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*Original Artcle*

# Evaluation of the anti-glycation effect and the safety of a vinegar beverage containing indigestible dextrin and a mixed herbal extract – A placebo-controlled, double-blind study –

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## Abstract

***Objective*****:** We evaluated the inhibitory effect of the long-term consumption of a vinegar beverage containing indigestible dextrin and a mixed herbal extract (the test diet) on the production of advanced glycation end products (AGEs) in a placebo-controlled, randomized, double-blind, parallel-group comparison study design.

***Methods*****:** A total of 109 post-menopausal women previously diagnosed with an increased blood glucose or hemoglobin A1c (HbA1c) level, with an abdominal circumference of 90 cm or more, were subjected to an oral rice ingestion test followed by measurement of fluorescence intensity of skin AGEs. Among them, 23 women (mean age 57.4 ± 3.9) with high levels of 60-minute postprandial blood glucose and skin AGE deposition were enrolled and assigned to either the test group (n = 11) or the control group (n =11). The subjects in the test group took 240 mL of the test diet (corresponding to 840 mg/day of acetic acid, 5 g/day of indigestible dextrin, and 100 mg/day of mixed herbal extract) daily divided into two equal doses taken before breakfast and dinner for a period of 8 consecutive weeks. Blood biochemistry parameters and glycation stress markers were measured before and at 8 and 12 weeks after the start of the test diet. The study was approved by an ethical review committee.

***Results*****:** A significant decrease in serum aspartate transaminase (AST/GOT) level in the test group compared to control was observed after 8 weeks of diet intake ($p<0.05$). With regard to glycation stress markers, no significant differences were observed between groups in the blood concentrations of fasting glucose, HbA1c, insulin, 3-deoxyglucosone (3DG), $N^{\varepsilon}$-(carboxymethyl) lysine (CML) or pentosidine. No significant intergroup difference was observed in the fluorescence intensity of skin AGE as measured with an AGE Reader. In the subclass analysis of the subjects with high postprandial blood glucose (>150 mg/dL at 60 minutes), CML content of the skin stratum corneum as determined by the tape stripping method was significantly decreased in the test group compared to control after 8 weeks of diet intake ($p<0.05$). No serious adverse event was observed during the study period.

***Conclusion*****:** The results of the present study suggest that the intake of the test diet causes a reduction in CML content in the skin stratum corneum, a marker of glycation stress, in people with relatively high glycation stress. Combined with the demonstrated safety of the test diet, this observation indicates a potential for the use of the test diet as a functional food.

***KEY WORDS*****:** Glycation stress, vinegar beverage, advanced glycation end products (AGEs), $N^{\varepsilon}$-(carboxymethyl) lysine (CML), tape stripping technique

## Introduction

Glycation stress is a series of reactions triggered when reducing sugars, organic acids or aldehydes react with protein-derived amino acids to produce post-translational modification products, such as carbonyl compounds, succinyl compounds, racemic compounds, or advanced glycation end products (AGEs). Subsequent accumulation of such compounds causes stress to cells and tissues and leads to the functional decline of proteins which can bind to receptors for AGEs, inducing or aggravating inflammation via cytokine production [1-3]. Glycation stress is considered a major risk factor for accelerated aging.

Although diabetes, including the prediabetic state, is a major predisposing factor for glycation stress, glycation can also occur in the absence of diabetes. This type of glycation is referred to as normoglycemic glycation or non-hyperglycemic glycation and is caused most commonly by postprandial hyperglycemia or, less commonly, by hypertriglyceridemia, uremic toxin, excessive alcohol intake or excessive fructose intake. Smoking and a lack of sleep are also known to promote AGE production [4]. These factors should be eliminated to reduce glycation stress.

Recent studies have identified and evaluated various substances that inhibit the production of AGEs, promote the degradation of AGEs or antagonize receptors for AGEs. Previously, we have evaluated the *in vitro* inhibitory effect of roman chamomile (*Anthemis nobilis*), doku-dami (*Houttuynia cordata*), hawthorn (*Crataegus laevigata* (*C. oxyacantha*)), grape leaf (*Vitis vinifera*), and a mixture of these herbs [5], *Chrysanthemum morifolium* [6,7], *Sasa senanensis* [8], and extracts






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of various types of healthy tea [9] on AGE production. We have also performed a clinical evaluation of a herbal mixture [10], *Chrysanthemum morifolium* [7], and a food product containing lingonberry (*Vaccinium vitis-idaea*) and cherry blossom (*Prunus lannesiana*) as main ingredients [11]. The objective of the present study was to evaluate the inhibitory effect on AGE production and the safety of a vinegar beverage containing indigestible dextrin and a mixed herbal extract administered for 8 consecutive weeks in a placebo-controlled, randomized, double-blind design.

## Methods

### *Subjects*

Eligible subjects were selected according to the following criteria. A total of 109 post-menopausal women aged between 50-65 years, previously diagnosed with increased blood glucose or hemoglobin A1c (HbA1c) levels and an abdominal circumference of 90 cm or more, were recruited. After explanation of the study, written informed consent to study participation was obtained from all women. An oral rice ingestion test and skin AGE deposition analysis were performed as screening tests on all potential subjects. In the oral rice ingestion test, subjects were instructed to eat 200 g of rice (294 kcal, 67.8 g carbohydrate and 4.2 g protein) with 2.5 g of rice seasoning condiment (11 kcal, 1.0 g carbohydrate and 0.6 g protein). Blood glucose levels were measured at 0, 30, 60, and 120 minutes after consumption according to the unified procedure proposed by the Japanese Association for the Study of Glycemic Index. A total of 23 women (mean age 57.4 ± 3.9 years) shown to have high levels of 60-minute blood glucose and skin AGE deposition were included in the full analysis set (FAS) of the study. Except for one subject who was withdrawn during the study period, no subject met any of the exclusion criteria, and all of the remaining 22 subjects completed the study and complied with the protocol (per protocol set; PPS). The subject was withdrawn because she experienced acid reflux when taking the test diet, which was determined to be unrelated to study diet intake. Subjects taking drugs or supplements that may affect the blood glucose level, smokers or those with a short sleep duration (< 5 hours) were excluded. Those meeting the last two criteria were excluded because these lifestyle factors are known to increase AGE deposition in the skin [4].

### *Study design*

The study was conducted in a two-group (control and test group), placebo-controlled, randomized, double-blind study design. Subjects were given either a placebo (control group) or a vinegar beverage containing indigestible dextrin and a mixed herbal extract as the test diet (test group) for 8 consecutive weeks. A clinical evaluation consisting of an interview, a physical examination, a blood/urine tests, and a skin function test was performed at 0, 8, and 12 weeks.

Subjects were instructed to take 120 ml of the control or test diet twice daily, *i.e*., before breakfast and dinner. The daily intake amounted to 840 mg of acetic acid, 5 g of indigestible dextrin and 100mg of mixed herbal extract. Both the placebo and the test diet were given for 8 weeks, with a follow-up period of 4 weeks. Initially, the placebo/test diet intake period was set at 12 weeks. However, later it was shortened to 8 weeks after foreign matter contamination was detected in the manufacturing process of the test diet. Subjects were instructed to take the test diet even if they were not having a meal. The mean intake rate was 97.1% (88.3%) in the control group, 98.3% (97.3%) in the test group, and 97.7% (92.8%) in the entire population, with values in parenthesis representing mean rates up to week 8.

The study was conducted between September 2012 and December 2012 at TES Holdings Co., Ltd. (Bunkyo-ku, Tokyo, Japan). After the subjects were given sufficient explanation regarding purpose and details of the study and participants' rights, they provided written informed consent. They were also told that early withdrawal from the study would not be a detriment.

### *Test diet*

The compositions of placebo and test diet are shown in ***Table 1***.

***Table 1. Composition of placebo and test diet.***

| Ingredient (%) | Placebo diet | Test diet |
|---|---|---|
| Acetic acid | – | 0.35 |
| Citric acid | – | 0.1 |
| Indigestible dextrin | – | 2.1 |
| Mixed herbal extract 1) | – | 0.042 |
| Tien-cha extract 2) | – | 0.01 |
| α-G-rutin | – | 0.0125 |
| Sucralose | 0.004 | 0.008 |
| Acesulfame potassium | 0.003 | 0.006 |
| Salt | – | 0.04 |
| Condensed tomato juice | – | 0.05 |
| Other ingredients | flavoring water | flavoring water |

1) Mixed herbal extract contains *Anthemis nobilis, Houttuynia cordata, Crataegus laevigata*, and *Vitis vinifera* of leaf.
2) Tien-cha extract contains *Rubus suavissimus*.

AG herb mix™, a functional food ingredient developed and marketed by ARKRAY Inc. (Kyoto, Japan), is a mixture of powdered hot water extracts of herbs belonging to different taxonomic groups, including *Houttuynia cordata, Crataegus laevigata* (*C.oxyacantha*), *Anthemis nobilis*, and *Vitis vinifera*. This herbal extract and its formulated product have been shown to inhibit AGE production *in vitro*, both in a diabetes model in rats and in randomized controlled trials (RCTs) in humans [10-13]. The herbal extract is considered safe as it is composed of raw materials that have long been consumed as food ingredients, and because it is produced with the same extraction method as used for herb tea. *Anthemis nobilis* has been shown to cause an allergic reaction in people with multiple allergies, and *Houttuynia cordata*, used as a folk medicine, has been reported to cause photosensitivity and hyponatremia when consumed in large amounts for long periods of time. However, the daily intake of the mixed herbal extract contained in the test diet used in this study was only 100 mg, thus it was not likely to cause any problems. Moreover, the safety of the mixed herbal extract has been demonstrated in various studies, including a rec-assay (lethal sensitivity test), a reverse mutation test, an acute oral toxicity study using male and female rats, and an overdose test in humans (3,000 mg/day, corresponding to 5 times the regular dose, for 4 weeks).

Fibersol-2H was developed and marketed by Matsutani Chemical Industry (Hyogo, Itami, Japan). The compound affects the control of hyperglycemia after a meal and is used as a functional food for specified health use. Indigestible dextrin in the test diet has been associated with diarrhea and other gastrointestinal symptoms when consumed in large amounts at a time. Given that the maximum non-effect level for diarrhea in women is ≥1.0 g/kg, the daily intake of indigestible dextrin was set at 5 g in this study.

An inhibitory action on AGE production of tien-cha extract, containing *Rubus suavissimus* extract, was confirmed *in vitro* [9]. Rutin, Citric acid, sucralose, and potassium acesulfame are food additives, and their content was within accepted limits. Acetic acid, salt, and concentrated tomatoes have a long history as ingredients in various foods and were added to the test diet at concentrations smaller than found in commercially available food items. These facts assured a sufficient level of safety of the test diet. A list of safety studies of the active ingredients present in the test diet is shown in ***Table 2***.

To ensure product quality, the manufacturing process of the vinegar beverage containing indigestible dextrin and mixed herbal extract is strictly controlled from the reception of raw materials to the packaging of the end product. Among other acceptance criteria, the end product has to contain less than $10^3$/mL viable cells and no detectable coliform bacteria to pass quality control.

## Test procedure

### *Subjective symptoms*

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 [7,10,11,14,15].

### *Anthropometry and physical examination*

Body height, body weight, blood pressure, and body composition as measured by bioelectrical impedance analysis with a body composition analyzer (BC-118D Tanita Corp., Tokyo, Japan) as described previously [14,15], were determined.

### *Vascular function test*

Blood concentrations of endothelin, vascular endothelial growth factor (VEGF), and nitric oxide (NO) were measured as parameters for evaluating arteriosclerosis. Fingertip acceleration pulse wave was also measured with a plethysmometer (SDP-100, Fukuda Denshi, Tokyo, Japan) to estimate vascular age [16-18]. Briefly, the second derivative of plethysmogram aging index (SDPTGAI) was calculated from parameters b/a, c/a, d/a, and e/a, and vascular age was calculated using the following formulas:

Men: Vascular age = 43.50 × SDPTGAI + 65.90

Women: Vascular age = 41.67 × SDPTGAI + 61.75

### *Glycation stress markers*

Insulin resistance was evaluated by fasting plasma glucose (FPG), insulin, and HbA1c levels. For analysis of AGEs and glycation intermediates, serum concentrations of 3-deoxyglucosone (3DG), $N^{\varepsilon}$-(carboxymethyl) lysine (CML), and pentosidine were measured as described previously [7,10,11].

Skin AGE deposition was measured in the medial aspect of the right upper arm (at 10 cm from the olecranon toward the shoulder) with an AGE Reader (DiagnOptic, Netherland) as described previously [4,19]. CML content in the skin stratum corneum (in the medial aspect of the right upper arm) was

***Table 2. List of safety studies.***

| Name of ingredient | Study details | Results |
|---|---|---|
| Indigestible dextrin (as Fibersol-2H) | Reverse mutation test | Negative |
| | A single-dose oral toxicity study in rats (male and female) | No sign of toxicity at 10 g/kg |
| | A repeated-dose oral toxicity study in rats | NOAEL: > 5.0 g/kg |
| | A study to find the maximum non-effect level in humans at solid content of 0.4, 0.5, 0.6, 0.8 and 1.0 g/kg | Maximum non-effect level for diarrhea Men: 0.8 g/kg, Women: > 1.0 g/kg |
| Mixed herbal extract (as AG herb mix) | Rec-assay (lethal sensitivity test) | Negative |
| | Reverse mutation test | Negative |
| | An acute oral toxicity study in rats (male and female) | $LD_{50}$: > 2,000 mg/kg |
| | An overdose study (in humans) 3,000 mg/day (5 times the regular dose) for 4 weeks | No adverse event was observed. |
| Tien-cha extract (Tien-cha Extract M Powder) | An acute oral toxicity study in mice (male and female) | $LD_{50}$: > 5,000 mg/kg |
| | A 28-day repeated-dose oral toxicity study in rats (male and female) | NOAEL: 600 mg/kg/day |
| Rutin (as glucosyl rutin) | Reverse mutation test | Negative |
| | Micronucleus test | No mutation was detected. |
| | An acute oral toxicity study in mice (male and female) | $LD_{50}$: > 42,000 mg/kg |
| | A 28-day subacute oral toxicity study 50, 200 and 1,000 mg/kg/day | No subacute oral toxicity was observed. |

Abbreviations: NOAEL, no observable adverse effect level; LD50, 50% lethal dose measured as described previously[20]. Briefly, an adhesive film was firmly applied to the skin to collect a sample of the stratum corneum (tape stripping technique). Three samples were collected from the same site and CML content was measured. Samples are typically collected from the left cheek (center portion between the bottom of the ear lobe and the lip end) or from the medial aspect of the right upper arm (at 10 cm from the olecranon toward the shoulder).

### *Oxidation stress markers*

Concentrations of 8-hydroxydeoxyguanosine (8-OHdG) and isoprostane in urine samples collected during the night were measured as markers for oxidation stress[21-25]. These parameters were measured by Mitsubishi Chemical Medience Corporation (Minato-ku, Tokyo, Japan). In addition, concentrations of 8-OHdG, isoprostane, and creatinine in the first urine collected in the early morning were measured to calculate creatinine-adjusted concentrations of 8-OHdG (8-OHdG/CRE) and isoprostane (isoprostane/CRE).

### *Immune stress markers*

Serum and plasma concentrations of high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6), both inflammatory markers, were measured as immune stress markers.

### *Skin function test*

The properties and function of the skin were evaluated by measuring color difference, moisture content, and melanin/erythema content, as well as by the identification of wrinkles and spots based on imaging analysis. These tests were performed in a room with constant temperature and humidity (25°C, 50%) after a 20-minute conditioning period.

For the imaging analysis of facial skin, the VISIA Evolution System (Canfield Imaging Systems, Fairfield, NJ, USA)[26] was used to examine pores, spots (visualized as light spots), melanin (brown spots), hemoglobin (red spots), wrinkles, color unevenness (texture), porphyrin, and latent spots (UV spots). This test was performed on the left cheek of each subject.

Skin elasticity was evaluated using a cutometer (MPA580; Courage & Khazaka, Kern, Germany)[29-31]. The skin surface was drawn by negative pressure into the aperture of the probe, and the length of the skin drawn into the aperture was measured by the prism. This test was performed on the left cheek (center portion between the bottom of the ear lobe and the lip end) in the supine position or on the medial aspect of the right upper arm (at 10 cm from the olecranon toward the shoulder) in a sitting position. Results were expressed as skin elasticity index R2 or R7, respectively.

Skin moisture content was measured in the left cheek with a moisture meter (Corneometer, CM825; Courage & Khazaka)[32]. For skin color analysis, a spectrophotometer (CM-2600d, Konica Minolta Sensing, Osaka, Japan) was used to measure L*, a*, b*, melanin index (melanin content), hemoglobin content (Hb index), and blood oxygen saturation (Hb $SO_2$ index) of the left cheek as described previously[28].

### *Safety test*

For safety evaluation, the following parameters were measured for the test diet and placebo before (0 weeks) and at 8 and 12 weeks after intake: total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglyceride (TG), atherogenic index (AI), total bilirubin (TB), aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (γ-GTP), creatine phosphokinase (CPK), uric acid (UA), urea nitrogen (BUN), creatinine (CRE), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), total protein (TP), albumin (ALB), albumin/globulin ratio (A/G), and iron (Fe). Urine samples collected during the night were also analyzed for concentrations of sodium (Na), potassium (K), and calcium (Ca) to detect possible excessive electrolyte intake.

Analysis of glycation stress markers, *i.e.*, 3DG and CML, was performed by SRL Inc. (Shinjuku-ku, Tokyo, Japan). Analysis of endothelin, a marker for vascular endothelial function, and CML content in the skin stratum corneum was performed at the Life & Medical Science Investigation Center of A-kit Corporation (Kyotanabe, Kyoto, Japan). The remaining blood/urine parameters were measured by Mitsubishi Chemical Medience Corporation.

### *Ethical considerations*

This study was conducted at a third-party institution 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, Labor and Welfare, Ordinance No. 28 of March 27, 1997). The study protocol was reviewed for ethical aspects and appropriateness of the study and approved by the human research ethics committee of the institutional review board at Tokyo Synergy Clinic (Chuo-ku, Tokyo, Japan). The study was conducted according to the approved protocol.

The principal investigator and sub investigators, in cooperation with a contract research organization, explained the details of the study to and obtained written consent from each subject based on her free will before initiating the study.

### *Statistical analysis*

All results were expressed as mean ± standard deviation. Dunnett's test was used to compare data obtained before and at 8 and 12 weeks after diet intake, while unpaired Mann-Whitney U-test was used for comparisons between two groups. In addition, a subclass analysis was performed in the group of subjects with a 60-minute postprandial blood glucose level of 150 mg/dL or more (7 subjects from the control group and 10 from the test group), as determined by screening examination.

All analyses were performed using IBM SPSS Statistics 20 software (IBM Japan, Tokyo, Japan), with a two-sided significance level of 5%. Safety evaluation was based on blood parameter assessment and the occurrence of individual adverse events.

## Results

### *Subjective and objective symptoms*

No significant changes or differences were observed in the scores for physical and mental symptoms, as assessed by the AAQol questionnaire, within or between the control and test arms over the study period (data not shown).

### *Physical examination*

No significant changes or differences were observed in body height, body weight, body composition, basal metabolic rate, blood pressure or pulse rate within or between the control and test arms over the study period (***Table 3***).

### *Blood chemistry*

The results of blood chemistry are summarized in ***Table 4***. The AST (GOT) level in the test arm was significantly decreased compared to control at week 8, but returned to baseline level after discontinuation of the test diet. No significant intergroup difference was observed in any other parameter.

### *Vascular function test*

No significant intergroup difference was observed in estimated vascular age as determined by fingertip acceleration pulse wave analysis or by serum concentrations of endothelin, VEGF, and $NO_x$ (***Table 5***).

### *Glycation stress parameters*

Glycation stress parameters are summarized in ***Table 6***. No significant intergroup difference was observed in FPG or HbA1c levels during the study period. A significantly increased insulin level in the test group compared to control was observed at week 12, but was within the range of physiological variation.

No significant intergroup differences were observed in the serum concentrations of 3DG, CML, or pentosidine or in the CML content in the skin stratum corneum during the study period. The fluorescence intensity of skin AGEs was similar between the two groups at week 8, but significantly higher in the test group compared to the control group at week 12, *i.e.*, after discontinuation of the test diet.

A subclass analysis was performed in 10 subjects from the test group and 7 from the control group with a 60-minute postprandial blood glucose level of 150 mg/dL or more. CML content in the skin stratum corneum in the test group was significantly decreased compared to control at week 8 ($p < 0.05$) and remained at a low level even after discontinuation of the test diet, although no significant difference was observed at 12 weeks (***Fig. 1***).

### *Oxidation stress parameters*

In the test group, a significant increase in urine 8-OHdG concentration was observed at weeks 8 and 12 as compared to before diet intake ($p = 0.009$ and $p = 0.009$, respectively) (***Table 7***). Meanwhile, no significant difference was found in this parameter between the test and control group. No significant changes or differences were observed in urine isoprostane concentrations within or between groups.

***Table 3. Anthropometry and physical examination.***

| Parameter | Unit | Reference range | Diet | n | 0W | 8W | Dunnett | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| Age | years | – | Control diet | 11 | 57.45 ± 4.44 | – ± – | – | – ± – | – | – | – |
| | | | Test diet | 11 | 57.27 ± 3.72 | – ± – | – | – ± – | – | | |
| Body height | cm | – | Control diet | 11 | 152.43 ± 4.00 | – ± – | – | – ± – | – | – | – |
| | | | Test diet | 11 | 153.18 ± 6.49 | – ± – | – | – ± – | – | | |
| Body weight | kg | – | Control diet | 11 | 59.16 ± 5.20 | 59.18 ± 5.20 | 1.000 | 58.88 ± 5.45 | 0.989 | 0.907 | 0.568 |
| | | | Test diet | 11 | 60.05 ± 8.79 | 60.10 ± 8.57 | 1.000 | 60.12 ± 8.01 | 1.000 | | |
| Body fat | % | – | Control diet | 11 | 35.05 ± 3.75 | 35.06 ± 3.67 | 1.000 | 34.59 ± 3.89 | 0.943 | 0.949 | 0.683 |
| | | | Test diet | 11 | 35.80 ± 5.15 | 35.84 ± 5.13 | 1.000 | 35.55 ± 5.11 | 0.990 | | |
| Fat mass | kg | – | Control diet | 11 | 20.87 ± 3.87 | 20.88 ± 3.79 | 1.000 | 20.52 ± 3.97 | 0.969 | 0.959 | 0.727 |
| | | | Test diet | 11 | 21.80 ± 5.61 | 21.83 ± 5.56 | 1.000 | 21.63 ± 5.24 | 0.996 | | |
| Fat-free mass | kg | – | Control diet | 11 | 38.28 ± 2.10 | 38.30 ± 2.12 | 1.000 | 38.35 ± 2.21 | 0.995 | 0.881 | 0.404 |
| | | | Test diet | 11 | 38.23 ± 4.04 | 38.27 ± 3.94 | 1.000 | 38.49 ± 3.75 | 0.983 | | |
| Muscle mass | kg | – | Control diet | 11 | 36.09 ± 1.92 | 36.11 ± 1.93 | 0.999 | 36.16 ± 2.02 | 0.994 | 0.923 | 0.442 |
| | | | Test diet | 11 | 36.05 ± 3.68 | 36.08 ± 3.59 | 1.000 | 36.27 ± 3.43 | 0.984 | | |
| BMI | $kg/m^2$ | – | Control diet | 11 | 25.45 ± 1.89 | 25.46 ± 1.95 | 1.000 | 25.34 ± 1.99 | 0.985 | 0.837 | 0.460 |
| | | | Test diet | 11 | 25.48 ± 2.57 | 25.52 ± 2.56 | 0.999 | 25.55 ± 2.47 | 0.997 | | |
| Basal metabolic rate | kcal | – | Control diet | 11 | 1128.36 ± 70.40 | 1129.00 ± 71.21 | 1.000 | 1128.64 ± 74.24 | 1.000 | 0.934 | 0.470 |
| | | | Test diet | 11 | 1133.55 ± 130.05 | 1134.55 ± 126.05 | 1.000 | 1139.18 ± 118.86 | 0.992 | | |
| Systolic blood pressure | mmHg | 100-139 | Control diet | 11 | 114.73 ± 14.80 | 113.36 ± 16.23 | 0.969 | 113.18 ± 15.27 | 0.961 | 0.509 | 0.750 |
| | | | Test diet | 11 | 118.82 ± 12.68 | 114.41 ± 12.42 | 0.667 | 118.55 ± 15.37 | 0.998 | | |
| Diastolic blood pressure | mmHg | 50-80 | Control diet | 11 | 71.55 ± 10.32 | 71.27 ± 10.83 | 0.997 | 71.64 ± 11.09 | 1.000 | 0.216 | 0.375 |
| | | | Test diet | 11 | 76.05 ± 8.51 | 71.91 ± 8.03 | 0.461 | 73.73 ± 10.28 | 0.772 | | |
| Pulse rate | bpm | 40-80 | Control diet | 11 | 71.64 ± 7.13 | 67.50 ± 7.79 | 0.335 | 68.18 ± 7.38 | 0.455 | 0.497 | 0.598 |
| | | | Test diet | 11 | 71.91 ± 10.00 | 65.73 ± 7.66 | 0.181 | 67.00 ± 8.10 | 0.321 | | |

Abbreviations: BMI; body mass index

***Table 4. Serum parameters.***

| Parameter | Unit | Reference range | Diet | n | 0W | 8W | Dunnett's | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| Total protein | g/dL | 6.7 - 8.3 | Control diet | 11 | 7.35 ± 0.33 | 7.57 ± 0.32 | 0.292 | 7.54 ± 0.44 | 0.413 | 0.407 | 0.549 |
| | | | Test diet | 11 | 7.34 ± 0.37 | 7.50 ± 0.39 | 0.525 | 7.60 ± 0.38 | 0.202 | | |
| Albumin | g/dL | 3.8 - 5.3 | Control diet | 11 | 4.36 ± 0.14 | 4.46 ± 0.17 | 0.355 | 4.43 ± 0.23 | 0.639 | 0.419 | 0.650 |
| | | | Test diet | 11 | 4.37 ± 0.15 | 4.43 ± 0.22 | 0.698 | 4.47 ± 0.18 | 0.346 | | |
| A/G | | 1.1 - 2.0 | Control diet | 11 | 1.49 ± 0.18 | 1.45 ± 0.18 | 0.770 | 1.45 ± 0.17 | 0.844 | 0.726 | 0.821 |
| | | | Test diet | 11 | 1.49 ± 0.15 | 1.45 ± 0.21 | 0.811 | 1.45 ± 0.16 | 0.764 | | |
| AST(GOT) | IU/L/37°C | 10 - 40 | Control diet | 11 | 19.91 ± 2.95 | 21.18 ± 4.83 | 0.604 | 21.27 ± 2.10 | 0.564 | 0.029 | 0.117 |
| | | | Test diet | 11 | 24.36 ± 6.09 | 20.80 ± 4.39 | 0.307 | 23.45 ± 7.03 | 0.913 | | |
| ALT(GPT) | IU/L/37°C | 5 - 45 | Control diet | 11 | 16.82 ± 4.77 | 16.91 ± 5.11 | 0.999 | 17.64 ± 4.70 | 0.893 | 0.333 | 0.830 |
| | | | Test diet | 11 | 21.73 ± 7.51 | 19.30 ± 5.58 | 0.678 | 22.00 ± 8.67 | 0.995 | | |
| LD(LDH) | IU/L/37°C | 120 - 240 | Control diet | 11 | 204.55 ± 53.95 | 196.55 ± 37.52 | 0.871 | 198.45 ± 34.25 | 0.922 | 0.729 | 0.767 |
| | | | Test diet | 11 | 216.36 ± 19.73 | 205.70 ± 18.32 | 0.380 | 206.82 ± 21.59 | 0.438 | | |
| γ-GT | IU/L/37°C | ≤30 | Control diet | 11 | 26.82 ± 17.55 | 23.64 ± 9.05 | 0.782 | 21.73 ± 9.63 | 0.548 | 0.537 | 0.634 |
| | | | Test diet | 11 | 40.55 ± 34.52 | 28.40 ± 19.57 | 0.561 | 38.55 ± 32.98 | 0.982 | | |
| Total bilirubin | mg/dL | 0.2 - 1.2 | Control diet | 11 | 0.75 ± 0.25 | 0.79 ± 0.19 | 0.879 | 0.76 ± 0.16 | 0.992 | 0.723 | 0.451 |
| | | | Test diet | 11 | 0.80 ± 0.29 | 0.89 ± 0.28 | 0.773 | 0.88 ± 0.43 | 0.799 | | |
| CPK | IU/L/37°C | 40 - 150 | Control diet | 11 | 109.64 ± 53.84 | 115.18 ± 65.40 | 0.953 | 94.18 ± 23.19 | 0.699 | 0.891 | 0.153 |
| | | | Test diet | 11 | 84.27 ± 32.42 | 84.60 ± 26.60 | 1.000 | 94.36 ± 36.21 | 0.687 | | |
| Creatinine | mg/dL | 0.47 - 0.79 | Control diet | 11 | 0.67 ± 0.09 | 0.63 ± 0.09 | 0.479 | 0.66 ± 0.08 | 0.971 | 0.288 | 0.597 |
| | | | Test diet | 11 | 0.64 ± 0.07 | 0.62 ± 0.07 | 0.876 | 0.64 ± 0.07 | 0.984 | | |
| Urea nitrogen | mg/dL | 8.0 - 20.0 | Control diet | 11 | 12.84 ± 3.34 | 13.57 ± 3.44 | 0.826 | 13.34 ± 3.26 | 0.915 | 0.660 | 0.734 |
| | | | Test diet | 11 | 12.34 ± 2.18 | 12.64 ± 2.22 | 0.938 | 12.57 ± 2.58 | 0.960 | | |
| Uric acid | mg/dL | 2.5 - 7.0 | Control diet | 11 | 5.12 ± 1.50 | 4.83 ± 1.21 | 0.855 | 4.95 ± 1.64 | 0.951 | 0.271 | 0.735 |
| | | | Test diet | 11 | 4.74 ± 1.25 | 4.76 ± 1.03 | 0.998 | 4.49 ± 1.10 | 0.833 | | |
| Total cholesterol | mg/dL | 120 - 219 | Control diet | 11 | 234.36 ± 29.69 | 238.27 ± 38.58 | 0.950 | 235.45 ± 34.37 | 0.996 | 0.910 | 0.731 |
| | | | Test diet | 11 | 238.09 ± 31.48 | 245.70 ± 30.84 | 0.829 | 243.09 ± 38.51 | 0.917 | | |
| HDL cholesterol | mg/dL | 40 - 95 | Control diet | 11 | 63.91 ± 14.58 | 71.73 ± 20.07 | 0.459 | 68.45 ± 15.73 | 0.756 | 0.146 | 0.555 |
| | | | Test diet | 11 | 62.36 ± 10.59 | 66.10 ± 11.09 | 0.645 | 65.27 ± 10.47 | 0.752 | | |
| LDL cholesterol | mg/dL | 65 - 139 | Control diet | 11 | 148.09 ± 26.63 | 147.36 ± 36.81 | 0.998 | 148.00 ± 34.92 | 1.000 | 0.931 | 0.880 |
| | | | Test diet | 11 | 154.45 ± 29.54 | 156.10 ± 29.17 | 0.989 | 155.91 ± 34.48 | 0.991 | | |
| TG (triglyceride) | mg/dL | 30 - 149 | Control diet | 11 | 102.82 ± 52.17 | 84.55 ± 37.62 | 0.525 | 88.64 ± 40.40 | 0.670 | 0.145 | 0.172 |
| | | | Test diet | 11 | 102.00 ± 24.08 | 104.60 ± 39.88 | 0.984 | 111.00 ± 51.40 | 0.821 | | |
| Sodium | mgEq/L | 137 - 147 | Control diet | 11 | 142.00 ± 1.26 | 140.55 ± 1.21 | 0.016 | 141.18 ± 1.17 | 0.215 | 0.116 | 0.697 |
| | | | Test diet | 11 | 142.18 ± 1.33 | 141.70 ± 1.57 | 0.619 | 141.64 ± 1.03 | 0.530 | | |
| Potassium | mgEq/L | 3.5 - 5.0 | Control diet | 11 | 4.30 ± 0.24 | 4.37 ± 0.27 | 0.722 | 4.40 ± 0.24 | 0.551 | 0.716 | 0.307 |
| | | | Test diet | 11 | 4.32 ± 0.27 | 4.44 ± 0.39 | 0.562 | 4.29 ± 0.24 | 0.968 | | |
| Chloride | mgEq/L | 98 - 108 | Control diet | 11 | 104.64 ± 1.57 | 103.45 ± 1.04 | 0.079 | 103.55 ± 1.29 | 0.109 | 0.180 | 0.897 |
| | | | Test diet | 11 | 104.91 ± 1.45 | 104.90 ± 2.13 | 1.000 | 103.91 ± 1.51 | 0.303 | | |
| Calcium | mg/dL | 8.4 - 10.4 | Control diet | 11 | 9.77 ± 0.30 | 9.81 ± 0.30 | 0.946 | 9.76 ± 0.33 | 0.997 | 0.854 | 1.000 |
| | | | Test diet | 11 | 9.83 ± 0.25 | 9.84 ± 0.27 | 0.991 | 9.82 ± 0.27 | 0.995 | | |
| Serum iron | μg/dL | 40 - 180 | Control diet | 11 | 98.09 ± 34.67 | 99.91 ± 35.80 | 0.988 | 94.64 ± 28.39 | 0.957 | 0.812 | 0.119 |
| | | | Test diet | 11 | 94.00 ± 27.04 | 96.70 ± 21.80 | 0.961 | 105.73 ± 29.13 | 0.483 | | |
| AI (atherogenic index) | | <4 | Control diet | 11 | 2.82 ± 0.97 | 2.50 ± 0.91 | 0.633 | 2.59 ± 0.87 | 0.787 | 0.167 | 0.584 |
| | | | Test diet | 11 | 2.89 ± 0.65 | 2.81 ± 0.81 | 0.957 | 2.78 ± 0.79 | 0.919 | | |

Abbreviations: AST/GOT, aspartate aminotransferase; ALT/GPT, alanine aminotransferase; LD/LDH, lactate dehydrogenase; γ-GT, gamma-glutamyl transpeptidase; CPK creatine phosphokinase

***Table 5. Vascular endothelial function parameters.***

| | Parameter | Unit | Diet | n | 0W mean ± SD | 8W mean ± SD | Dunnett's vs. 0W | 12W mean ± SD | Dunnett's vs. 0W | Independent samples t-test At 8W | Independent samples t-test At 12W |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fingertip acceleration pulse wave | Estimated vascular age | Years | Control diet | 11 | 60.50 ± 11.33 | 58.32 ± 8.58 | 0.831 | 62.95 ± 10.12 | 0.793 | 0.530 | 0.110 |
| | | | Test diet | 11 | 65.27 ± 8.13 | 60.77 ± 12.01 | 0.414 | 61.68 ± 6.23 | 0.559 | | |
| | SDPTGAI | – | Control diet | 11 | -0.01 ± 0.27 | -0.07 ± 0.20 | 0.812 | 0.04 ± 0.24 | 0.849 | 0.519 | 0.156 |
| | | | Test diet | 11 | 0.11 ± 0.23 | -0.01 ± 0.30 | 0.420 | 0.02 ± 0.17 | 0.594 | | |
| | b/a | – | Control diet | 11 | -0.48 ± 0.07 | -0.51 ± 0.08 | 0.647 | -0.45 ± 0.10 | 0.647 | 0.392 | 0.262 |
| | | | Test diet | 11 | -0.42 ± 0.12 | -0.49 ± 0.13 | 0.272 | -0.44 ± 0.07 | 0.878 | | |
| | c/a | – | Control diet | 11 | -0.20 ± 0.11 | -0.15 ± 0.08 | 0.333 | -0.17 ± 0.09 | 0.669 | 0.481 | 0.943 |
| | | | Test diet | 11 | -0.21 ± 0.05 | -0.18 ± 0.08 | 0.600 | -0.17 ± 0.09 | 0.490 | | |
| | d/a | – | Control diet | 11 | -0.37 ± 0.11 | -0.38 ± 0.11 | 0.984 | -0.40 ± 0.11 | 0.699 | 0.257 | 0.032 |
| | | | Test diet | 11 | -0.42 ± 0.11 | -0.38 ± 0.11 | 0.539 | -0.36 ± 0.10 | 0.295 | | |
| | e/a | – | Control diet | 11 | 0.11 ± 0.04 | 0.09 ± 0.03 | 0.634 | 0.08 ± 0.04 | 0.261 | 0.749 | 0.874 |
| | | | Test diet | 11 | 0.10 ± 0.06 | 0.08 ± 0.06 | 0.566 | 0.07 ± 0.03 | 0.397 | | |
| | b-a | ms | Control diet | 11 | 95.45 ± 19.06 | 86.73 ± 12.34 | 0.322 | 88.18 ± 14.01 | 0.444 | 0.655 | 0.625 |
| | | | Test diet | 11 | 93.64 ± 9.71 | 87.09 ± 12.44 | 0.356 | 88.73 ± 13.92 | 0.544 | | |
| | c-a | ms | Control diet | 11 | 173.27 ± 18.38 | 168.00 ± 11.70 | 0.647 | 163.27 ± 15.98 | 0.244 | 0.731 | 0.067 |
| | | | Test diet | 11 | 164.91 ± 14.60 | 161.45 ± 14.67 | 0.824 | 167.09 ± 17.24 | 0.924 | | |
| | d-a | ms | Control diet | 11 | 222.55 ± 14.89 | 225.09 ± 15.06 | 0.878 | 224.18 ± 12.02 | 0.947 | 0.431 | 0.539 |
| | | | Test diet | 11 | 226.73 ± 21.60 | 222.36 ± 6.12 | 0.736 | 222.91 ± 14.73 | 0.789 | | |
| | e-a | ms | Control diet | 11 | 304.36 ± 21.03 | 303.64 ± 16.90 | 0.993 | 303.09 ± 15.83 | 0.980 | 0.542 | 0.696 |
| | | | Test diet | 11 | 311.09 ± 28.29 | 304.73 ± 9.60 | 0.668 | 306.00 ± 16.10 | 0.769 | | |
| | a-a | ms | Control diet | 11 | 914.36 ± 99.64 | 918.55 ± 87.08 | 0.991 | 928.18 ± 72.64 | 0.905 | 0.403 | 0.924 |
| | | | Test diet | 11 | 896.18 ± 94.47 | 926.18 ± 86.91 | 0.676 | 906.00 ± 99.70 | 0.957 | | |
| | PTGAI | – | Control diet | 11 | 1.14 ± 0.12 | 1.20 ± 0.12 | 0.449 | 1.25 ± 0.16 | 0.109 | 0.308 | 0.052 |
| | | | Test diet | 11 | 1.24 ± 0.21 | 1.22 ± 0.20 | 0.956 | 1.22 ± 0.16 | 0.979 | | |
| Endothelin | | pg/mL | Control diet | 11 | 1.25 ± 0.23 | 1.39 ± 0.29 | 0.251 | – – | – | 0.357 | – |
| | | | Test diet | 11 | 1.27 ± 0.38 | 1.27 ± 0.38 | 0.985 | – – | – | | |
| VEGF (vascular endothelial growth factor) | | pg/mL | Control diet | 11 | 19.68 ± 6.25 | 27.48 ± 12.47 | 0.179 | 29.42 ± 12.69 | 0.080 | 0.109 | 0.091 |
| | | | Test diet | 11 | 27.11 ± 13.12 | 63.53 ± 59.29 | 0.089 | 58.94 ± 38.02 | 0.135 | | |
| NO | | µmol/L | Control diet | 11 | 32.29 ± 24.71 | 48.41 ± 26.44 | 0.229 | 31.14 ± 22.28 | 0.991 | 0.917 | 0.622 |
| | | | Test diet | 11 | 26.65 ± 13.29 | 42.70 ± 19.37 | 0.039 | 22.05 ± 12.00 | 0.699 | | |

Abbreviations: SDPTGAI, second derivative of plethysmogram aging index; PTGAI, plethysmogram aging index; NO, nitric oxide

***Table 6. Glycation stress-related parameters.***

| Parameter | Unit | Reference range | Diet | n | 0W mean ± SD | 8W mean ± SD | Dunnett's vs. 0W | 12W mean ± SD | Dunnett's vs. 0W | Independent samples t-test At 8W | Independent samples t-test At 12W |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose | mg/dL | 70 - 109 | Control diet | 11 | 87.91 ± 5.74 | 88.73 ± 6.59 | 0.934 | 90.36 ± 6.41 | 0.562 | 0.951 | 0.468 |
| | | | Test diet | 11 | 90.45 ± 9.11 | 92.60 ± 11.42 | 0.814 | 91.18 ± 6.31 | 0.975 | | |
| Insulin | µU/mL | 1.7 - 10.4 | Control diet | 11 | 3.80 ± 2.65 | 3.48 ± 1.61 | 0.919 | 3.95 ± 2.17 | 0.982 | 0.087 | 0.026 |
| | | | Test diet | 11 | 3.15 ± 0.98 | 4.04 ± 1.25 | 0.245 | 4.65 ± 1.73 | 0.028 | | |
| HbA1c/NGSP | % | 4.6 - 6.2 | Control diet | 11 | 5.53 ± 0.34 | 5.54 ± 0.27 | 0.996 | 5.40 ± 0.24 | 0.487 | 0.329 | 0.328 |
| | | | Test diet | 11 | 5.65 ± 0.35 | 5.59 ± 0.37 | 0.903 | 5.45 ± 0.45 | 0.366 | | |
| HbA1c/JDS | % | 4.3 - 5.8 | Control diet | 11 | 5.15 ± 0.31 | 5.15 ± 0.26 | 1.000 | 5.03 ± 0.21 | 0.483 | 0.516 | 0.339 |
| | | | Test diet | 11 | 5.26 ± 0.34 | 5.22 ± 0.34 | 0.948 | 5.07 ± 0.42 | 0.382 | | |
| 3-deoxyglucosone | ng/mL | 3.76 - 18.14 | Control diet | 11 | 16.82 ± 3.57 | 24.81 ± 6.89 | 0.001 | 19.05 ± 3.660 | 0.474 | 0.741 | 0.599 |
| | | | Test diet | 11 | 16.73 ± 4.13 | 25.89 ± 5.13 | 0.000 | 19.94 ± 4.289 | 0.185 | | |
| CML | µg/mL | – | Control diet | 11 | 4.45 ± 0.64 | 4.78 ± 1.04 | 0.616 | 5.78 ± 1.045 | 0.004 | 0.613 | 0.820 |
| | | | Test diet | 11 | 4.40 ± 0.77 | 4.39 ± 0.64 | 1.000 | 5.61 ± 0.824 | 0.001 | | |
| Pentosidine | pmol/mL | – | Control diet | 11 | 94.52 ± 15.60 | 73.43 ± 27.35 | 0.038 | 87.26 ± 15.221 | 0.613 | 0.824 | 0.081 |
| | | | Test diet | 11 | 89.13 ± 26.33 | 66.29 ± 15.30 | 0.028 | 93.98 ± 16.634 | 0.798 | | |
| Skin AGE deposition (AF level) | – | – | Control diet | 11 | 2.30 ± 0.25 | 2.09 ± 0.25 | 0.129 | 2.06 ± 0.268 | 0.071 | 0.077 | 0.017 |
| | | | Test diet | 11 | 2.24 ± 0.22 | 2.18 ± 0.23 | 0.755 | 2.25 ± 0.276 | 0.990 | | |
| CML content in stratum corneum | µg/mg protein | – | Control diet | 11 | 103.31 ± 24.99 | 100.71 ± 18.17 | 0.933 | 94.08 ± 14.458 | 0.450 | 0.176 | 0.246 |
| | | | Test diet | 11 | 112.12 ± 24.13 | 93.85 ± 27.17 | 0.182 | 89.52 ± 25.502 | 0.085 | | |

Abbreviations: HbA1c/NGSP, hemoglobin A1c/National Glycohemoglobin Standardization Program; HbA1c/JDS, hemoglobin A1c/ Japan Diabetes Society; CML, carboxymethyl lysine; AGE, advanced glycation end products; AF, autofluorescence

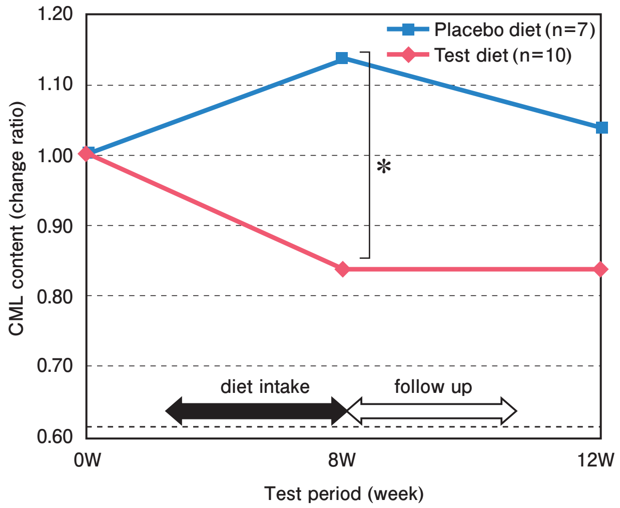


***Fig. 1.*** **Change in carboxymethyl lysine (CML) content in the stratum corneum.**

Subclass analysis of the subjects with high postprandial blood glucose (>150 mg/dL at 60 minutes).

CML content in the stratum corneum was measured by the tape stripping method. The change ratio was calculated as follows:

* p < 0.05, Mann-Whitney test.

### *Inflammation-related parameters*

No significant changes or differences were observed in hsCRP or IL-6 levels within or between groups over the study period (***Table 8***).

### *Urine electrolytes*

Concentrations of sodium, potassium, and calcium present in urine samples collected during the night are summarized in ***Table 9***. No significant changes or differences were observed in urine electrolyte concentrations within or between groups over the study period.

### *Skin function test*

No significant changes or differences were observed in the results of the imaging analysis with the VISIA Evolution system, moisture content analysis, or skin elasticity test within or between groups over the study period (***Table 10***).

In the color difference test, the Hb $SO_2$ index (blood oxygen saturation) in the test group (measured in the left cheek) was significantly higher than that in the control group at week 8, and it remained at a similar level until week 12, *i.e.*, after discontinuation of the test diet, although no significant intergroup difference was observed. No significant intergroup difference was observed in other parameters.

***Table 7. Oxidation stress-related parameters.***

| Parameter | Unit | Diet | n | 0W | 8W | Dunnett's | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| 8OHdG | pg/mg・Cr | Control diet | 11 | 7.21 ± 2.06 | 9.52 ± 2.23 | 0.063 | 10.21 ± 2.95 | 0.014 | 0.504 | 0.956 |
| | | Test diet | 11 | 6.98 ± 2.38 | 10.03 ± 2.06 | 0.009 | 10.05 ± 2.58 | 0.009 | | |
| Urine isoprostane | pg/mg・Cr | Control diet | 11 | 267.09 ± 102.38 | 291.55 ± 97.15 | 0.754 | 287.36 ± 69.30 | 0.822 | 0.634 | 0.898 |
| | | Test diet | 11 | 308.36 ± 111.31 | 346.73 ± 93.29 | 0.508 | 323.09 ± 55.08 | 0.898 | | |

Abbreviations: 8OHdG, 8-hydroxydeoxyguanosine

***Table 8. Inflammation-related parameters***

| Parameter | Unit | Diet | n | 0W | 8W | Dunnett's | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| High-sensitivity CRP | mg/dL | Control diet | 11 | 0.06 ± 0.05 | 0.05 ± 0.02 | 0.522 | – ± – | – | 0.530 | – |
| | | Test diet | 11 | 0.08 ± 0.07 | 0.07 ± 0.07 | 0.856 | – ± – | – | | |
| Interleukin-6 | pg/mL | Control diet | 11 | 0.93 ± 0.62 | 0.84 ± 0.51 | 0.712 | – ± – | – | 0.529 | – |
| | | Test diet | 11 | 0.99 ± 0.47 | 0.88 ± 0.39 | 0.550 | – ± – | – | | |

Abbreviations: CRP, C-reactive protein

***Table 9. Results of urine electrolyte analysis.***

| Parameter | Unit | Diet | n | 0W | 8W | Dunnett's | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| Urine sodium | mEq/L | Control diet | 11 | 94.45 ± 45.96 | 93.18 ± 51.38 | 0.998 | 97.73 ± 56.44 | 0.984 | 0.168 | 0.168 |
| | | Test diet | 11 | 77.91 ± 42.80 | 103.55 ± 41.20 | 0.328 | 110.36 ± 52.09 | 0.183 | | |
| Urine potassium | mEq/L | Control diet | 11 | 20.20 ± 12.26 | 27.75 ± 21.92 | 0.532 | 27.67 ± 19.39 | 0.538 | 0.992 | 0.992 |
| | | Test diet | 11 | 17.85 ± 15.50 | 25.45 ± 14.36 | 0.439 | 28.65 ± 17.94 | 0.213 | | |
| Urine calcium | mg/dL | Control diet | 11 | 7.65 ± 3.93 | 12.18 ± 7.61 | 0.123 | 8.79 ± 4.67 | 0.851 | 0.894 | 0.894 |
| | | Test diet | 11 | 12.55 ± 9.51 | 16.53 ± 13.86 | 0.589 | 14.38 ± 7.51 | 0.888 | | |

***Table 10. Skin function test.***

| Parameter | | | Diet | n | 0W | 8W | Dunnett's | 12W | Dunnett's | Independent samples t-test | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD | mean ± SD | vs. 0W | mean ± SD | vs. 0W | At 8W | At 12W |
| Imaging analysis by VISIA (score) | Brown spot | ×100 | Control diet | 11 | 7.52 ± 1.93 | 7.69 ± 2.27 | 0.976 | 7.76 ± 2.39 | 0.953 | 0.443 | 0.572 |
| | | | Test diet | 11 | 6.94 ± 2.58 | 7.46 ± 2.08 | 0.808 | 7.45 ± 2.01 | 0.816 | | |
| | Pore | ×100 | Control diet | 11 | 1.26 ± 0.52 | 1.50 ± 0.54 | 0.542 | 1.52 ± 0.71 | 0.498 | 0.922 | 0.583 |
| | | | Test diet | 11 | 1.59 ± 1.25 | 1.84 ± 1.42 | 0.871 | 1.77 ± 1.36 | 0.924 | | |
| | Porphyrin | ×100 | Control diet | 11 | 0.30 ± 0.30 | 0.25 ± 0.29 | 0.867 | 0.26 ± 0.23 | 0.918 | 0.263 | 0.712 |
| | | | Test diet | 11 | 0.15 ± 0.21 | 0.15 ± 0.14 | 0.999 | 0.13 ± 0.15 | 0.947 | | |
| | Red spot | ×100 | Control diet | 11 | 1.26 ± 0.45 | 1.37 ± 0.32 | 0.751 | 1.31 ± 0.51 | 0.934 | 0.894 | 0.715 |
| | | | Test diet | 11 | 1.40 ± 0.64 | 1.51 ± 0.85 | 0.930 | 1.52 ± 0.76 | 0.913 | | |
| | Spot | ×100 | Control diet | 11 | 2.38 ± 1.00 | 2.59 ± 1.16 | 0.856 | 2.48 ± 1.02 | 0.970 | 0.645 | 0.327 |
| | | | Test diet | 11 | 2.11 ± 0.79 | 2.27 ± 0.91 | 0.879 | 2.32 ± 0.86 | 0.789 | | |
| | Texture | ×100 | Control diet | 11 | 1.14 ± 0.43 | 1.22 ± 0.51 | 0.909 | 1.28 ± 0.62 | 0.765 | 0.538 | 0.872 |
| | | | Test diet | 11 | 1.63 ± 1.57 | 1.81 ± 1.67 | 0.953 | 1.80 ± 1.71 | 0.959 | | |
| | UV spot | ×100 | Control diet | 11 | 2.51 ± 1.64 | 2.64 ± 1.81 | 0.985 | 3.25 ± 2.71 | 0.630 | 0.524 | 0.516 |
| | | | Test diet | 11 | 2.32 ± 1.52 | 2.56 ± 1.66 | 0.912 | 2.66 ± 1.59 | 0.834 | | |
| | Wrinkle | ×100 | Control diet | 11 | 2.79 ± 2.28 | 2.86 ± 2.75 | 0.996 | 2.77 ± 1.98 | 1.000 | 0.946 | 0.387 |
| | | | Test diet | 11 | 3.54 ± 3.02 | 3.55 ± 2.66 | 1.000 | 3.09 ± 2.62 | 0.900 | | |
| Color difference analysis | L* | – | Control diet | 11 | 61.77 ± 2.34 | 62.39 ± 2.56 | 0.774 | 62.86 ± 2.39 | 0.474 | 0.911 | 0.668 |
| | | | Test diet | 11 | 61.88 ± 1.89 | 62.44 ± 2.15 | 0.714 | 62.76 ± 1.63 | 0.457 | | |
| | a* | – | Control diet | 11 | 7.01 ± 1.21 | 6.75 ± 1.30 | 0.856 | 6.74 ± 1.40 | 0.849 | 0.287 | 0.551 |
| | | | Test diet | 11 | 7.18 ± 1.02 | 7.32 ± 1.57 | 0.957 | 7.09 ± 1.30 | 0.979 | | |
| | b* | – | Control diet | 11 | 17.82 ± 1.60 | 17.34 ± 1.93 | 0.778 | 16.92 ± 2.14 | 0.444 | 0.603 | 0.226 |
| | | | Test diet | 11 | 18.01 ± 1.96 | 17.75 ± 2.26 | 0.941 | 17.69 ± 2.13 | 0.912 | | |
| | Melanin Index | – | Control diet | 11 | 1.04 ± 0.17 | 0.99 ± 0.21 | 0.772 | 0.94 ± 0.21 | 0.409 | 0.749 | 0.248 |
| | | | Test diet | 11 | 1.05 ± 0.15 | 1.01 ± 0.18 | 0.780 | 0.99 ± 0.16 | 0.624 | | |
| | Hb Index | – | Control diet | 11 | 0.92 ± 0.15 | 0.92 ± 0.17 | 1.000 | 0.95 ± 0.18 | 0.904 | 0.396 | 0.626 |
| | | | Test diet | 11 | 0.94 ± 0.19 | 0.98 ± 0.24 | 0.855 | 0.94 ± 0.20 | 0.998 | | |
| | Hb $SO_2$ Index | – | Control diet | 11 | 56.45 ± 6.11 | 57.02 ± 7.57 | 0.968 | 58.82 ± 5.20 | 0.593 | 0.036 | 0.152 |
| | | | Test diet | 11 | 56.49 ± 5.67 | 60.23 ± 3.95 | 0.169 | 61.25 ± 5.56 | 0.068 | | |
| Moisture content (%) | Moisture content | Apex of left cheek | Control diet | 11 | 63.57 ± 6.77 | 60.72 ± 8.61 | 0.545 | 59.05 ± 5.54 | 0.247 | 0.534 | 0.344 |
| | | | Test diet | 11 | 59.71 ± 3.73 | 54.81 ± 6.89 | 0.093 | 57.87 ± 5.93 | 0.671 | | |
| Skin viscoelasticity | R2 | Medial aspect of right upper arm | Control diet | 11 | 0.81 ± 0.03 | 0.80 ± 0.06 | 0.640 | 0.80 ± 0.04 | 0.678 | 0.842 | 0.465 |
| | | | Test diet | 11 | 0.81 ± 0.05 | 0.80 ± 0.05 | 0.761 | 0.81 ± 0.04 | 0.967 | | |
| | R7 | Medial aspect of right upper arm | Control diet | 11 | 0.60 ± 0.05 | 0.56 ± 0.06 | 0.250 | 0.57 ± 0.06 | 0.534 | 0.849 | 0.488 |
| | | | Test diet | 11 | 0.59 ± 0.06 | 0.56 ± 0.08 | 0.426 | 0.59 ± 0.05 | 0.944 | | |
| | R2 | Left cheek | Control diet | 11 | 0.69 ± 0.07 | 0.71 ± 0.07 | 0.830 | 0.75 ± 0.06 | 0.129 | 0.563 | 0.089 |
| | | | Test diet | 11 | 0.73 ± 0.07 | 0.72 ± 0.05 | 0.995 | 0.72 ± 0.06 | 0.986 | | |
| | R7 | Left cheek | Control diet | 11 | 0.33 ± 0.06 | 0.35 ± 0.04 | 0.570 | 0.38 ± 0.06 | 0.098 | 0.926 | 0.212 |
| | | | Test diet | 11 | 0.33 ± 0.06 | 0.35 ± 0.04 | 0.453 | 0.34 ± 0.05 | 0.714 | | |

Abbreviations: 8OHdG, 8-hydroxydeoxyguanosine

### Safety test

No serious adverse events related to the test diet were observed. One adverse event was assessed as probably related to the test diet, but it was mild and did not require study discontinuation. The event occurred in early October and was resolved by November 22. The study was continued, and no aggravation of symptoms was observed. In brief, a 51-year-old woman reported a tendency for gas retention, possibly due to a transient disturbance of the intestinal bacterial flora caused by indigestible dextrin. With no serious adverse event observed during the study period, the test diet was considered safe.

## Discussion

In this study we investigated the long-term effects of the intake of a vinegar beverage containing indigestible dextrin and a mixed herbal extract on a panel of glycation stress markers. A main result of the study showed that intake of the test diet caused a reduction in CML content in the skin stratum corneum, a maker of glycation stress, in people with a relatively high level of glycation stress, indicating the diet has an anti-glycation effect. As a second result of the study we found the test diet to be safe for long-term use as no serious adverse events could be observed during the study period. Indigestible dextrin acts by suppressing hyperglycemia after a meal. The tien-cha extract containing *Rubus suavissimus* has been shown to inhibit AGE production *in vitro* [9]. AG herb mix™ has been shown to inhibit AGE production in *in vitro* studies, experimentally induced diabetic rats, and RCTs in humans [5,10].

While no significant intergroup difference was observed in any of the glycation stress markers evaluated in the entire population, a subclass analysis involving 17 subjects with a 60-minute postprandial blood glucose level of 150 mg/dL or more showed a significant improvement in CML content in the stratum corneum after intake of the test diet ($p<0.05$). The pentosidine level was unchanged in the control arm and decreased in the test group. Although no significant difference was observed between groups, this observation also supports the inhibitory effect of the test diet on AGE production. The tape stripping technique [20], used in this study for determining CML content in the stratum corneum, is promising as a non-invasive method for evaluating glycation stress in the skin.

No significant changes were observed in the blood concentrations of 3DG, CML or skin AGE deposition in the test group. A previous report showed a significant improvement in blood CML content in a subclass analysis [10]. This discrepancy is likely due to the difference in the amounts of mixed herbal extract used in the two studies, *i.e.*, 600 mg/day in the previous study [10,12] versus 100 mg/day in the present study.

With regard to other parameters, a significant decrease in the AST (GOT) level in the test group compared to the control group was observed in the whole population analysis ($p = 0.029$). In our previous study, a decrease in GOT level was also observed after the intake of balsamic vinegar (15 mL), from 25.9 ± 13.7 U/L at baseline to 20.7 ± 6.0 U/L at 8 weeks after intake (-13.0%, $p = 0.040$, $n = 20$) [25]. This effect may be mediated by the action of acetic acid contained in both rice vinegar and balsamic vinegar. In view of the known suppressive effect of acetate Ringer's solution on protein catabolism [33,34] and metabolic acidosis [35,36], it is reasonable to supplement acetic acid in people who tend to be acidotic due to strong glycation stress or in those with a high lactate level.

Vinegar, the ingredient of the test diet, has a long history of consumption as food and thus should have no safety concern, as confirmed in this study. The intake of a typical amount (20-100 mL) of vinegar (corresponding to 150-750 mg of acetic acid) has been shown to cause no adverse event [37-39]. Moreover, the intake of a large amount of vinegar (300 mL, corresponding to 2,250 mg of acetic acid) for 4 weeks has been shown to cause no serious adverse event [40]. However, since the direct exposure of teeth to a high concentration of acetic acid may lead to dissolution of the tooth enamel [41], the mouth should be rinsed after the intake of vinegar. Gargling after vinegar intake or taking vinegar before a meal is therefore recommended.

## Conclusion

The results of the present study suggest that the intake of the test diet causes a reduction in CML content in the skin stratum corneum, a marker of glycation stress, in people with a relatively high level of glycation stress. Combined with the demonstrated safety of the test diet, this observation indicates a potential for the use of the test diet as a functional food.

## Conflict of interest

The authors have no conflict of interest in this study.

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