

Improvement in Skin Conditions by Consumption of Traditional Japanese Miso Soup and Its Mechanism

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Abstract

The present study was undertaken to determine the beneficial effects of miso soup consumption on the skin. The women were divided into two groups: the first group consumed three bowls of miso soup per day (miso-consuming group) and the second group consumed three bowls of soup per day without miso (non-miso-consuming group or control group). The women in both groups continued their respective routines every day. Before and after the 2-week trial, the stratum corneum water content, elasticity, sebum amount and skin texture of both their cheek and corner of their eye were analyzed. The skin texture was measured according to the images taken through a digital microscope. The women in the miso-consuming group had a significantly higher the stratum corneum water content, and their skin elasticity and texture improved compared with those in the non-miso-consuming group. There was no significant difference in the sebum amount between the two groups. The questionnaire on individual skin conditions showed that consumption of miso soup tended to improve smooth make-up application. In addition, miso extracts were prepared to examine the effect on ceramide synthesis of epidermal keratinocytes using cultured cells. The findings revealed that miso extracts increased mRNA expression and activity of β -glucocerebrosidase, an enzyme involved in the synthesis of ceramides in the stratum corneum. These results suggest that daily intake of miso soup may improve skin moisture and some ingredients in miso may stimulate the increase of ceramides in the stratum corneum.

Keywords: Shinshu white miso soup; Rice malt; Skin moisture; Ceramide

Introduction

Miso, a fermented seasoning, contains several ingredients, such as soybeans and rice malt, and has been traditionally consumed as a highly nutritious food in Japan. The Japanese proverb 'three basics of miso' refers to the following three benefits of miso consumption: taste, life and beauty [1]. The amino acids in soybeans, which are the major components of miso, stimulate digestion through the action of enzymes and create balance within the digestive system. Miso contains valuable ingredients such as fatty acids, vitamins (E, B2, B12, etc.), isoflavones, lecithin and brown pigments, and it is often used as a macrobiotic seasoning [1].

In Japan, there are many other proverbs that signify miso's positive effects on health [1]. For example, there is a saying 'Miso soup kills a doctor'; those who eat miso soup every day do not fall sick often, so doctors are turned adrift [1]. In addition, the phrase 'a bowl of miso soup equals the power of 7 miles' implies that a bowl of miso soup provides inexhaustible energy to walk 7 miles [1]. Lastly, 'Miso soup is a morning detoxifier' means that a bowl of miso soup in the morning detoxifies the body [1].

Moreover, some studies have shown that miso is effective in preventing breast cancer and hypertension. According to the Japan Public Health Center (JPHC)-based prospective study, mainly conducted by the National Cancer Center, the incidence rate of breast cancer decreases by 40% in Japanese women who eat three or more bowls of miso soup per day [2]. Furthermore, sufficient intake of isoflavones after menopause markedly decreases the incidence of breast cancer. The group with the highest level of genistein had a one-third lower risk of breast cancer than the group with the lowest level of genistein. In this study, women with high isoflavone levels had a decreased risk of breast cancer [2]. A diet rich in isoflavones from soy products reduces the risk of postmenopausal

breast cancer, particularly in populations with comparatively high excretion of phytoestrogens [3]. Another study investigating possible life-style factors that may cause hypertension in normotensive subjects found that consumption of two or more bowls of miso soup per day had a significant effect on the control of high blood pressure [4]. In addition, traditional Japanese miso soup has been shown to attenuate salt-induced hypertension and subsequent organ damage in Dahl salt-sensitive rats [5]. Miso (Japanese soybean paste) soup also attenuates salt-induced sympathoexcitation and left ventricular dysfunction in mice with chronic pressure overload [6].

Improved quality of life and advances in medical treatment contribute to the longevity of our society; the average life expectancy for Japanese women has exceeded 85 years. In a survey of the elderly on 'beauty and health', almost all women wished to maintain their youth and beauty and considered both their physical and mental health as the basics of beauty. People hope to have a comfortable life, both physically and mentally, and many consume beauty-boosting foods not only for external but also for internal health and beauty. There is a proverb in Japan as follows: "three sources, five powers" of miso (in Japanese, the saying is 'Miso no miso gokyo'; "miso" is the traditional Japanese seasoning but is also the Japanese word for 'three sources'). According

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to this saying, “beauty” is one of the effects gained from consuming miso. Miso is considered rich in beauty-essential ingredients. However, to the best of our knowledge, there has been no scientific study to date on the beauty benefits of miso. The present study investigated the molecular basis of the benefits of miso. Several indicators were examined including the stratum corneum water content, skin elasticity, sebum amount and skin texture. In addition, we examined the effect of miso extracts on ceramide synthesis in skin cells. The results of these investigations are presented in this paper.

Materials and Methods

Effects of miso consumption on human cheek skin

Subjects: The principle investigator explained the objectives and details of the trial to each subject, and subjects provided written consent to participate.

Exclusion criteria: Participants were excluded from this trial if they met the following criteria: (1) develops an abnormality (any disorder that may impact the evaluations of the trial such as atopic dermatitis) on the facial regions to be examined during the course of the trial; (2) has had previous contact dermatitis caused by a cosmetic product or atopic dermatitis; (3) takes vitamins, supplements or specific foods on a daily basis that could affect the evaluations of this trial, including vitamin B2, vitamin B6, vitamin C, tranexamic acid and skin-care products (collagen drinks, ceramide products, soybean- or soymilk-related products and konjac-related products); (4) regularly consumes foods fortified with the ingredients related to this trial (rice malt and soybean); (5) is unable to limit the intake of products containing rice malt, soybean and miso; (6) currently takes a medication every day for a disease or history of a serious disease requiring drug administration; (7) carries a risk of allergic reaction during this trial; (8) has significantly different test result(s) from the standard value at baseline before miso consumption; (9) is participating in another clinical trial at the beginning of this trial; (10) is pregnant; and (11) is deemed inappropriate for participation by the investigators for other reasons.

Informed consent from the subjects: The principal investigator explained the objectives and details of this trial to each subject, and subjects provided written consent to participate.

Regions to be examined: Skin examinations (stratum corneum water content, elasticity, sebum amount and skin texture) were performed on the cheek and outer corner of the eye. In addition, the subjects completed self-assessments of specific skin conditions.

Feeding methods: The subjects were provided miso soup sets for two weeks. One bowl of miso soup can be made by adding 160 cc of hot water to packets of Shinshu white miso and dried ingredients. Table 1 shows the composition of the trial food (per meal). In order to adjust to the same salt concentration as in the Miso-consuming group, salt was added to the Non-miso-consuming group (control). The ingredients of the food used in this experiment were not indicated on the packaging. The package of sample was not labelled, however, it managed which sample was made taking to eat in a subject's number.

Room temperature and humidity at the time of examination: Room temperature and humidity were measured using a thermohygrometer (A&D Co., Ltd., Tokyo, Japan) during the examination of each participant before and after the 2-week consumption. In Figure 1, data show that there was no significant difference in the temperature or humidity at the time of skin examinations between the two groups before and after the 2-week trial period.

Ingredients (g)	Miso-consuming group	Non-miso-consuming group (control)
Shinshu white miso	13.83	0.00
Water	2.71	9.78
Alcohol	0.81	0.84
Soup stock	0.60	0.60
Salt	0.02	1.75
Sodium L-glutamate etc.	0.34	0.34

Table 1. Composition of the trial food (per meal).

The table shows the composition of both the miso-containing and non-miso-containing (control) foods. Only Shinshu white miso was used. To obtain the same salt concentration of the food eaten by the miso-consuming group, salt was added to the food of the non-miso-consuming group (control). The water and alcohol in both trial food was set at the same concentration. The participants consumed the soup with their preferred dried ingredients, either seaweed containing seaweed, wheat-containing fu (bread-like dried wheat gluten), leek, antioxidant (vitamin E) and pH regulator, or long leek containing leek and seaweed.

Grouping and measuring methods: Intake of food containing miso was limited for at least one week prior to obtaining the baseline skin measurements. The stratum corneum water content, skin elasticity, sebum amount and skin texture of the cheek and outer corner of the eye were determined using specified devices after the subjects washed their faces using a solid soap and waited for 30 minutes at room temperature of $20.6 \pm 0.5^\circ\text{C}$ with relative humidity of $34.2\% \pm 1.4\%$. These measurements were used to select a pair of participants with identical skin parameters and classify them into two separate groups with no difference in the skin parameters. The subjects were divided into two groups, the miso-consuming group and the non-miso-consuming group. After two weeks, the skin parameters (stratum corneum water content, skin elasticity, sebum amount and skin texture) were determined in the same manner as described above. The technicians performing the measurements were blinded and had no way of identifying if a subject belonged to the miso-consuming or non-miso-consuming group.

Skicon-200EX (IBS Japan Co., Ltd, Shizuoka, Japan), Cutometer MPA 580 (Courage + Khazaka electronic GmbH Electronic GmbH, Köln, Germany), Sebumeter (Courage + Khazaka electronic GmbH) and Digital Microscope VHX-100 (Keyence corporation) were used to determine the stratum corneum water content, skin elasticity, sebum amount and skin texture, respectively. The stratum corneum water content was independently measured and recorded six times for each subject. Skin elasticity and amount of sebum were also measured and recorded three and four times, respectively. Skin texture was evaluated using images of the surface of the skin that were magnified 50 times, and the texture was rated on a VAS (visual analogue scale) from 1 to 4. The subjects completed self-assessments of the skin's smoothness for make-up application, moisture, radiance (shininess) and translucence on a VAS from 0 to 10.

The Skicon-200EX device was used to measure conductance (unit: μS) in a high-frequency alternating current to determine the water content of the stratum corneum (stratum corneum water content). Conductance refers to the ability of current to flow between metal electrodes in contact with the skin. In short, this measurement uses the ability of electricity to flow through water in the human body. Thus, an increase in the stratum corneum water content leads to higher conductance (μS).

The Cutometer MPA 580 was used to determine skin elasticity by non-stop suction and sudden release of negative pressure. In this trial, the elastic ratio (overall elasticity = U_a/U_f) was set as the parameter to

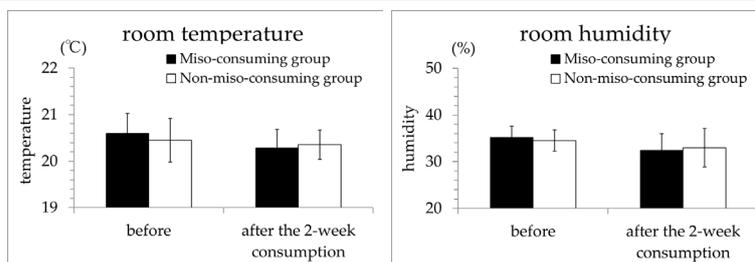


Figure 1: Room temperature (left) and room humidity (right) at the time of examination. The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups before and after the 2-week trial period (n=14).

measure skin elasticity and was defined as the immediate retraction of the skin after sudden release of negative pressure (U_a) to the maximum skin suction height after non-stop suction using negative pressure (U_f). An increase in skin elasticity is indicated by a higher elastic ratio.

Restrictions: During the study, the subjects were required to adhere to the following: (1) avoid exaggerated sunburn (apply a sunscreen when going out for long periods of time); (2) refrain from excessive exercise, dieting or overeating and try to obtain adequate sleep; (3) comply with the prescribed trial rules; (4) eat three bowls of the specified miso soup every day for a designated period and avoid consuming any other miso-containing products; (5) try not to consume other products made of soybeans or rice malt; (6) squeeze out the packet of the trial food completely and eat everything in the bowl; and (7) avoid consumption of alcohol, obtain adequate sleep and eat an adequate diet on the day of the skin examinations.

Premature termination of the trial: In cases of premature termination of the trial due to development of a skin abnormality or for any other reason, the date and reason(s) of termination were recorded.

Handling of trial cases: Data were excluded from the analysis in the following cases:

- (1) The subject discontinued the study because they
 1. Voluntarily withdrew from the trial for any reason or
 2. Could not continue participation in the trial because of health issues.
- (2) The subjects were eliminated if they seriously violated the trial protocol.

Ethics: This trial was conducted with the approval of an ethics committee. (1) The prospective subjects' health conditions, age, ability to provide consent, participation in another clinical trial, and the exclusion criteria per the study protocol were carefully considered, which were in line with the objectives of this trial from an ethical viewpoint. If a potential subject may unduly incur any disadvantages from participation in the trial or if the subject considered refusing participation in the trial, the options were carefully discussed, and the consent was obtained only if the subject voluntarily decided to participate. (2) The principal investigator was required to take necessary measures to ensure that adequate medical care was provided to a subject in the case of any adverse events. In addition, the principal investigator was required to compensate the subject for any trial-related adverse events; however, this was not applicable if the subject was deemed at fault for the adverse event or if the adverse event was deliberate in nature. (3) Personal information was carefully protected by managing identifiable data and study documents separately in electronic documents.

Effect of miso extracts on ceramide synthesis in cultured epidermal keratinocytes

Preparation of miso extracts: After 45 g of ethanol (99.5%) was added to 5 g of Shinshu white miso, the mixture was kept in the dark at room temperature for one week. The extract essence was filtered, and a rotary evaporator was used to distill the solvent and obtain a solid extract. The solid extract was then dissolved in 40 mg/mL dimethyl sulfoxide (DMSO) and diluted to 4 mg/mL and 0.4 mg/mL.

Culture of human epidermal keratinocytes: A carbon dioxide incubator was used to culture and grow human keratinocytes with 10% FBS-DMEM in a 75-cm² flask. Human keratinocytes were seeded at 10,000 cells/well in each 96-well plate and cultured for a day in DMEM GlutaMAX (Life Technologies Japan, Tokyo, Japan) medium containing 2% bovine serum (2% FBS-DMEM). After 1 μ L of the miso extract prepared according to the specific densities or DMSO was added and cultured for three days in a carbon dioxide incubator, the number of cells and β -glucocerebrosidase (β GCase) activity were determined. To determine the mRNA expression of ceramide synthases and the amount of ceramides, human keratinocytes were seeded at 300,000 cells/well and 2,000,000 cells/well in 35-mm and 100-mm dishes, respectively. After they were cultured for a day in DMEM GlutaMAX medium containing 2% bovine serum (2% FBS-DMEM), 1 μ L of the miso extract prepared according to the specific densities or DMSO was added and cultured for three days in a carbon dioxide incubator.

Measurement of the number of cells: Cell Counting Kit-8 (Doujindo Laboratories) was used to identify the number of cells in each well, and 10 μ L of the Cell Counting Kit solution was added to the wells. After 2 hours of reaction in a carbon dioxide incubator, the absorbance was determined at 450 nm using a microplate reader.

mRNA expression of ceramide synthases: mRNA expression of enzymes related to ceramide synthesis, including serine palmitoyltransferase 2 (SPTLC2), acid sphingomyelinase (aSMase) and β GCase, were examined in cultured human keratinocytes. Glycerinaldehyde-3-phosphate dehydrogenase (G3PDH) was used as the control housekeeping gene. RNA extraction was performed using the RNeasy Protect Mini Kit (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions.

RT-PCR was performed according to the manufacturer's instructions using One Step SYBR[®] Prime Script[™] RT-PCR Kit II (Takara Bio Inc., Shiga, Japan). A reaction solution was prepared with the reagents provided in the kits and stored on ice. Furthermore, 2 μ L/well of the forward and reverse primers and 4 μ L/well of the extracted sample were introduced in a 96-well plate for PCR. Finally, mRNA expressions were determined using real-time PCR (ABI PRISM

7900HT, Applied Biosystems Co., Ltd., Forster City, CA, USA) after 42 μ L of the reaction solution was added. The primers used are listed in Table 2.

Measurement of β GCCase activity: β GCCase activity was determined using 4-methylumbelliferyl β -D-glucopyranoside [7]. The human keratinocytes cultured for three days in the 96-well plate were washed twice with PBS, and 30 μ L of 0.01 mol/L acetate buffer (pH 5.0) and 40 μ L of a 5 mmol/L 4-methylumbelliferyl β -D-glucopyranoside solution were added. After 1 hour of reaction in an incubator, 50 μ L of 0.1 mol/L glycine sodium hydrate buffer solution (pH 10.7) was added to determine the fluorescence using a microplate reader. The measurement was performed at 360 nm of excitation wavelength and 460 nm of fluorescence wavelength.

Measurement of ceramides: Quantification of ceramides was performed using sphingolipid ceramide N-deacylase (SCDase; Takara Bio Inc.) [8,9]. Ceramides were dissolved at 0.02 and 0.2 μ g/mL in the medium for the 3D-cultured human epidermal model (LabCyte EPI-MODEL24; Japan Tissue Engineering Co., Ltd., Aichi, Japan). In addition, 500 μ L of the medium was added separately and cultured in a CO₂ incubator at 37°C for three days. After removing the medium and washing the 3D-cultured human epidermal model with PBS, they were transferred into micro-test tubes, and 400 μ L of a solvent for lipid extraction (chloroform:methanol = 2:1) and 20 μ L of 10 μ mol/L C17-sphingosine (internal standard) were added and maintained at 37°C for 2 hours. Centrifugal separation was performed after vortexing and ultrasound processing to collect the supernatants. The collected supernatants were dried using a centrifugal thickener. Furthermore, 27 μ L of a sodium acetate buffer solution containing 5 mmol/L CaCl₂ and 2% Triton-X-100 and 2 μ L of SCDase (200 mU/mL) were added to the lipid extracts. After 1 hour of reaction at 37°C, 200 μ L of chloroform-methanol mixtures (2:1) were added to stop the reaction. Centrifugal separation was performed again after adding and stirring 15 μ L of pure water to collect the lower layers and distill the chloroform-methanol mixtures using a centrifugal thickener. To re-dissolve the lower layers, 120 μ L of ethanol was added. Furthermore, 15 μ L of ortho-phthalaldehyde solution [0.5 mg/mL, boric acid (3% (w/v), pH 10.5), 0.5 μ L 2-mercaptoethanol] was added, and the reaction was continued for 1 hour at 70°C under light-shielded conditions. The number of ceramides in the samples was determined using high-performance liquid chromatography (HPLC; LC6 system, Shimadzu Co., Kyoto, Japan) under the conditions as outlined below. For the samples, phytosphingosine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), D-sphingosine (LKT Laboratories, Inc., St. Paul, MN, USA) and D-erythro-dihydrosphingosine (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) were used.

Preparation of the OPA solution: Ten milligrams of ortho-phthalaldehyde (OPA) was dissolved in 0.1 mL of ethanol and 20 μ L of 2-mercaptoethanol. Subsequently, 9.9 mL of 3% (w/v) boric acid buffer solution (pH 10.5) was added and stirred, and the solution was incubated at 70°C for 20 minutes. Light was blocked using aluminum

foils.)

HPLC measurement conditions: Column used: CAPCELLPAK C18 (Shiseido Co., Ltd., Tokyo, Japan), Fluid velocity: 1 mL/min

0–20 min: methanol 80%, 0.1% acetic acid 20%

20–25 min: methanol 100%

25–35 min: methanol 80%, 0.1% acetic acid 20%

Detection wavelength: excitation 335 nm, emission 440 nm

Statistical methods

Measured values were analyzed for significant differences using a t-test function (two-tailed) in Microsoft Excel. Paired t-tests were performed to compare the means and the Δ values of the means from two paired samples. In the paired t-test, a two-sided p value <5% was considered significant (': $p < 0.05$, '": $p < 0.01$, '": $p < 0.001$) and $p \geq 5\%$ was considered not significant (n.s.). In addition, the paired t-test was performed to evaluate the VAS scores from the self-assessments by the subjects. In this test, a two-sided p value <5% was considered significant (': $p < 0.05$, '": $p < 0.01$, '": $p < 0.001$), 5% < $p < 10\%$ was considered marginally significant (': $p < 0.10$), and $p > 10\%$ was considered not significant (n.s.).

Results

Effects of miso consumption on human cheek skin

Analyzed subjects: Each group had 14 participants, and no dropout was noted. In addition, data on water content, skin elasticity, amount of sebum, and the skin texture of the participants in each group were analyzed.

Water content of the stratum corneum: Figure 2 shows the variations in the stratum corneum water content of the cheek and outer corner of the eye for the miso-consuming and non-miso-consuming groups. The stratum corneum water content of the cheek and outer corner of the eye increased by 1.4 and 1.2 times ($p < 0.05$ and $p < 0.1$), respectively, in the miso-consuming group. For the non-miso-consuming group, no significant difference was observed for the cheek, while the stratum corneum water content of outer corner of the eye was significantly reduced ($p < 0.05$). Furthermore, the Δ values in the stratum corneum water content of the cheek and outer corner of the eye over the two-week trial period were significantly different between the miso-consuming and non-miso-consuming groups ($p < 0.05$ and $p < 0.01$, respectively) (Figure 3).

Skin elasticity: Figure 4 shows the variations in the elasticity of the skin of the cheek and outer corner of the eye for the miso-consuming and non-miso-consuming groups. The subjects of the miso-consuming group showed a significant ($p < 0.05$) increase in the elasticity of the skin in outer corner of the eye, whereas the subjects of the non-miso-consuming group did not show a significant difference in elasticity before and after consumption of miso. Regarding skin elasticity of the cheek, no significant differences were observed before and after consumption of miso in both groups. In addition, the Δ values in the elasticity of the skin of the cheek and outer corner of the eye over the two weeks were not significantly different between the two groups (Figure 5).

Amount of sebum: There was no significant difference in the sebum amount in the skin of the cheek or outer corner of the eye between the miso-consuming group and the control group before and after consumption of miso for two weeks (Figure 6). The inter-individual variations in the sebum amount over the two weeks were not significantly different between the two groups (Figure 7).

mRNA	Sequence
human SPTLC2	Forward 5'-AGCCGCCAAAGTCCTTGAG-3' Reverse 5'-CTTGTCCAGGTTTCCAATTTCC-3'
human aSMase	Forward 5'-GCTGGCTCTATGAAGCGATGGC-3' Reverse 5'-AGAGCCAGAAGTTCTCACGGGA-3'
Human β GCCase	Forward 5'-TGGCATTGCTGTACATTGG-3' Reverse 5'-CGTCTCTGACTGGCAACC-3'
human G3PDH	Forward 5'-ATGGTGGTGAAGACGCCAGT-3' Reverse 5'-GCACCGTCAAGGCTGAGAAC-3'

Table 2. Primer names and sequences.

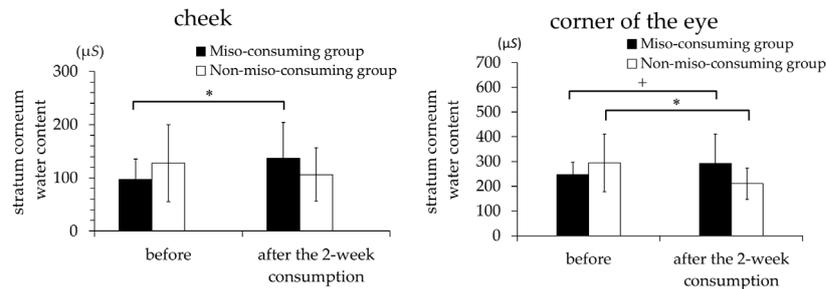


Figure 2: Increase in the stratum corneum water content in the skin of the cheek (left) and outer corner of the eye (right) because of miso consumption is shown in the figures. The water content in the stratum corneum was determined based on the electrical conductivity (μS). The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze between the miso-consuming and non-miso-consuming groups before and after the 2-week trial period ($n=14$): $^* p < 0.1$, $^* p < 0.05$.

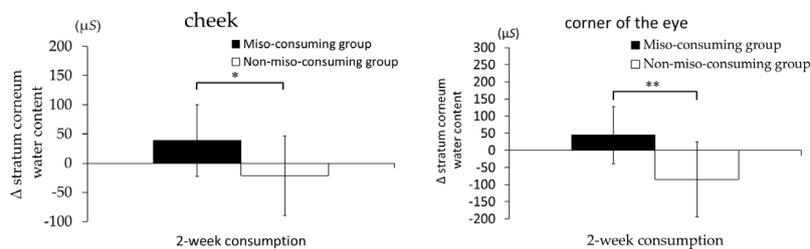


Figure 3: Variations in the stratum corneum water content in the skin of the cheek and outer corner of the eye because of miso consumption. The figures show the changes in the stratum corneum water content obtained by subtracting the values before the consumption from the values after the consumption, for the cheek and outer corner of the eye (left and right, respectively). The stratum corneum water content was determined by the electrical conductivity (μS). The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups ($n=14$): $^* p < 0.05$, $^{**} p < 0.01$.

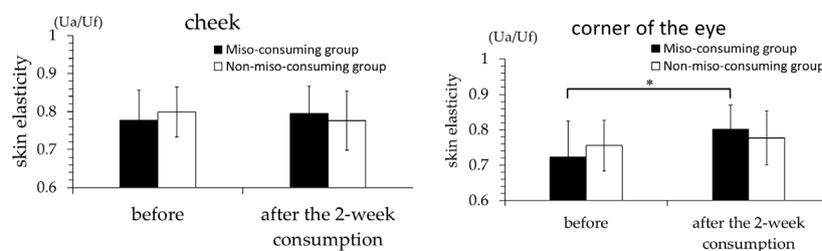


Figure 4: Increase in skin elasticity of the cheek (left) and outer corner of the eye (right) because of miso consumption is shown in the figures. The values of elasticity were determined from the ratio of immediate retraction after suction, U_a/U_f (R2). The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups before and after the 2-week trial period ($n=14$): $^* p < 0.05$.

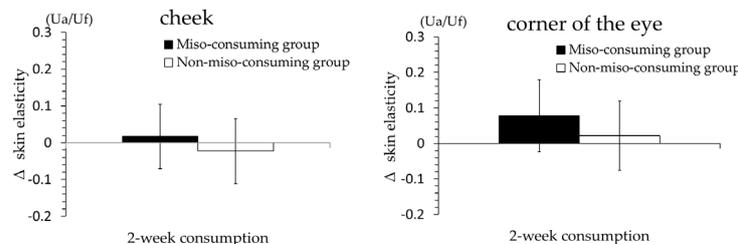


Figure 5: Variations in skin elasticity of the cheek and outer corner of the eye because of miso consumption. The figures show the change in skin elasticity obtained by subtracting the values before consumption from the values after consumption, for the cheek and outer corner of the eye (left and right, respectively). The values of elasticity were determined from the ratio of immediate retraction after suction, U_a/U_f (R2). The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups ($n=14$).

Evaluation of skin texture: Figure 8 shows the mean VAS scores and standard deviations for skin texture of the cheek and outer corner of the eye for the miso-consuming and non-miso-consuming groups. The miso-consuming group showed significantly improved skin texture of the cheek and outer corner of the eye compared to the baseline ($p < 0.01$ and $p < 0.05$, respectively), whereas the control group did not show any significant differences. Furthermore, the Δ values in the skin texture of the cheek and outer corner of the eye over the two weeks were significantly different between the miso-consuming and non-miso-consuming groups ($p < 0.01$ and $p < 0.05$, respectively) (Figure 9).

Effect of miso extracts on the ceramide synthesis in cultured epidermal keratinocytes

mRNA expression of ceramide synthases: Miso extracts (0.2 μ g/

mL) were prepared to examine the effect of miso on ceramide synthesis in epidermal keratinocytes using cultured cells. The findings revealed that miso extracts increased mRNA expression of β GCase, an enzyme involved in ceramide synthesis in the stratum corneum (Figure 10).

Measurement of β GCase activity: Miso extracts (0.02, 0.2 μ g/mL) were prepared to examine the effect of miso on ceramide synthesis in epidermal keratinocytes using cultured cells. The findings revealed that miso extracts increased β GCase activity (Figure 11).

Measurement of ceramides: Miso extracts (0.02, 0.2 μ g/mL) were prepared to examine the effect of miso on ceramide synthesis in epidermal keratinocytes using the 3D epidermal model. The findings revealed that miso extracts increased the phytosphingosine- and sphingosine-type ceramides (Figure 12).

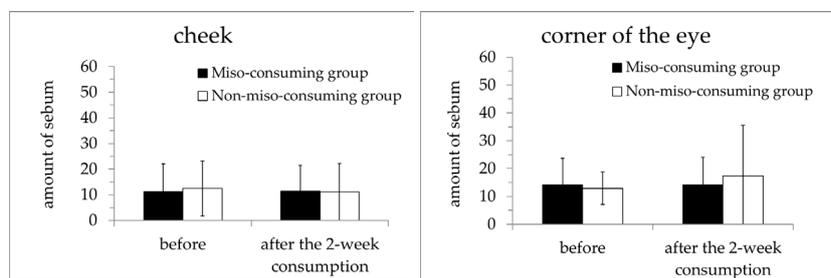


Figure 6: The amount of sebum on the skin of the cheek (left) and outer corner of the eye (right) because of miso consumption is shown in the figures. The relative amount of sebum on the skin surface was measured by the Sebumeter. The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups before and after the 2-week trial period (n=14).

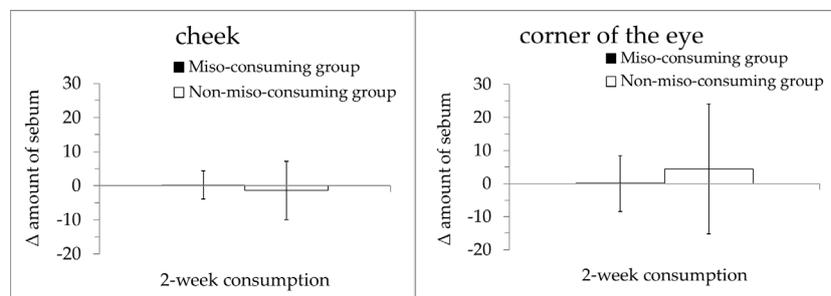


Figure 7: Variations in the amount of sebum on the skin surface of the cheek and outer corner of the eye because of miso consumption. The figures show the changes in the amount of skin surface sebum obtained by subtracting the values before consumption from the values after consumption, for the cheek and outer corner of the eye (left and right, respectively). The relative amount of sebum on the skin surface was measured by the Sebumeter. The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups (n=14).

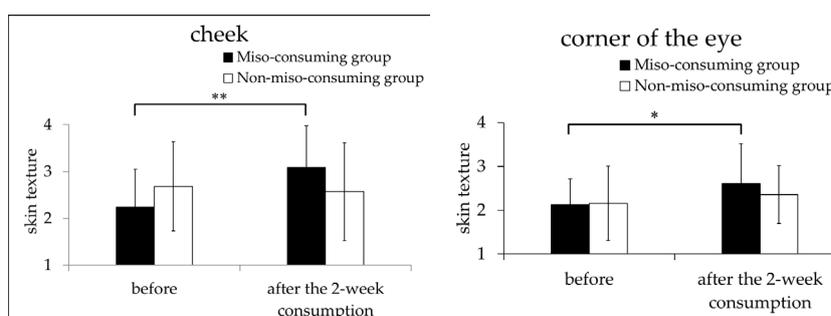


Figure 8: Improvement in skin texture of the cheek (left) and outer corner of the eye (right) because of miso consumption is shown in the figures. The texture was evaluated on a 4-point VAS using images of the skin. The measurements are presented as mean value \pm standard deviation. A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups before and after the 2-week trial period (n=14). *: $p < 0.05$, **: $p < 0.01$.

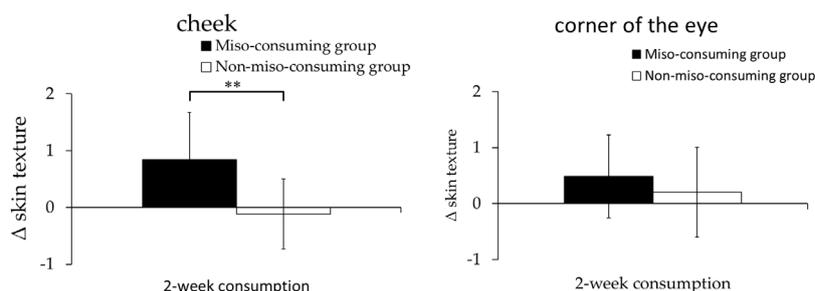


Figure 9: Variations in skin texture of the cheek and outer corner of the eye due to miso consumption. The figures show the change in skin texture obtained by subtracting the values before the consumption from the values after the consumption (left and right, respectively). The texture was evaluated on a 4-point VAS using images of the skin. The measurements are presented as mean value \pm standard deviation. The significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups (n=14). *: $p < 0.01$.

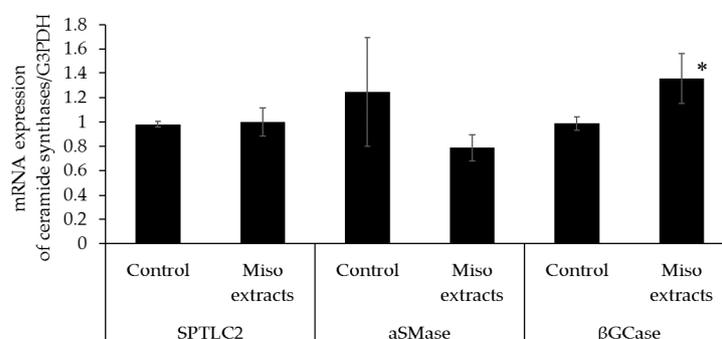


Figure 10: The effect of miso extracts on mRNA expression of ceramide synthases. Keratinocytes were cultured for three days to examine the effect of miso extracts (0.2 μ g/mL) on the mRNA expression of enzymes involved in the synthesis of ceramides in the stratum corneum. The figure shows mRNA expression of serine palmitoyltransferase 2 (SPTLC2), acid sphingomyelinase (aSMase) and β -glucocerebrosidase (β GCCase) compared to the mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The measurements shown are the mean value \pm standard deviation. *: $p < 0.05$, n = 3.

Discussion

The stratum corneum is important for skin moisturization and constitutes a barrier from the environment. Moisturization of the skin is defined by the skin's capacity to retain water in the stratum corneum to protect the skin from becoming dry, and the barrier function of the skin keeps out irritants and prevents loss of moisture from the skin. In this study, we clarified, for the first time to our knowledge, that women who consumed miso soup had a significantly higher stratum corneum water content and that their skin elasticity and texture improved compared with those who did not consume miso soup. There was no significant difference in the sebum amount between the two groups.

The subjects did not experience any skin problems during the trial period. Beautiful skin is defined as skin that is "elastic and shiny", "moisturized and translucent" and "pimple-, spot- or freckle-free". The skin consists of the epidermis, dermis and subcutis. The epidermis completely regenerates every 4 to 6 weeks. The stratum corneum of the epidermis specifically plays a role in skin moisturization, and epidermal turnover is essential to maintain the quality of the stratum corneum. For instance, in the case of skin inflammation due to long exposure to sunlight, the 4- to 6-week cycle may be shorter as the epidermis undergoes repair. A shorter turnover time leads to the incomplete regeneration of the corneocytes and lack of intercellular lipids in the stratum corneum. This weak epidermal barrier often causes dry skin and skin irritation. Conversely, if the epidermal turnover is delayed

because of aging or cold weather, the old stratum corneum is retained over a longer period and makes the skin appear dull and devoid of smoothness and shininess. This means that normal skin with a regular turnover cycle leads to moisturized, smooth and shiny skin with a smooth texture and a bright tone.

Maintaining a healthy body is important. The skin constitutes the barrier of the human body from the external environment and is the farthest from the center of the body. Therefore, it is essential to include beauty-boosting nutrients in the daily diet and provide stable delivery of nutrients to the skin. The proverb, "three sources, five powers" of miso defines beauty as one of the three constituents. To verify the scientific basis of this saying, this study compared the skin properties between miso-consuming subjects and non-miso-consuming subjects. Our results showed that women in the miso-consuming group had higher water content in the stratum corneum and improved skin elasticity and texture compared to those in the non-miso-consuming group. Figure 13 shows the mean VAS scores and standard deviations of the self-assessments for skin smoothness for make-up application, moisture, translucence and radiance (shininess) of the 14 female subjects in the miso-consuming and non-miso-consuming groups. The scores from the subjects in the miso-consuming group significantly improved ($p < 0.05$) in terms of skin smoothness for make-up application, moisture, skin radiance (shininess) and translucence compared to those in the miso-non-consuming group ($p < 0.01$ and $p < 0.05$, respectively). The subjects' self-assessments of the skin parameters using the VAS revealed that

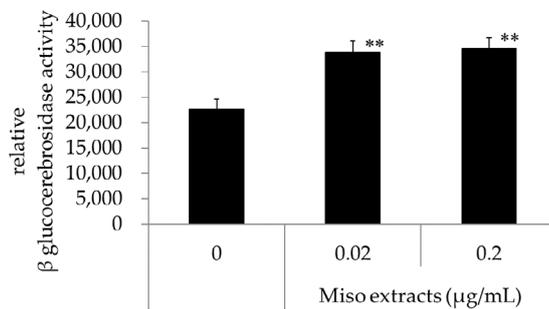


Figure 11: The effect of miso extracts on β GCCase activity. Keratinocytes were cultured for three days to examine the effect of miso extracts (0.02, 0.2 $\mu\text{g/mL}$) on β GCCase activity. The procedure for determining β GCCase activity is described in the Materials and Methods section. The measurements shown are mean value \pm standard deviation. **: $p < 0.01$, $n = 3$.

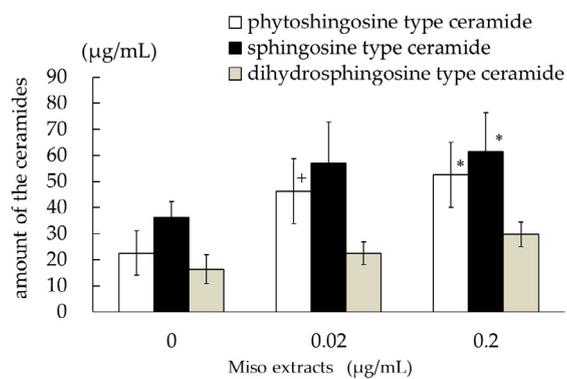


Figure 12: The effect of miso extracts on the amount of ceramides in the epidermis using the 3D epidermal model. The 3D epidermal model was cultured for three days to examine the effect of miso extracts (0.02, 0.2 $\mu\text{g/mL}$) on the phytosphingosine-, sphingosine- and dihydrosphingosine-type ceramides. The measurements shown are mean value \pm standard deviation. *: $p < 0.1$, **: $p < 0.05$, $n = 3$.

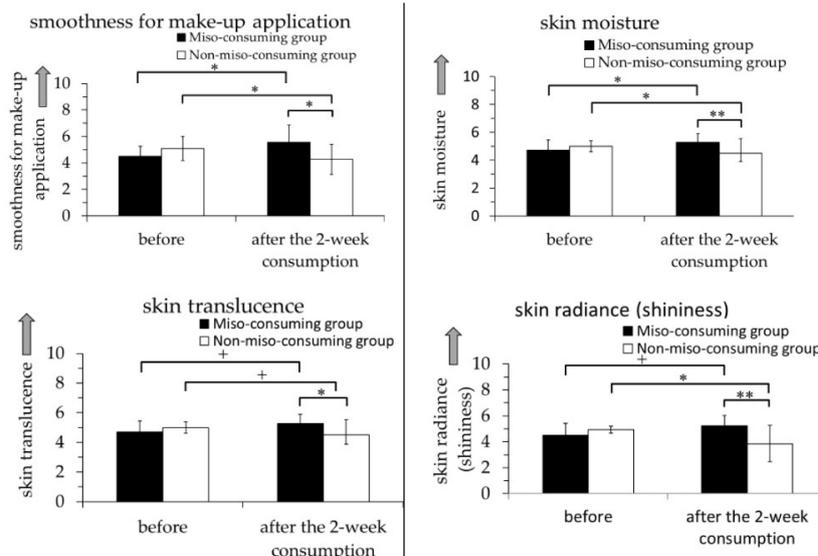


Figure 13: Self-assessments after miso consumption for skin smoothness for make-up application, moisture, translucence and radiance (shininess). The figures are shown as follows: the skin is smooth for make-up application (upper left), the skin is moisturized (upper right), the skin is translucent (lower left) and the skin is shiny (lower right). A significance test (t-test) was used to analyze the differences between the miso-consuming and non-miso-consuming groups before and after consumption. The measurements are presented as mean value \pm standard deviation ($n=14$). *: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.01$.

the intake of miso improved skin smoothness for make-up application, moisture, radiance (shininess) and translucence. The daily quantity of the rice miso used in this trial was 41.49 g, and the subjects consumed three bowls of typical miso soup in a day. Intake of miso soup in the daily diet may help maintain beautiful skin.

The intercellular lipids of the stratum corneum play a role in the moisture and barrier functions of the skin and are composed of 50% ceramides, 15% fatty acids, 10% cholesterol and 10% cholesterol ester with the ceramides playing a key role in the barrier function. Ceramides belong to a large molecular family and have different chemical structures. They are linearly arranged in the lipid bilayer, and the interlayer has the capacity to hold water within the stratum corneum [10-13]. Ceramides are generated from the following process: SPTLC2 catalyzes the reaction between serine and palmitoyl-CoA to form 3-ketosphinganine, and subsequently, it generates sphingomyelin, glucosylceramide and acylglucosylceramide in the granular cells with the acyl group of non-hydroxy fatty acid, α -hydroxy fatty acid and esterified ω -hydroxy fatty acid. Glucosylceramide and acylglucosylceramide are converted to ceramides by β GCase and are secreted into the extracellular space to form the intercellular lipids of the stratum corneum [14]. In addition, the plasma membranes in the stratum corneum are thick and are reinforced by the structures beneath the membranes, which are called resistant plasma membranes (cornified cell envelope: CE). Outside the CE required for cornification, ω -hydroxyceramides are linked to form the stratum corneum barrier. As described above, ceramides in the normal stratum corneum play an important role in maintaining the moisture and barrier functions of the skin [15,16]. Ceramides in the stratum corneum are classified into phytosphingosine-type, sphingosine-type and dihydrosphingosine-type [17-19]. Therefore, we investigated the effect of miso extracts on the expression of enzymes for ceramide synthesis and on the activity of the enzymes that were specifically induced by miso extracts. This trial showed that miso extracts increase mRNA expression and the activity of β GCase. Furthermore, this trial showed that miso increases the production of phytosphingosine- and sphingosine-type ceramides in the cultured 3D epidermal model.

In the miso-brewing process, the enzymes from a wide range of anaerobic microorganisms play various functional roles. For example, rice miso is rich in functional substances such as essential fatty acids (linoleic acid and linolenic acid) and acylphosphatidic acid produced by the rice malt bacteria (*Rhizopus oryzae*) [20]. Because isoflavones in soybeans control cell growth and induce apoptosis, they are expected to inhibit the growth of cancer cells. Miso is prepared from fermented soybeans, which makes it unique from original soybeans. In particular, mature miso has been considered to have a greater effect compared to isoflavones because isoflavone glucosides are converted to aglycones. Moreover, the fermenting and maturing processes reduce allergens in soybeans or rice and improve miso's antioxidant effects.

The results of this study clearly showed that the daily intake of miso soup can improve skin moisture. Furthermore, certain miso ingredients produced by fermenting both rice and soybean stimulate ceramide synthesis in the stratum corneum. We are now investigating the active components in miso and fermented rice that can improve skin moisture.

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Authors' Contributions

Kazuhiisa Maeda designed the study and performed the experiments as well as the data analysis. Kazuhiisa Maeda interpreted the data and drafted the manuscript. Keiji Nakata, Ayano Nakamura, Manabu Kitagawa and Seiki Ito were engaged in manufacture of the test samples.

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