1.85 ± 0.45 | **<0.001** | 1.86 ± 0.41 | **<0.001** | | | | | | | |
| sRAGE (serum) | pg/mL | Test group | 12 | 798.00 ± 384.57 | 878.25 ± 471.09 | 0.078 | 944.83 ± 528.76 | **0.001** | 0.262 | 0.178 | 0.815 | 0.621 | 0.206 | 0.167 | 0.143 |
| | | Control | 11 | 627.00 ± 209.99 | 660.36 ± 188.02 | 0.431 | 686.91 ± 197.86 | 0.097 | | | | | | | |
| esRAGE (serum) | ng/mL | Test group | 12 | 0.37 ± 0.18 | 0.40 ± 0.22 | 0.231 | 0.44 ± 0.25 | **0.003** | 0.153 | 0.168 | 0.328 | 0.526 | 0.682 | 0.381 | 0.343 |
| | | Control | 11 | 0.33 ± 0.19 | 0.33 ± 0.15 | 0.907 | 0.36 ± 0.14 | 0.452 | | | | | | | |
| sRAGE – esRAGE | pg/mL | Test group | 12 | 432.17 ± 236.87 | 481.58 ± 278.83 | **0.040** | 507.33 ± 312.86 | **0.002** | 0.459 | 0.299 | 0.392 | 0.425 | 0.281 | 0.220 | 0.189 |
| | | Control | 10 | 329.70 ± 187.25 | 357.60 ± 145.02 | 0.465 | 360.10 ± 149.03 | 0.409 | | | | | | | |
| Skin corneum CML | μg/mg protein | Test group | 12 | 46.68 ± 30.91 | | | 27.92 ± 22.16 | 0.102 | | 0.460 | | 0.484 | 0.962 | | 0.238 |
| | | Control | 10 | 46.10 ± 28.70 | | | 37.71 ± 15.57 | 0.359 | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. For skin corneum CML, the paired t-test was used to compare data obtained before diet intake with those obtained 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation; AGEs, advanced glycation end products; 3DG, 3-deoxyglucosone; CML, carboxymethyl lysine; RAGE, receptor for AGE; sRAGE, soluble RAGE; esRAGE, endogenous secreted RAGE.

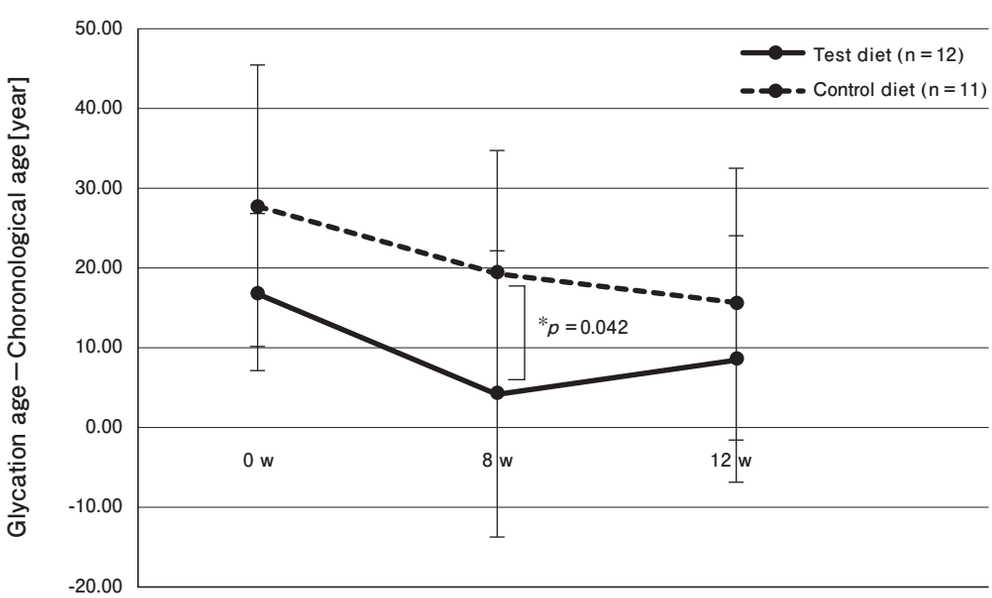


***Fig 1.*** **Difference between glycation age and chronological age.**
Results are expressed as mean ± stndaed deviation. Student's t test.

***Table 8. AGE-related parameters (subclass analysis)***

| Parameter | Unit | Group | n | 0w | 8w | | 12w | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean SD | Mean SD | p values | Mean SD | p values | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Skin AGE fluorescence | | Test group | 11 | 2.25 ± 0.23 | 2.00 ± 0.33 | **0.002** | 2.09 ± 0.31 | **0.049** | 0.402 | 0.395 | 0.255 | 0.506 | 0.229 | 0.097 | 0.616 |
| | | Control | 8 | 2.40 ± 0.28 | 2.24 ± 0.21 | **0.037** | 2.16 ± 0.18 | **0.003** | | | | | | | |
| Glycation age | year | Test group | 11 | 65.08 ± 12.89 | 51.00 ± 18.68 | **0.002** | 56.08 ± 17.58 | **0.049** | 0.402 | 0.395 | 0.167 | 0.612 | 0.229 | 0.097 | 0.616 |
| | | Control | 8 | 73.39 ± 16.18 | 64.27 ± 11.91 | **0.037** | 59.64 ± 10.05 | **0.003** | | | | | | | |
| Glycation age – chronological age | year | Test group | 11 | 16.44 ± 10.12 | 2.37 ± 17.63 | **0.002** | 7.45 ± 15.86 | **0.049** | 0.402 | 0.395 | 0.270 | 0.207 | 0.134 | 0.068 | 0.490 |
| | | Control | 8 | 25.89 ± 16.11 | 16.77 ± 13.05 | **0.037** | 12.14 ± 11.77 | **0.003** | | | | | | | |
| Pentosidine (serum) | pmol/mL | Test group | 11 | 71.78 ± 14.31 | 90.94 ± 23.73 | **0.016** | 119.68 ± 32.22 | **<0.001** | 0.669 | 0.741 | 0.501 | 0.823 | 0.626 | 0.490 | 0.966 |
| | | Control | 8 | 76.21 ± 24.53 | 99.53 ± 29.38 | **0.030** | 120.26 ± 24.43 | **<0.001** | | | | | | | |
| 3-deoxyglucosone (serum) | ng/mL | Test group | 11 | 19.51 ± 14.44 | 28.48 ± 25.82 | 0.476 | 29.29 ± 12.22 | 0.420 | 0.495 | 0.299 | 0.305 | 0.396 | 0.920 | 0.456 | 0.250 |
| | | Control | 8 | 20.12 ± 10.39 | 20.47 ± 17.00 | 0.998 | 22.18 ± 13.70 | 0.942 | | | | | | | |
| CML (serum) | μg/mL | Test group | 11 | 1.15 ± 0.32 | 1.53 ± 0.39 | **0.003** | 1.52 ± 0.31 | **0.003** | 0.608 | 0.266 | 0.931 | 0.810 | 0.188 | 0.130 | **0.019** |
| | | Control | 8 | 1.40 ± 0.49 | 1.85 ± 0.50 | **0.001** | 1.95 ± 0.41 | **<0.001** | | | | | | | |
| sRAGE (serum) | pg/mL | Test group | 11 | 815.91 ± 398.06 | 905.36 ± 484.16 | 0.061 | 977.55 ± 541.68 | **0.001** | 0.140 | 0.155 | 0.367 | 0.327 | 0.409 | 0.275 | 0.249 |
| | | Control | 8 | 687.38 ± 182.28 | 704.88 ± 142.11 | 0.861 | 739.25 ± 173.30 | 0.324 | | | | | | | |
| esRAGE (serum) | ng/mL | Test group | 11 | 0.38 ± 0.19 | 0.41 ± 0.22 | 0.204 | 0.45 ± 0.25 | **0.003** | 0.069 | 0.107 | 0.075 | 0.186 | 0.904 | 0.618 | 0.555 |
| | | Control | 8 | 0.39 ± 0.19 | 0.36 ± 0.15 | 0.591 | 0.40 ± 0.13 | 0.918 | | | | | | | |
| sRAGE – esRAGE | pg/mL | Test group | 11 | 440.45 ± 246.60 | 495.36 ± 288.13 | **0.030** | 523.91 ± 322.55 | **0.001** | 0.348 | 0.341 | 0.495 | 0.522 | 0.436 | 0.310 | 0.302 |
| | | Control | 7 | 354.14 ± 178.69 | 375.86 ± 100.43 | 0.783 | 387.00 ± 121.62 | 0.588 | | | | | | | |
| Skin corneum CML | μg/mg protein | Test group | 11 | 45.45 ± 32.10 | | | 28.95 ± 22.93 | 0.174 | | 0.775 | | 0.829 | 0.952 | | 0.577 |
| | | Control | 7 | 46.31 ± 28.60 | | | 34.45 ± 17.24 | 0.289 | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. For CML, the paired t-test was used to compare data obtained before diet intake with those obtained 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation; AGEs, advanced glycation end products; 3DG, 3-deoxyglucosone; CML, carboxymethyl lysine; RAGE, receptor for AGE; sRAGE, soluble RAGE; esRAGE, endogenous secreted RAGE.

*Fig 2-a*

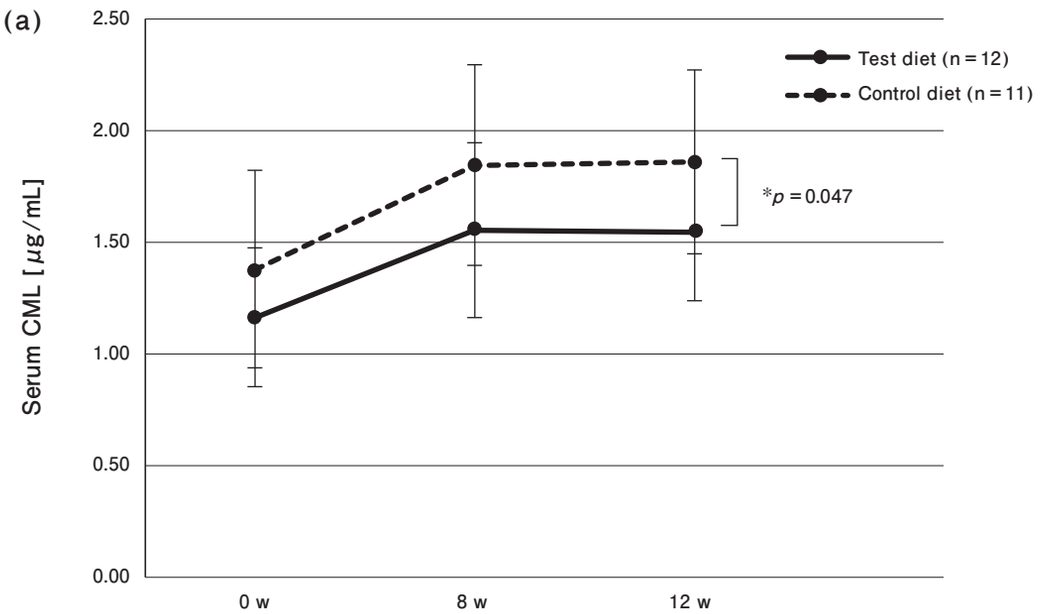


*Fig 2-b*

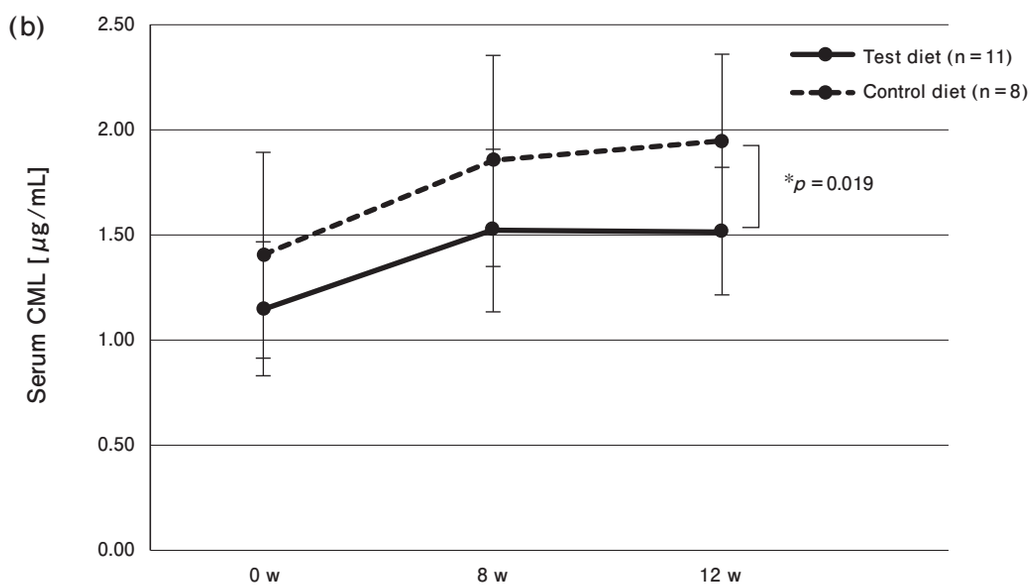


***Fig 2.*** **CML change**

a: Total analysis, b: Subclass analysis. Results are expressed as mean ± stndaed deviation. Student t test. CML, carboxymethyl lysine.

***Table 9. Skin-related parameters***

| Parameter | | Unit | Group | n | 0w | 8w | | 12w | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Skin color difference (Upper arm) | Melanin Index | | Test group | 12 | 0.70 ± 0.08 | 0.67 ± 0.10 | **0.016** | 0.69 ± 0.08 | 0.545 | 0.273 | 0.438 | 0.252 | 0.452 | 0.197 | 0.405 | 0.102 |
| | | | Control | 11 | 0.64 ± 0.12 | 0.63 ± 0.12 | 0.854 | 0.61 ± 0.13 | 0.202 | | | | | | | |
| | Hb Index | | Test group | 12 | 0.86 ± 0.19 | 0.91 ± 0.24 | 0.547 | 0.93 ± 0.30 | 0.335 | 0.324 | 0.791 | 0.535 | 0.554 | 0.990 | 0.348 | 0.809 |
| | | | Control | 11 | 0.87 ± 0.18 | 0.83 ± 0.13 | 0.845 | 0.95 ± 0.21 | 0.357 | | | | | | | |
| | Hb SO2 Index | (%) | Test group | 12 | 59.45 ± 4.52 | 56.05 ± 7.17 | 0.154 | 55.70 ± 5.83 | 0.109 | 0.591 | 0.252 | 0.702 | 0.270 | 0.894 | 0.492 | 0.220 |
| | | | Control | 11 | 59.04 ± 9.03 | 53.73 ± 8.71 | 0.054 | 51.72 ± 9.06 | **0.008** | | | | | | | |
| | L* | | Test group | 12 | 69.42 ± 1.49 | 69.57 ± 2.01 | 0.827 | 69.42 ± 2.20 | 1.000 | 0.883 | 0.982 | 0.841 | 0.997 | 0.233 | 0.226 | 0.372 |
| | | | Control | 11 | 70.37 ± 2.18 | 70.61 ± 1.96 | 0.855 | 70.35 ± 2.71 | 0.999 | | | | | | | |
| | a* | | Test group | 12 | 5.55 ± 0.92 | 5.47 ± 1.03 | 0.938 | 5.51 ± 1.29 | 0.983 | 0.238 | 0.694 | 0.263 | 0.724 | 0.608 | 0.122 | 0.492 |
| | | | Control | 11 | 5.37 ± 0.70 | 4.85 ± 0.80 | 0.182 | 5.16 ± 1.12 | 0.713 | | | | | | | |
| | b* | | Test group | 12 | 14.41 ± 1.51 | 14.10 ± 1.83 | 0.357 | 14.67 ± 1.73 | 0.466 | 0.063 | 0.789 | 0.058 | 0.685 | 0.176 | 0.687 | 0.269 |
| | | | Control | 11 | 13.41 ± 1.91 | 13.78 ± 1.90 | 0.438 | 13.81 ± 1.92 | 0.389 | | | | | | | |
| Skin color diffrerence (Cheak) | Melanin Index | | Test group | 12 | 1.12 ± 0.15 | 1.09 ± 0.13 | 0.240 | 1.10 ± 0.16 | 0.481 | 0.681 | 0.588 | 0.638 | 0.654 | 0.239 | 0.257 | 0.135 |
| | | | Control | 11 | 1.04 ± 0.14 | 1.03 ± 0.12 | 0.671 | 1.01 ± 0.11 | 0.171 | | | | | | | |
| | Hb Index | | Test group | 12 | 1.12 ± 0.18 | 1.17 ± 0.24 | 0.503 | 1.20 ± 0.20 | 0.179 | 0.698 | 0.953 | 0.777 | 0.765 | 0.351 | 0.377 | 0.511 |
| | | | Control | 11 | 1.18 ± 0.14 | 1.26 ± 0.24 | 0.171 | 1.26 ± 0.23 | 0.159 | | | | | | | |
| | Hb SO2 Index | (%) | Test group | 12 | 52.99 ± 4.73 | 57.17 ± 3.00 | **＜0.001** | 58.82 ± 5.02 | **＜0.001** | 0.359 | 0.308 | 0.387 | 0.367 | 0.482 | 0.083 | 0.176 |
| | | | Control | 11 | 51.71 ± 3.79 | 54.49 ± 4.02 | **0.019** | 56.05 ± 4.43 | **＜0.001** | | | | | | | |
| | L* | | Test group | 12 | 64.72 ± 1.61 | 64.86 ± 2.06 | 0.898 | 64.74 ± 2.45 | 0.999 | 0.965 | 0.406 | 0.983 | 0.395 | 0.448 | 0.505 | 0.279 |
| | | | Control | 11 | 65.35 ± 2.24 | 65.47 ± 2.23 | 0.829 | 65.80 ± 2.08 | 0.124 | | | | | | | |
| | a* | | Test group | 12 | 8.86 ± 0.89 | 9.16 ± 1.60 | 0.464 | 9.49 ± 1.35 | 0.055 | 0.814 | 0.474 | 0.750 | 0.465 | 0.916 | 0.852 | 0.726 |
| | | | Control | 11 | 8.91 ± 0.95 | 9.28 ± 1.45 | 0.149 | 9.29 ± 1.32 | 0.130 | | | | | | | |
| | b* | | Test group | 12 | 18.49 ± 1.49 | 18.24 ± 1.33 | 0.437 | 18.15 ± 1.31 | 0.242 | 0.323 | 0.318 | 0.336 | 0.339 | 0.091 | 0.342 | 0.341 |
| | | | Control | 11 | 17.42 ± 1.40 | 17.61 ± 1.73 | 0.791 | 17.54 ± 1.65 | 0.911 | | | | | | | |
| Skin elesticity (Upper arma) | R2 | | Test group | 12 | 0.91 ± 0.02 | 0.91 ± 0.02 | 0.998 | 0.90 ± 0.02 | **0.031** | 0.866 | 0.259 | 0.869 | 0.261 | 0.345 | 0.639 | 0.681 |
| | | | Control | 11 | 0.92 ± 0.01 | 0.92 ± 0.02 | 0.947 | 0.90 ± 0.02 | **0.010** | | | | | | | |
| | R6 | | Test group | 12 | 0.26 ± 0.05 | 0.25 ± 0.03 | 0.165 | 0.22 ± 0.04 | **＜0.001** | 0.168 | 0.281 | 0.229 | 0.447 | 0.181 | 0.922 | 0.658 |
| | | | Control | 11 | 0.29 ± 0.05 | 0.25 ± 0.04 | 0.019 | 0.23 ± 0.04 | **0.001** | | | | | | | |
| | R7 | | Test group | 12 | 0.71 ± 0.04 | 0.71 ± 0.05 | 0.209 | 0.69 ± 0.05 | **0.003** | 0.165 | 0.056 | 0.176 | 0.069 | 0.275 | 0.962 | 0.843 |
| | | | Control | 11 | 0.73 ± 0.02 | 0.71 ± 0.03 | 0.015 | 0.69 ± 0.03 | **＜0.001** | | | | | | | |
| Skin elesticity (Cheak) | R2 | | Test group | 12 | 0.81 ± 0.06 | 0.78 ± 0.06 | 0.137 | 0.78 ± 0.06 | 0.116 | 0.414 | 0.337 | 0.408 | 0.360 | **0.030** | 0.328 | 0.247 |
| | | | Control | 11 | 0.86 ± 0.04 | 0.80 ± 0.05 | 0.003 | 0.80 ± 0.04 | **0.003** | | | | | | | |
| | R6 | | Test group | 12 | 0.40 ± 0.07 | 0.38 ± 0.05 | 0.384 | 0.41 ± 0.08 | 0.922 | **0.028** | 0.839 | 0.063 | 0.984 | **0.023** | 0.384 | 0.086 |
| | | | Control | 11 | 0.34 ± 0.04 | 0.36 ± 0.05 | 0.245 | 0.36 ± 0.06 | 0.371 | | | | | | | |
| | R7 | | Test group | 12 | 0.41 ± 0.05 | 0.39 ± 0.07 | 0.334 | 0.40 ± 0.06 | 0.972 | 0.112 | 0.192 | 0.198 | 0.277 | **0.025** | 0.375 | 0.214 |
| | | | Control | 11 | 0.46 ± 0.06 | 0.41 ± 0.06 | **0.004** | 0.44 ± 0.06 | 0.107 | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation.

***Table 10. Skin-related parameters (subclass analysis)***

| Parameter | | Unit | Group | n | 0w | 8w | | 12w | | Inter-group analysis by change values | | Inter-group analysis by % change | | Inter-group analysis by measured values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean SD | Mean SD | p values | Mean SD | p values | 8w | 12w | 8w | 12w | 0w | 8w | 12w |
| Skin color difference (Upper arm) | Melanin Index | | Test group | 11 | 0.70 ± 0.08 | 0.67 ± 0.10 | **0.020** | 0.69 ± 0.08 | 0.542 | 0.303 | 0.646 | 0.323 | 0.680 | 0.195 | 0.400 | 0.121 |
| | | | Control | 8 | 0.63 ± 0.14 | 0.63 ± 0.13 | 0.945 | 0.61 ± 0.14 | 0.480 | | | | | | | |
| | Hb Index | | Test group | 11 | 0.85 ± 0.19 | 0.88 ± 0.23 | 0.698 | 0.87 ± 0.24 | 0.808 | 0.711 | 0.125 | 0.887 | 0.147 | 0.759 | 0.600 | 0.272 |
| | | | Control | 8 | 0.83 ± 0.11 | 0.83 ± 0.16 | 0.998 | 1.00 ± 0.22 | 0.063 | | | | | | | |
| | Hb SO2 Index | (%) | Test group | 11 | 59.68 ± 4.67 | 56.85 ± 6.92 | 0.285 | 56.72 ± 4.88 | 0.255 | 0.263 | 0.064 | 0.271 | 0.070 | 0.242 | 0.691 | 0.307 |
| | | | Control | 8 | 62.66 ± 6.07 | 55.30 ± 9.83 | **0.032** | 53.13 ± 9.79 | **0.007** | | | | | | | |
| | L* | | Test group | 11 | 69.48 ± 1.55 | 69.75 ± 2.00 | 0.400 | 69.74 ± 1.99 | 0.442 | 0.496 | 0.180 | 0.522 | 0.182 | 0.317 | 0.560 | 0.968 |
| | | | Control | 8 | 70.41 ± 2.41 | 70.33 ± 2.17 | 0.978 | 69.78 ± 3.00 | 0.360 | | | | | | | |
| | a* | | Test group | 11 | 5.51 ± 0.95 | 5.36 ± 1.00 | 0.750 | 5.29 ± 1.08 | 0.529 | 0.458 | 0.528 | 0.498 | 0.475 | 0.777 | 0.355 | 0.748 |
| | | | Control | 8 | 5.39 ± 0.79 | 4.95 ± 0.82 | 0.387 | 5.46 ± 1.19 | 0.976 | | | | | | | |
| | b* | | Test group | 11 | 14.51 ± 1.53 | 14.26 ± 1.83 | 0.463 | 14.94 ± 1.54 | 0.152 | 0.212 | 0.813 | 0.198 | 0.967 | 0.106 | 0.354 | 0.070 |
| | | | Control | 8 | 13.15 ± 1.95 | 13.41 ± 2.01 | 0.781 | 13.44 ± 1.83 | 0.742 | | | | | | | |
| Skin color difference (Cheak) | Melanin Index | | Test group | 11 | 1.12 ± 0.15 | 1.09 ± 0.14 | 0.252 | 1.10 ± 0.16 | 0.622 | 0.986 | 0.357 | 0.886 | 0.440 | 0.389 | 0.314 | 0.166 |
| | | | Control | 8 | 1.05 ± 0.16 | 1.03 ± 0.12 | 0.438 | 1.01 ± 0.10 | 0.118 | | | | | | | |
| | Hb Index | | Test group | 11 | 1.11 ± 0.18 | 1.16 ± 0.25 | 0.498 | 1.20 ± 0.21 | 0.173 | 0.717 | 0.976 | 0.767 | 0.832 | 0.653 | 0.590 | 0.751 |
| | | | Control | 8 | 1.14 ± 0.13 | 1.22 ± 0.26 | 0.248 | 1.23 ± 0.23 | 0.224 | | | | | | | |
| | Hb SO2 Index | (%) | Test group | 11 | 53.37 ± 4.77 | 57.19 ± 3.15 | **0.001** | 58.90 ± 5.25 | **＜0.001** | 0.422 | 0.446 | 0.460 | 0.512 | 0.677 | 0.153 | 0.366 |
| | | | Control | 8 | 52.48 ± 4.13 | 55.12 ± 2.72 | **0.036** | 56.85 ± 3.95 | **0.001** | | | | | | | |
| | L* | | Test group | 11 | 64.85 ± 1.63 | 64.89 ± 2.15 | 0.989 | 64.71 ± 2.57 | 0.908 | 0.626 | 0.279 | 0.602 | 0.266 | 0.738 | 0.613 | 0.390 |
| | | | Control | 8 | 65.16 ± 2.41 | 65.43 ± 2.35 | 0.609 | 65.70 ± 2.13 | 0.186 | | | | | | | |
| | a* | | Test group | 11 | 8.85 ± 0.94 | 9.11 ± 1.67 | 0.577 | 9.49 ± 1.42 | 0.072 | 0.885 | 0.431 | 0.832 | 0.436 | 0.934 | 0.980 | 0.596 |
| | | | Control | 8 | 8.81 ± 1.09 | 9.13 ± 1.66 | 0.394 | 9.13 ± 1.46 | 0.394 | | | | | | | |
| | b* | | Test group | 11 | 18.51 ± 1.56 | 18.22 ± 1.39 | 0.409 | 18.14 ± 1.37 | 0.254 | 0.420 | 0.374 | 0.416 | 0.372 | 0.108 | 0.295 | 0.273 |
| | | | Control | 8 | 17.28 ± 1.54 | 17.43 ± 1.78 | 0.917 | 17.38 ± 1.51 | 0.960 | | | | | | | |
| Skin elesticity (Upper arma) | R2 | | Test group | 11 | 0.92 ± 0.02 | 0.92 ± 0.02 | 0.993 | 0.90 ± 0.02 | 0.059 | 0.789 | 0.052 | 0.794 | 0.051 | 0.708 | 0.913 | 0.070 |
| | | | Control | 8 | 0.92 ± 0.01 | 0.92 ± 0.02 | 0.873 | 0.89 ± 0.02 | **0.010** | | | | | | | |
| | R6 | | Test group | 11 | 0.26 ± 0.04 | 0.24 ± 0.03 | 0.207 | 0.22 ± 0.04 | **0.001** | 0.241 | 0.357 | 0.216 | 0.565 | 0.110 | 0.571 | 0.423 |
| | | | Control | 8 | 0.29 ± 0.05 | 0.25 ± 0.05 | 0.071 | 0.23 ± 0.04 | **0.009** | | | | | | | |
| | R7 | | Test group | 11 | 0.72 ± 0.04 | 0.71 ± 0.04 | 0.229 | 0.70 ± 0.05 | **0.007** | 0.207 | **0.013** | 0.221 | **0.016** | 0.667 | 0.611 | 0.213 |
| | | | Control | 8 | 0.73 ± 0.02 | 0.70 ± 0.03 | **0.021** | 0.68 ± 0.03 | **＜0.001** | | | | | | | |
| Skin elesticity (Cheak) | R2 | | Test group | 11 | 0.82 ± 0.06 | 0.78 ± 0.06 | 0.097 | 0.77 ± 0.06 | 0.071 | 0.723 | 0.630 | 0.733 | 0.687 | **0.049** | 0.141 | 0.151 |
| | | | Control | 8 | 0.87 ± 0.04 | 0.82 ± 0.04 | **0.027** | 0.81 ± 0.04 | **0.018** | | | | | | | |
| | R6 | | Test group | 11 | 0.41 ± 0.08 | 0.38 ± 0.06 | 0.428 | 0.42 ± 0.08 | 0.875 | 0.076 | 0.983 | 0.135 | 0.868 | **0.011** | 0.152 | **0.046** |
| | | | Control | 8 | 0.33 ± 0.03 | 0.34 ± 0.05 | 0.510 | 0.34 ± 0.06 | 0.652 | | | | | | | |
| | R7 | | Test group | 11 | 0.41 ± 0.05 | 0.39 ± 0.07 | 0.274 | 0.40 ± 0.06 | 0.726 | 0.335 | 0.708 | 0.517 | 0.892 | 0.065 | 0.322 | 0.145 |
| | | | Control | 8 | 0.47 ± 0.07 | 0.43 ± 0.06 | **0.041** | 0.45 ± 0.07 | 0.506 | | | | | | | |

Differences were considered significant for $p < 0.05$. Dunnett's test was used to compare data obtained before diet intake with those obtained 8 and 12 weeks after diet intake. The independent samples t-test (the unpaired t-test on SPSS) was used to compare absolute and percent changes in values from baseline (before diet intake) between groups. SD, standard deviation.

*Fig 3 - a*

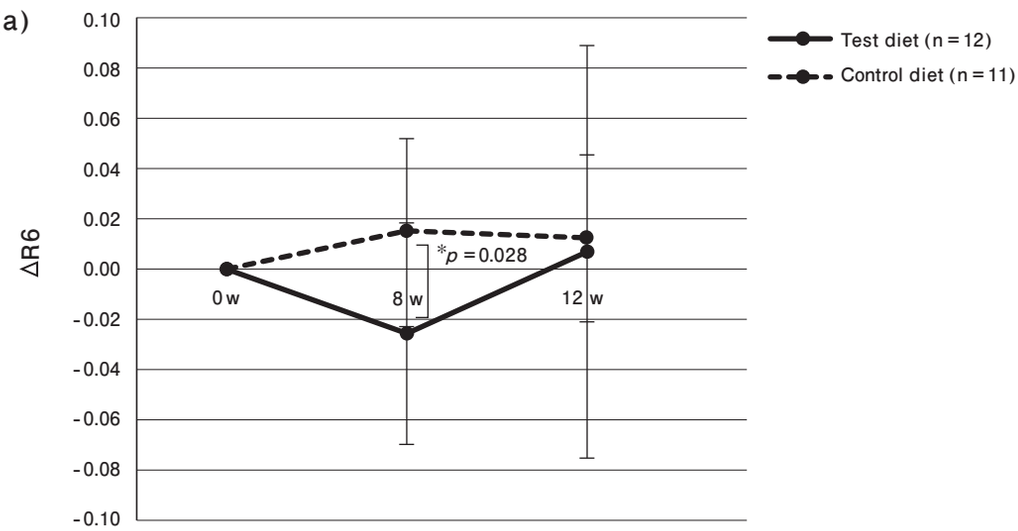


*Fig 3 - b*

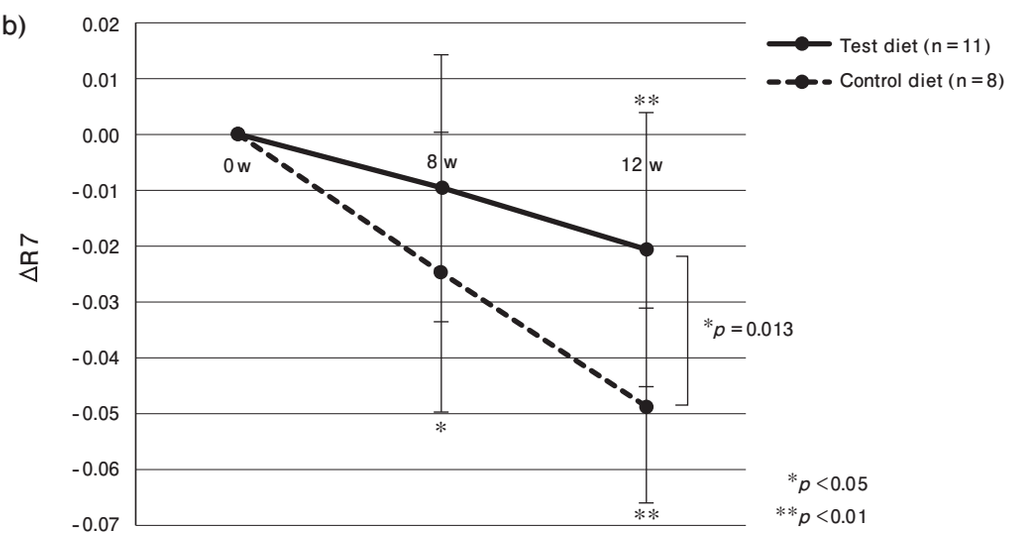


*Fig 3 - c*

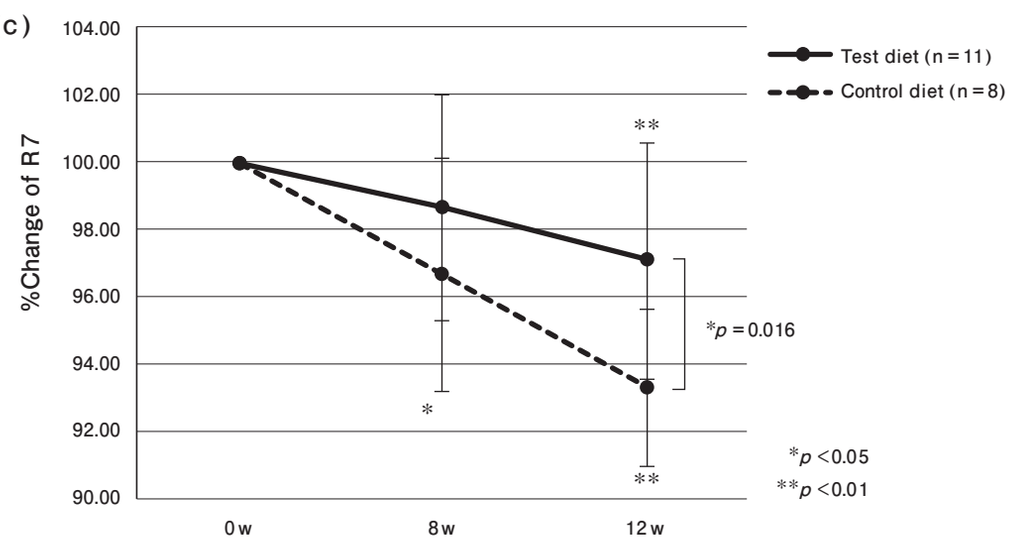


***Fig 3.*** **Skin viscoelasticity change**

a: Difference in R6 by total analysis (Cheak), b: Difference in R7 by subclass analysis (Upper arm), c: % change of R7 by subclass analysis (Upper arm). Results are expressed as mean ± stndaed deviation. *$p$ < 0.05, **$p$ < 0.01, intra-group analysis vs.0w by Dunnett's test, inter-group analysis by Student's t test.

### *Safety evaluation*

Safety evaluation was based on the FAS (n = 25). No serious adverse event was observed during the study period (from September 10 to December 18, 2013). One subject in the control group had a skin rash after the completion of assessments at week 8 and was prematurely withdrawn from the study at the investigator's discretion.

## *Discussion*

### *Data summary*

The objective of this study was to evaluate the anti-glycation effect of the test diet. Individuals with high glycation stress were selected by screening and assigned to receive either a functional soymilk beverage, the test diet, or a control beverage for 12 weeks in a randomized controlled trial (RCT). The results showed that the following parameters were significantly improved in the test group compared to the control group: increased exercise as determined by a questionnaire survey, decreased CML (an AGE), and improved skin elasticity index R6. A subclass analysis in the subjects with a BMI of ≥ 25 showed a slower increase in plasma glucose level and a slower decrease in skin elasticity index R7 in the test group. Moreover, HbA1c, a marker of glucose metabolism, sRAGE, esRAGE, [sRAGE - esRAGE], and melanin index for skin color difference in the upper arm was significantly improved after diet intake only in the test group, with no significant difference between groups.

### *Beneficial effects of rice bran extracts*

Rice bran extracts contain a variety of substances and have various biological activities, such as anti-oxidative [5,21-23], anti-inflammatory [24], cholesterol-lowering [4,25,26] and glucose metabolism-improving [27] actions, as well as providing dietary fibers that inhibit absorption and promote the excretion of intestinal toxins [1,28,29].

The ethanol-soluble fraction of the hot water extract of rice bran contains phenols that have a strong antioxidative capability, such as protocatechuic acid, caffeic acid, ferulic acid and gentisic acid [5], as well as dietary fiber, glucosylceramide and arabinoxylan. We have also demonstrated that rice bran extracts inhibit AGE production in an *in vitro* model of the human serum albumin-glucose reaction [8].

Extracts and processed products of rice bran have been tested in animals. The oral administration of rice bran-derived dietary fiber in rats pretreated with polychlorinated biphenyl (PCB) has been shown to inhibit the absorption of polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-para-dioxin (PCDD) by the small intestine and promote their excretion in feces [1,28,29]. This effect has also been demonstrated in humans; a combination of rice bran fiber and cholestyramine has been shown to promote the excretion of PCDF and PCB in Taiwanese Yu-Cheng patients [30]. Rice bran oil has been shown to reduce the serum cholesterol level in rats [4]. Rice bran oil extracts fermented with *Saccharomyces cerevisiae* (IFO2346) have demonstrated anti-stress and anti-fatigue activities when administered orally in rats and mice [31]. Rice bran fluid treated with enzymes and *Bifidobacterium bifidum* has been shown to reduce the area of stress-induced gastric ulcers and ameliorate elevated serum levels of GOT and GPT [32]. Hairless mice (HR-AD) fed a diet containing rice bran-derived glucosylceramide (GluCer) showed better preservation and restoration of skin barrier function chronically or acutely disrupted by repeated tape stripping, as compared to control mice [33]. Furthermore, modified arabinoxylan extracted from rice bran (MGN-3) and its fractions have been shown to suppress D-galactosamine (D-GalN)-induced liver damage and IL-18 expression in rats [34].

In cell culture systems, the methanol extract of rice bran has been shown to significantly suppress the proliferation of mouse splenic lymphocytes stimulated with concanavalin A (ConA) or lipopolysaccharide (LPS) and suppress ConA-induced interferon-gamma production and LPS-induced interleukin-6 (IL-6) production [35]. The extract has also been shown to suppress the production of IL-1-alpha and tumor necrosis factor (TNF)-alpha from LPS-stimulated mouse macrophage lineage cells [35].

Interesting results have been reported regarding the effect of rice bran on glucose metabolism [36,37]. Treatment of 26 patients with type 1 diabetes with the soluble fraction of rice bran for 60 days resulted in decreased HbA1c (11%), decreased FPG (29%) and increased insulin (4%) levels [36]. When the same treatment was given to 31 patients with type 2 diabetes, the results showed decreased HbA1c (15%), decreased FPG (33%) and increased serum insulin (4%). In that study, the fiber fraction of rice bran was also administered to 26 patients with type 2 diabetes and decreases in total cholesterol (12%), LDL-cholesterol (15%) and tryglyceride (8%) were reported [36]. In a double-blind study in which 28 patients with type 2 diabetes were divided into 2 groups and treated with stabilized rice bran extract at a dosage of 20 g/day for 12 weeks, the treatment resulted in a decreased peak postprandial plasma glucose level (14.4%), decreased area under the glucose level-time curve (15.7%), decreased HbA1c, decreased total cholesterol (9.2%), decreased LDL-cholesterol (13.7%), increased free fatty acid (20%) and increased adiponectin (40%) levels [37]. These findings suggest that rice bran contains ingredients that have beneficial effects on glycolipid metabolism, with its soluble and fiber fractions contributing to its effects on glucose and lipid metabolism, respectively.

Clinical experience with BioBran, a rice bran arabinoxylan derivative, has been reported from case reports [38,39], non-controlled open-label studies [40-42] and RCTs [43,44].

A case report has been published in which a 62-year-old female patient with umbilical metastasis of recurrent colon cancer (Sister Mary Joseph's Nodule, SMJN) was treated with BioBran in a combination therapy; she achieved a long-term survival of at least 2 years and 1 month [38]. In another case report, a 64-year-old male patient with surgically untreatable terminal pancreatic cancer with distant metastasis was treated with BioBran in combination with a low-toxicity chemotherapy; he exhibited temporary relief of ascites, increased appetite, increased body weight, and reduced tumor markers; he was able to engage in a normal social life for about 17 months after the discovery of cancer [39].

In an uncontrolled open-label study, 8 patients with chronic rheumatoid arthritis were treated with BioBran as a supportive treatment to steroids, analgesics and other medications for 6 to 12 months, and 3 of them showed evidence of improvement in symptoms and QOL [40]. In that study, 5 of the 8 patients used steroids and analgesics and the remaining 3 patients used analgesics and Chinese herbal medicines as well as heat therapy. No rice bran-related

adverse event was reported. A 6-month treatment with BioBran in 16 patients with advanced cancer treated with standard cancer therapies resulted in increased body weight in 10 (63%) patients, increased white blood cell count in 9 (56%), increased activity of natural killer (NK) cells in all patients, and reduced tumor markers in 10 (63%) [41]. No adverse event was observed. Another study in which BioBran was administered in combination with other therapies to 5 cancer patients also reported no adverse event [42].

Two controlled trials of rice bran-containing products have been reported. In a double-blind cross-over study in which hydrolyzed rice bran (HRB) extract, prepared by partially hydrolyzing soluble dietary fiber extracted from rice bran (main ingredient: arabinoxylan) with a shiitake-derived carbohydrate-degrading enzyme, was administered to 36 elderly subjects (aged 70-95 years, including 9 males) for 6 weeks to evaluate its effect on preventing common cold symptoms, the treatment shortened the duration of common cold symptoms, reduced the worsening of symptoms and the need for symptomatic treatment, and relieved the physical burden of acute-phase respiratory infection [43]. In a RCT in which 152 patients with stage IIIb to IV cancer who experienced metastasis after undergoing surgery or failed to have their tumor completely resected were treated with (96 patients; combination group) or without (56 patients; control group) BioBran in addition to a standard alternative/supportive treatment, the 18-month survival rates in the combination and control groups were 54.2% and 35.8%, respectively [44]. Moreover, many of the patients in the combination group showed unchanged or increased NK cell activity. Among the subjective symptoms evaluated (*i.e.* pain, malaise, queasy and decreased appetite), a particularly notable improvement in appetite was observed in the combination group.

Potential benefits of topically applied rice bran oil on atopic dermatitis have also been reported. Topical application of a milky bath agent comprised mainly of rice bran oil in 18 patients with relatively mild atopic dermatitis that mainly manifests as dry skin either every day or every other day for 2-7 weeks resulted in improvement of dry skin in 14 of 18 patients, as well as resolution or improvement of itching in 87.5% and improvement of scales in 83.3% of patients [45]. The treatment caused no adverse reaction and was highly satisfied by patients.

## *Interpretation of data*

An increased physical activity as indicated by the questionnaire survey in this study is consistent with the previous observation of a prolonged swimming time and anti-fatigue effect in rats treated with rice bran extracts [31]. With no significant change in any of the lipid metabolism parameters tested, the present study failed to verify in humans the previously-reported cholesterol-lowering action of rice bran.

The observed decrease in plasma CML level, a measure of glycation stress, may suggest that an AGE production-inhibitory action of the test diet, which has been demonstrated *in vitro*, was also exerted *in vivo*. Given that CML is abundant in the skin, this might have led to an improvement in skin elasticity index R6.

Significant increases were observed in sRAGE, esRAGE, and [sRAGE - esRAGE] only in the test group, with no significant difference between groups. sRAGE and esRAGE are decoy receptors that bind to AGEs in blood and interstitial fluid and promote their degradation and excretion. The difference between sRAGE and esRAGE reflects the level of sRAGE in a narrow sense (an enzyme-cleaved fragment of transmembrane RAGE). The present data suggest that there was no difference between the behavior of sRAGE in a narrow sense and that of sRAGE. Thus, the increases in sRAGE, esRAGE and the difference between sRAGE and esRAGE indicate increased functioning of these decoy receptors. Although no factor has been identified that affects soluble RAGE levels, our results provide an interesting implication that rice bran contains functional ingredients that affect soluble RAGE levels.

## *Considerations for soy allergy*

There are multiple types of soy allergy, including class 1 allergy, oral allergy syndrome (OAS = class 2 allergy) that occurs in association with pollinosis, and inhalation asthma [46-48]. Children are commonly affected by class 1 soy allergy, which is sensitized via the gastrointestinal tract [48]. A recent increase in health consciousness and subsequent increase in consumption of soy food have led to an increasing prevalence of soy allergy among adults. Young adults are increasingly affected by OAS, *i.e.* class 2 food allergy, primarily to soymilk and other soy products. By comparison, many of the soy-allergic adults experience pollinosis during the spring season. This is considered to be due to cross reactivity between soy allergens and alder pollens, as evidenced by the fact that many of the soy-allergic patients are tested positive for alder pollen-specific IgE [48].

Known soy allergens, *i.e.* class 1 food allergy-related antigens include beta-conglycinin (the major antigen), Glycinin A3 subunit, Gly mBd 30K, and Gly mBd 28K [46,48]. Kunitz soybean trypsin inhibitor (KSIT: 18-20 kDa) has been identified as a class 1 inhalation allergy-related allergen [46]. Class 2 allergens that have been identified include Gly m4, a homologue of major antigen PR-10, and Gly m3, a profilin. The oil body-binding protein, oleosin (23-24 kDa), has recently been identified as a novel allergen [47].

Increasing attention has been paid to food allergy that mainly manifests with gastrointestinal symptoms and commonly affects infants, especially newborns [49]. This type of allergy is characterized by a low incidence of IgE antibody positivity and the absence of immediate-type allergic reaction, suggesting the involvement of a cell-dependent allergy reaction. In fact, many of these patients test positive in an allergen-specific lymphocyte stimulation test aimed at evaluating cellular immunity. A majority of the cases are caused by milk, in particular formulated powdered milk, with fewer cases caused by breast milk and soymilk. Nearly half of these cases manifest allergic symptoms within 1 week after birth. Some post-weaning cases are caused by solid food, such as rice and soybeans. Eosinophilic gastroenteritis-like pathological features are observed in some cases, such as marked increase in eosinophils. There also are subtypes characterized by septicemia-like features, such as C-reactive protein positive, increased white blood cell count and collapse.

In the present study, 1 subject in the control group experienced skin rash, which was considered to have been caused by soymilk. Although class 1 allergy was suspected, no IgE test was performed. Soy allergy is characterized by the manifestation of diverse reactions depending on the mechanism of sensitization and the type of responsible allergens. Consistent with the fact that soybeans can cause not only class 1 but also class 2 allergy, cases have also been reported in which the ingestion of soymilk by pollinosis patients resulted in the manifestation of OAS and

anaphylaxis, among other cases of OAS caused by fruits [50]. However, the clinical symptoms and sensitization pathway have not been well characterized. There is an ongoing need for characterizing the sensitization mechanisms, pathogenesis and responsible allergens, as well as an increasing demand for low-allergic soy food taking into account the diversity of soy allergens and specific immunotherapies. It should always be kept in mind that any test diet that contains soymilk, even at a low volume, can evoke soy allergy.

### *Safety*

Rice bran and rice bran oil are considered safe food ingredients as they have long been consumed by people and the safety assessment information supports its safety. Residual pesticide testing has also detected none of the following nitrogen-containing pesticides from rice bran oil: eptam knoxweed torbin, metolachlor, thiobencarb, diethofencarb, pendimethalin, flutolanil, lenacil, alachlor, bitertanol, fenarimol and chlorpropham [51]. No serious adverse event suspected to be related to rice bran or rice bran oil has been reported in the present study, two pilot studies (13 subjects treated with the test diet at 16 g/day for 12 weeks, and 8 subjects treated at 20 g/day for 8 weeks, respectively), or the literature [36-42]. Given these considerations, we conclude that there is no concern about the safety of the test diet.

## *Conclusion*

In the present RCT in female subjects with high glycation stress, the ingestion of a test diet composed mainly of rice bran, which had demonstrated an AGE production inhibitory effect in an *in vitro* glycation model, resulted in significant reduction in plasma CML content and improved skin elasticity indexes, suggesting that the diet exerts an anti-glycation effect in humans. The safety of the test diet was also confirmed.

## *Acknowledgements*

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## *Conflict of interest*

A-Kit Corporation (Ogaki, Gifu, Japan) served as the clinical research coordinator in this study under a research fund commissioned by Sunstar Inc. (Takatsuki, Osaka, Japan).

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