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Original article

The function of amino acid mix foods in women with skin aging concerns: An exploratory study

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Abstract

***Objective*:** Firmness and elasticity of skin and sagging skin are great concerns for middle- and advanced-aged women. We conducted an exploratory study on the effectiveness of amino acid mixtures for these issues.

***Method*:** The research participants, 24 women with low levels of skin elasticity measurements (R7) and high levels of a glycative stress marker, skin autofluorescence (SAF), were chosen from 77 potential female participants who were aware of skin deterioration, *e.g.,* loss of elasticity and sagging. The 24 participants were classified into two groups, the His-Thr group and Orn-Thr group (each group: 12 women). Oral ingestions of amino acid mixtures were administrated for 8 weeks, with each group receiving: 1.65 g of His (histidine), 0.8 g of Orn (ornithine) and 1.65 g of Thr (threonine). Effects of skin elasticity, SAF, moisture retention (skin moisture and trans epidermal water loss [TEWL]) and skin color difference were examined after the 8-week oral administration. Both groups, the His-Thr group and Orn-Thr group, showed significant improvements in skin-related subjective symptoms, and indicators of skin elasticity, SAF and TEWL.

***Results*:** The His-Thr group significantly improved lightness assessments (L^*) in color difference tests and assessments of dry skin levels by doctors in comparison with the Orn-Thr group. It was judged that there were no safety issues as no adverse events were reported. Furthermore, both groups showed increases in plasma pentosidine concentration. Pentosidine is a product formed in the blood by the reaction of aldehydes with amino acids, peptides, or sugars. During this process, aldehyde modification of proteins can be prevented inside and outside vascular endothelial cells, and therefore it can thought that exogenous amino acids exert a bioprotection effect.

***Conclusion*:** The present study suggested possibilities that amino acid supplementation had favorable effects on the firmness, elasticity, and anti-sagging of skin for middle- and advanced-aged women. His is an amino acid required to maintain homeostasis of filaggrin, which is known for wound healing and moisturizing. We will aspire to conduct and develop further research on the administrations of His and Orn mixtures.

***KEY WORDS*:** skin moisturizing, elasticity, histidine, filaggrin, glycation

Introduction

Firmness and elasticity of the skin and sagging skin are great concerns for middle- and advanced-aged women. Aging changes in the skin include wrinkles, age spots, sagging, dull aspects, loss of firmness and elasticity, and radiance. Therefore, anti-skin aging to maintain young and beautiful skin would lead to a healthy life relieving their anxiety. It is recognized that glycative reactions are significantly related to skin aging.

The perceptions regarding glycative stress (GS) in humans have been changing [1,2]. At first, it was considered that reactions similar to the Maillard reaction could occur in tissues and organs of the human body; Maillard reaction, which occurs in food, is a chemical reaction between protein and reducing sugars with non-enzymatic browning to create glycative intermediates, which lead to the formation of advanced glycation end products (AGEs). Along with developed research, it has been confirmed that glycation in the human body is different from Maillard reaction in

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food. Glycation results from the excessive formation of carbohydrate-derived aldehydes and/or fatty-acid-derived aldehydes in the body. The aldehyde group of a ring-opening glucose, which is exposed in reducing glucose, and amino residue of proteins react to each other to form Schiff bases, which are basically similar reactions (reactions between aldehyde group and amino group). Aldehydes which are formed in the blood vessel permeate cell membranes, react with intercellular and extrasellar biological proteins, and induce post-translational modifications of proteins. AGEs are formed and deposited. Aldehydes react with lipid and DNA bases. As a result, various types of damage to tissues and cells are induced.

Glycation of protein, particularly in the skin, could cause collagen cross-link deficiency[3]. This is one of the reasons for sagging skin. As for food that acts against sagging skin, only "collagen," which is a material for skin collagen, is a major product. There is no food to protect glycation, which causes sagging. Therefore, it is required to identify functional foods to inhibit glycative reactions.

Previous studies[4,5] explored effective amino acid foods against glycation, which causes skin sagging, and detected two types of amino acid-rich food mixtures: a mixture of L-histidine (His) and L-threonine (Thr), and a mixture of L-ornithine hydrochloride (Orn) and Thr. It was confirmed *in vitro* reactions that these amino acid mixtures significantly suppressed the formation of pentosidine, which is an elasticity-decline-related glycated protein via the inhibition of collagen cross linking[6].

The following explanations are the validity of volumes contained in the two mixtures of this trial: L-carnosine, which is a type of imidazole dipeptides, is a dipeptide consisting of His and β-alanine. It was confirmed that consecutive administrations of L-carnosine of 1 g per day to humans significantly decreased serum pentosidine concentrations. Improvements in anti-glycation indicators in humans have been reported[7].

Many studies of *in vitro* and *in vivo* evaluation systems have reported anti-glycation effects of L-carnosine. The glycation inhibition ratio was approximately 50% in the *in vitro* evaluation system. The anti-glycation effects are higher via His than via L-carnosine, as is reported[8]. Therefore, it was assumed that the anti-glycation activities exerted in the present study would at least be equal to L-carnosine level of improvement and effectiveness regarding the human research evaluation indexes; the carnosine ingestion as the volume of His in the present study was 1.65 g per day, which was more than that of His in the above research.

Furthermore, the addition of Thr in our *in vitro* examination indicated increased effectiveness via synergistic effects than the single ingestion of His. The mixture of Orn and Thr had the same synergistic effects as the above-mentioned mixture[4,5].

As 1 g of administration of amino acid exerted anti-glycation effects *in vitro*, it was assumed that 1.65 g ingestion of His and Thr and 0.85 g of Orn per day would improve human evaluation indexes.

Moreover, anti-glycation effects in *in vitro* examinations are reported to affect not only anti-glycation indicators in humans but also index indicators of the skin in the study as follows.

The present study referred to previous research which was used for the pharmaceutical application of "Rhodanthenone B" (Nippon Shinyaku Co., Ltd.); "Rhodanthenone B" excels as a food with functional claims for skin quality improvement via the reduction of GS[9].

The purpose of this study was to clarify that the test foods (the food A of amino acid mixture and the food B of amino acid mixture) with a defined ingestion amount once a day would exert effectiveness on inhibitive activities of the pentosidine formation and skin-aging-related indexes.

Targets and Methods

1. Implementation structure of the trial

The present trial was conducted with deliberation and approval (approval number 2021-012, as of September 29th, 2021) of the Trial Review Board Committee concerning research involving human subjects of Ajinomoto Co., Inc and with deliberation and approval (approval number 2021-012, as of October 1st, 2021) of the Ethical Review Committee concerning research involving human subjects of General Incorporated Association Society for Glycative Stress Research; to ensure human rights and safety of research participants and the credibility of test data, consisting of a third party who was not related to the trial. The trial was performed from October to December of 2021 in Ueno Asagao Clinic (principal investigator of the trial: the president of the hospital, Ono Takahiro) based on the clinical trial protocol (UMIN trial ID: UMIN000045657). The present trial was conducted in compliance with ethical principles based on the Declaration of Helsinki (Amendment, 2013 WMA Fortaleza "Brazil") and Ethical Guideline for Medical and Biological Research Principles involving human subjects (Implemented in 2021, Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry. Notification: No.1).

2. Research Participants

In the recruitment of research participants, 77 participant candidates were provided with an explanation of the trial and underwent pre-screening. From these candidates who the investigator decided as appropriate participants based on the selection standard, satisfying the selection criteria and not matching the exclusion criteria, 24 research participant were selected for the present study. The number of cases was decided with grounds of setting as follows: The purpose of the present study was exploratory research. In a trial with comparison between before and after ingestion regarding primary endpoints, and between groups, 10 participates per group were required to perform statistical tests and estimations. With the consideration of risks for discontinuation/dropping out, the necessary number of cases was decided as 12 cases per group.

Key inclusion criteria for subjects as follows:

1) Women aged 40–59, when they agreed to participate in the test
2) Individuals who are healthy and do not have chronic physical illnesses, including skin disorders

3) Individuals who are aware that they have skin deterioration deterioration such as loss of firmness and sagging
4) Individuals who are fully informed regarding the purpose and contents of the test, has an ability to consent, voluntarily apply for participation with a full understanding, and agree to participate in the test with written informed consent
5) Individuals who can come on the designated examination date and receive the examination
6) Individuals judged appropriate for the study by the principal researcher

Key exclusion criteria for subjects as follows:
1) Individuals who have contracted diseases and receive medical treatment
2) Individuals who have skin disease, such as atopic dermatitis
3) Individuals who have wounds and/or inflammation at measurement sites
4) Individuals who are receiving/received medical drug treatment in the past month except for temporary relief medication for headaches, menstrual pain, common cold, *etc*.
5) Individuals who have a past or present medical history of serious diseases (uterine disease, hepatic, renal, cardiovascular, respiratory, hematologic, *etc*.)
6) Individuals who have a serious medical history of and/or current digestive disease and comorbidities
7) Individuals who have serious anemia
8) Individuals whose BMI is 30.0 kg/m^2 or more
9) Individuals who have allergic reactions to components of test foods and/or serious allergic reactions to other foods or drugs
10) Individuals who have a habit to ingest special health foods, foods with function claims, health food products and/or supplements containing collagen, His, Thr and/or Orn within the last 3 months
11) Individuals who have a habit to ingest special health foods, foods with function claims, health food products and/or supplements claiming to improve skin functions and/or anti-glycation within the last 3 months and/or are planning to ingest those foods during test periods (ingestion for health maintenance is accepted)
12) Individuals who have a habit to use medicine and/or quasi drug claiming improvements in skin functions within the last 3 months
13) Individuals who are pregnant, possibly pregnant, or are breastfeeding
14) Individuals whose alcohol intake is more than 60 g/day on average in terms of pure alcohol
15) Individuals who have psychiatric disorders
16) Individuals who have a smoking habit
17) Individuals who will have possible changes of life style during the test period
18) Individuals who will possibly develop seasonal allergy symptoms, such as pollinosis, and use an anti-allergic drug
19) Individuals who neglect skin care
20) Individuals who are unable to avoid direct sunlight exposure such as sunburn during test periods.
21) Individuals who have undergone medical treatments or beauty treatments (including beauty salons) on assessment sites within the last 6 months
22) Individuals who are participating and/or have participated in other clinical studies within the last 3 months
23) Individuals judged as not appropriate to this study by a responsible doctor.

3. Selection of research participants, randomization and blinding

From potential candidates who satisfied the inclusion criteria, did not match the exclusion criteria, and were judged as appropriate for this study by a responsible doctor at the pre-screening and pre-ingestion tests (SCR&Visit-1), 24 research participants who obtained higher total ranking scores in order, were selected for the present study through the process of the following rankings:

1) Ranking of R2 of elasticity measurement in ascending order
2) Ranking of R7 of elasticity measurement in ascending order
3) Ranking of skin autofluorescence (SAF) fluorescence advanced glycation end products (AGEs) level of AGE Reader measurement in descending order
4) Total ranking of these 3 rankings

Consequently, the stratification factors were age at the pre-screening and pre-ingestion tests (SCR&Visit-1) and total ranking at the subject selection. After randomly allocating participants into two groups by Stratified Block Randomization, it was finally confirmed that there were no significant differences between two allocated groups.

4. Test sample food

Test sample foods were two types of the test food A (His-Thr containing food) and the test food B (Orn-Thr containing food), which were provided by Ajinomoto Co., Inc. Each test food comprised two types of active ingredients. Subjects ingested test foods once a day, dissolving two types of powders in 100 ml of water or lukewarm water before intake. Ingredients of test foods are referred to ***Table 1***.

Table 1. Test food constituents.

Constituent Name	Test food A = His-Thr group (His-Thr containing food)	Test food B = Orn-Thr group (Orn-Thr containing food)
L-histidine (His)	1.65 g	—
L-threonine (Thr)	1.65 g	1.65 g
L-ornithine hydrochloride (Orn)	—	0.8 g

5. Study design

The present study employed an open trial (two groups).

Primary outcomes were skin-aging-related indicators (AGE Reader measurement with fluorescent AGEs: skin autofluorecence, SAF), skin elasticity, trans epidermal water loss (TEWL), skin moisture level, VISIA image analysis score, skin color difference measurement, observation for skin condition by dermatologists), as well as blood pentosidine levels. Furthermore, secondary outcomes were blood-sugar-related indicator biomarkers (fasting blood sugar level: GLU, glycoalbumin: GA, HbA1c) and impacts on QOL through Anti-Aging QOL Common Questionnaire survey[10,11]. Safety was examined by blood pressure/pulse, body weight/ body-fat percentage/BMI, subject diaries, interviews by a doctor and absence/presence of adverse effects occurrence (adverse event monitoring).

Alcohol consumption and excessive exercise were forbidden for trial participants and moreover, they were required to have sufficient sleep on the day before the test. During the test, trial participants were required to avoid irregular lifestyles such as insufficient sleep and excessive eating and drinking. In addition, they were supposed to maintain their habits in eating, doing exercise and sleeping quantitatively and qualitatively same as before the commencement of the test. Moreover, trial participants were forbidden to be directly exposed to sunlight for a long time such as exercise outdoor or farm work. The participants had to be attentive not have measurement sites of the skin exposed to ultraviolet radiation indoor and outdoor in their daily life. Basic skin-care products, cosmetics, cleansing products, and facial cleansers, which were in use, were prohibited to be changed or added during the test period. The participants continued, during the trial, to use same products, which had been used before the trial. When they needed inevitable changes, additions and usage, they must contact the consulting counter, recording the name of used products and its manufacturer and usage reasons in participant journals. The participants continued the same methods of facial care, which were performed before the trial. Therapies or treatments which were assumed to affect skin conditions were prohibited such as facial massage, facial esthetics, usage of facial care devices (electrical muscle stimulation, facial roller, *etc*.), hyaluronic acid injection, botox injection, chemical peeling, phototherapy and laser treatment. Medicine, including external agents, quasi-pharmaceutical products, and herbal medicine were prohibited in principle. For an unavoidable usage, they were required to report it to the consulting counter, recording the name of used products, its manufacture, and the reason for the usage in participant journal. It was banned to start using or ingesting a new health food products or beauty drink. When participants had regularly used health food products with the purpose of health maintenance before the participation in the trial, they were required to continue using them and not changing the method or quantity for usages of those products. In an unavoidable usage, they should record it in participant journal referring the name of used products, its manufacture, and the reason for the usage. It was prohibited for participants of the present trial to newly participate in another human clinical trial from the participation in the present study to the completion of the test.

6. Examination Items

AGE Reader measurement

Levels of Fluorescent AGEs: AF, accumulated AGEs, were measured, by an AGE Reader mu (Diagnoptics Technologies B.V., Netherlands)[12], on the center part of the left medial upperarm and the center part of the left medial forearm at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Skin elasticity

Employing a Cutometer MPA580® (Courage-Khazaka, Köln, Germany), the following areas of the left side of the face was measured 5 times: the center of the line connecting the bottom of the earlobe and the edge of the lip. With the standard of R2, the maximum and minimum values were eliminated and the mean value of 3 measurement values was obtained at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Trans epidermal water loss (TEWL)

Employing a Tewameter TM300® (Courage-Khazaka), consecutive measurements were performed with a one-second interval for longer than 60 seconds on the top head of the left zygomatic bone and the left medial upperarm. The mean value of the minimum standard deviation for the 30 seconds before the end was obtained at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Skin moisture level

Employing a Corneometer CM825® (Courage-Khazaka), the following areas were measured 5 times: on the top head of the left zygomatic bone and the left medial upperarm. The maximum and the minimum values were eliminated and the mean value of the 3 remaining measurements was obtained at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

VISIA image analysis

The left side of the face was captured by a VISIATM Evolution (VISIA-Evo) (Canfield, USA) to obtain scores of age spots, wrinkles, skin texture, pores, UV-related spots, brown spots, reddish regions, and porphyrin at SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Skin color difference measurement

Employing a Konika Minolta spectrophotometry device CM-2600d (KONICA MINOLTA, INC. Japan), the following areas of the left side of the face were measured 5 times: at the center of the lines connecting the bottom of the earlobe and the edge of the lip. With the standard of L*, the maximum and the minimum values were eliminated and the mean value of 3 measurement values was obtained at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Observation assessment (visual assessment) for skin conditions by dermatologists Assessment of skin texture

Visual assessments were performed using a DermLite DL100 (J. Hewitt, USA and a microscope (KH-1300/HXG-2016Z/HIROX, Japan). A 5-point scale rating was provided as follow: -2 (poor), -1 (slightly poor), 0 (average), 1 (slightly good), and 2 (good), at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Assessment of skin quality

Visual assessments were performed using a DermLite DL100 (J. Hewitt, USA) and a microscope (KH-1300/HXG-2016Z/HIROX, Japan). A 5-point scale rating was provided as follows: 0 (none, no symptoms were recognized), 1 (slight, slight symptoms were observed), 2 (mild, mild symptoms were observed, 3): (moderate, clear symptoms were observed), and 4 (serious, noticeable symptoms were observed) at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

Blood pentosidine levels

Pentosidine levels, fasting blood sugar levels: GLU, glycoalbumin: GA, and HbA1c* were measured by LSI Medience Corporation (Minato-ku, Tokyo, Japan) at pre-screening & pre-ingestion: SCR & Visit-1, 4 weeks after ingestion: Visit-2, and 8 weeks after ingestion: Visit-3.

HbA1c*: HbA1c assessments were performed at SCR & Visit-1 and 8 weeks after ingestion: Visit-3.

Adult ADHD Quality of Life Questionnaire (AAQOL)

Subjective symptoms were assessed using the Adult ADHD Quality of Life Questionnaire[10,11]. Research participants answered questions of physical conditions (33 questions) and mental conditions (21 questions) via a 5-point rating of 1: never, 2: rarely, 3: few, 4: moderate, and 5: highly frequent. Furthermore, participants answered 6 questions regarding life habits with a numerical rating scale.

Measurements were conducted in an environmental test lab(temperature: 21 ± 1 °C and humidity: 50 ± 5 %) including skin elasticity, TEWL, skin moisture levels, VISIA image analysis scores, and skin color difference measurements. Moreover, safety assessments were examined by blood pressure/pulse and body weight/ body-fat percentage/BMI.

Statistical analysis

As fundamental statistics, mean value, standard deviation, standard error, maximum value, and minimum value were calculated. Examination result data were compiled into a summary sheet using Microsoft Office Excel 2016 (Microsoft Corp.). Data results for analysis consisted of measured values and alternation quantity compared with values prior to ingestions of the test food products. Statistical analysis software, SAS 9.4 (SAS Institute Inc.) and SPSS Statistics 26 (IBM), were employed. The significance level of all tests was 5 % with a two-sided test. The tendency level was 10 %. Calculated data were expressed as the mean value ± standard error to record in tables and graphs.

A paired t-test was performed to compare the statistical data between groups, comparing measured values at points in time of commencement (before ingestion) and all observation points (during the observation period). Scores obtained from the AAQOL and visual assessments by dermatologists were regarded as nonparametric. For comparisons in groups, a Wilcoxon signed-rank test was performed.

Data comparing groups were analyzed via an unpaired t-test (two-sided test) to compare test food groups at examinations. Scores obtained from the AAQOL and visual assessments by dermatologists were regarded as nonparametric. For comparisons between groups, a Mann-Whitney U test was performed.

Results

Classification and content of trial participants

The present study started with 24 subjects and was completed with 24 subjects without canceled cases. The clinical case conference, which was held after the completion of the trial, confirmed that all 24 subjects initially enrolled in this randomized trial for the test food products were considered Intention To Treat (ITT) and the target of data analysis regarding primary and secondary endpoints as well as safety evaluation items were the target of analysis (***Table 2***).

The His-Thr group subjects, which consisted of 12 subjects (women) of the group ingesting His and Thr contained in the test foods, had an average age of 51.3 ± 5.4. The values of R2, R7, and SAF at the time of subject allocation were as follows: R2 = 0.682 ± 0.056, R7 = 0.317 ± 0.040, SAF on the center part of the left medial upperarm = 2.66 ± 0.58, and SAF on the center part of the left medial forearm = 2.35 ± 0.36.

The Orn-Thr group subjects, which consisted of 12 subjects (women) of the group ingesting Thr and Orn contained in the test foods had an average age of 51.9 ± 3.8. The values of R2, R7, and SAF at the time of subject allocation were as follows: R2 = 0.682 ± 0.055, R7 = 0.316 ± 0.049, SAF on the center part of the left medial upperarm

Table 2. Targets of ITT analysis.

Groups	Items		Women
His-Thr	The number of subjects		12
	Age		51.3 ± 5.4
	R2	(−)	0.682 ± 0.056
	R7	(−)	0.317 ± 0.040
	SAF (the center part of the left medial upper arm)	(−)	2.66 ± 0.58
	SAF (the center part of the left medial forearm)	(−)	2.35 ± 0.36
Orn-Thr	The number of subjects		12
	Age		51.9 ± 3.8
	R2	(−)	0.682 ± 0.055
	R7	(−)	0.316 ± 0.049
	SAF (the center part of the left medial upper arm)	(−)	2.66 ± 0.16
	SAF (the center part of the left medial forearm)	(−)	2.32 ± 0.34

Results are expressed as mean ± standard deviation. ITT, intention to treat; SAF, skin autofluorescence.

= 2.66 ± 0.16 and SAF on the center part of the left medial forearm = 2.32 ± 0.34. The ingestion rates of the test foods were 100 %.

Primary endpoints

AGE Reader measurement (***Table 3***)

Levels of SAF, which were measured on the center part of the left medial upperarm, were observed to significantly decrease in both of the His-Thr and the Orn-Thr groups at 4 weeks after ingestion in comparison with that of pre-ingestion ($p < 0.05$, ***Fig. 1***).

Compared to GS-related indices at 8 week, SAF levels of the His-Thr group showed greater improvements but there was no significant difference between groups (***Fig. 2***).

*Skin elasticity (**Table 4**).*

Levels of R2 of both His-Thr and Orn-Thr groups showed a significant increase at 8 week ($p < 0.01$, ***Fig. 3***) in comparison with that of pre-ingestion.

Levels of R5 of the His-Thr group showed a significant increase at 4 week ($p < 0.05$) and 8 week ($p < 0.01$) in comparison with that of pre-ingestion ($p < 0.01$) The Orn-Thr group showed a significant increase at 8 week ($p < 0.05$).

Levels of R6 of the Orn-Thr group showed a significant increase at 4 week ($p < 0.05$).

Levels of R7 of the His-Thr group showed a significant increase at 8 week in comparison with that of pre-ingestion ($p < 0.01$). Levels of R7 of the Orn-Thr group showed significant increase at 8 week ($p < 0.05$, ***Fig. 4***) in comparison with that of pre-ingestion.

Compared to the indices of skin elasticity at 8 week, both the His-Thr and the Orn-Thr groups showed significant improvements in R2, R5, and R7. There was no significant difference between groups (***Fig. 5***).

TEWL (***Table 5***) and levels of skin moisture (***Table 6***)

Levels of TEWL of the Orn-Thr group, which were measured on the top head of the left zygomatic bone, showed a significant increase at 4 week ($p < 0.01$) in comparison with that of pre-ingestion.

Levels of TEWL of the His-Thr group, which were measured on the left medial upperarm, showed a significant decrease at 8 week in comparison with that of pre-ingestion ($p < 0.05$).

Skin moisture levels on the left medial upperarm significantly decreased in the Orn-Thr group at 4 week ($p < 0.05$).

Compared to skin moisture indices at 8 week, TEWL on the cheek and moisture retention effectiveness seemed slightly larger in the His-Thr group. However, significant differences between groups were not observed (***Fig. 6***). We had to take it into account that this study was conducted from the middle of October to the middle of December, when the atmosphere was dry. It was suggested that on the cheeks of the face, which were exposed to outside air, TEWL increased and the volume of water evaporation increased. Therefore, it was interpreted that the skin moisture was retained well to a good extent.

However, TEWL decreased on the forearms, which were exposed to the outdoor air to a limited extent. The reasons for the decrease of skin moisture levels were uncertain.

VISIA image analysis (***Table 7***)

Age spots scores significantly deceased in the His-Thr group at 8 week ($p < 0.05$) in comparison with that of pre-ingestion.

Pores scores significantly increased in the His-Thr group at 4 week ($p < 0.05$) in comparison with that of pre-ingestion. Similarly, that of the Orn-Thr group significantly increased at 8 week ($p < 0.05$).

Scores of UV-related spots of the Orn-Thr group showed a significant increase at 4 ($p < 0.05$) and 8 week ($p < 0.01$).

Brown spot scores of the Orn-Thr group significantly decreased at 4 week ($p < 0.01$). In group comparisons with the His-Thr group, scores showed a significant difference at 4 week ($p < 0.05$).

Scores of reddish regions of the His-Thr group showed a significant increase at 8 week ($p < 0.05$).

In comparisons among VISIA indicators at 8 week, the His-Thr group seemed to have slightly higher degrees of maintenance and improvement in age spots and pores. Similarly, the His-Thr group seemed to maintain and improve wrinkles and skin textures better. However, there was no significant difference between groups (***Fig. 7***).

Skin color difference measurement (***Table 8***)

Significant increase was recognized in Lightness*: L* of the His-Thr group at 4 week ($p < 0.05$) compared with pre-ingestion. In group comparisons, Lightness* of the His-Thr group significantly increased at 8 week ($p < 0.05$).

Levels of redness (a*) significantly increased in the His-Thr group at 4 week ($p < 0.01$). Similarly, levels of redness (a*) of the Orn-Thr group significantly increased at 8 week ($p < 0.05$). In group comparisons, levels of the His-Thr group significantly increased at 4 week ($p < 0.05$).

Levels of yellowishness (b*) significantly decreased in the His-Thr group at 4 ($p < 0.05$) and 8 week ($p < 0.01$). In group comparisons, levels of the His-Thr group significantly decreased at 4 week ($p < 0.05$).

The Melanin Index significantly decreased in the His-Thr group at 4 and 8 week ($p < 0.01$).

The Hemoglobin (Hb) Index significantly increased in the His-Thr group at 4 ($p < 0.01$) and 8 week ($p < 0.05$) in comparison with pre-ingestion. Similarly, the Hb Index significantly increased in the His-Thr group at 8 week ($p < 0.05$) in comparison with pre-ingestion. In group comparisons, the His-Thr group significantly increased at 4 week ($p < 0.05$).

The Hemoglobin (Hb) SO_2 Index significantly increased in the His-Thr group at 4 ($p < 0.05$) and 8 week ($p < 0.01$). A significant increase of the Hemoglobin (Hb) SO_2 Index was recognized also in the Orn-Thr group at 8 week ($p < 0.05$).

In comparisons of skin color difference indices at 8 week, the degree of maintenance and improvement of L* (lightness*) of the His-Thr group was larger and a significant difference between groups was recognized (***Fig. 8***).

Observation (visual assessment) for skin conditions by dermatologists (***Table 9-a, b***)

Crista cutis significantly increased in the His-Thr group at 8 week ($p < 0.01$). Similarly, in the Orn-Thr group, crista cutis significantly increased at 4 week ($p < 0.05$).

Sulcus cutis significantly increased in the His-Thr group at 4 and 8 week ($p < 0.05$). Similarly, in the Orn-Thr group, crista cutis significantly increased at 8 week ($p < 0.01$).

In comprehensive evaluations, a significant increase was recognized in the His-Thr group at 8 week ($p < 0.05$) in comparison with pre-ingestion. Similarly, a significant increase was recognized in the Orn-Thr group at 4 and 8 week ($p < 0.01$).

Dryness significantly decreased in the Orn-Thr group at 4 ($p < 0.05$) and 8 week ($p < 0.01$). In comparison with the His-Thr group, a significant difference was recognized at 8 week ($p < 0.05$).

Erythema significantly decreased in the His-Thr group at 4 and 8 week ($p < 0.05$). Similarly, in the Orn-Thr group, significant decreases were recognized at 4 ($p < 0.05$) and 8 week ($p < 0.01$).

There were no significant differences recognized regarding scaly skin, feeling of stimulation and itching.

Blood pentosidine levels (***Table 10***)

Blood pentosidine levels significantly increased in the His-Thr group at 4 and 8 week ($p < 0.01$). Similarly, in the Orn-Thr group, significant decreases were recognized at 4 and 8 week ($p < 0.01$).

Table 3. SAF measured by AGE Reader.

Items	Groups	n	Before ingestion	p-value between groups	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
SAF (the center part of the left medial upper arm)	His-Thr	12	2.66 ± 0.58	0.974	2.50 ± 0.44	0.049 *	0.736	2.42 ± 0.35	0.118	0.122
	Orn-Thr	12	2.66 ± 0.16		2.54 ± 0.24	0.017 *		2.61 ± 0.23	0.405	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, a paired t-test was performed for comparisons with pre-ingestion. ITT, intention to treat; SAF, skin autofluorescence.

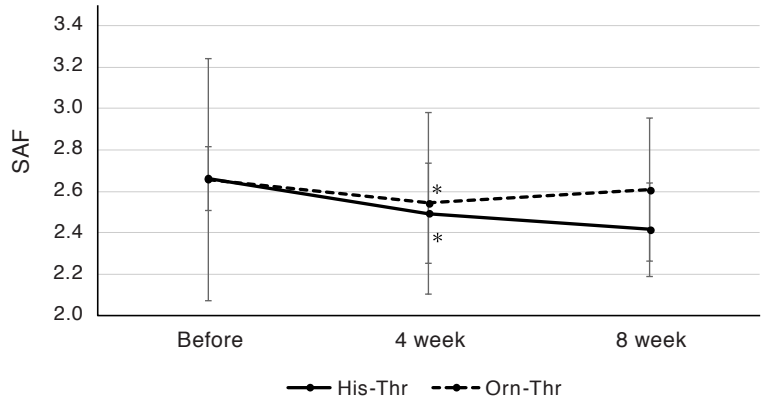

Fig. 1. **SAF measued by AGE Reader.**
Results are expressed as mean ± standard deviation, n = 12. * $p < 0.05$, a paired t-test was performed for comparisons with pre-ingestion.

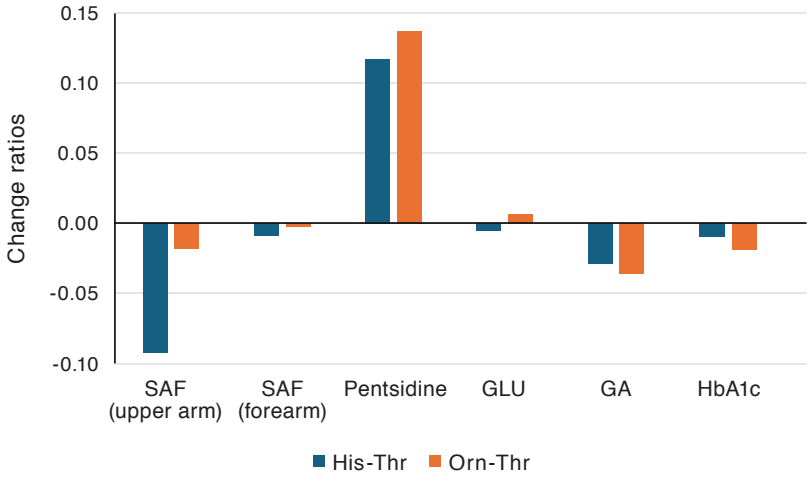

Fig. 2. **Change ratios of glycation-related indices at 8 week.**
Results are expressed as mean values of change ratios, n = 12. SAF, skin autofluorescence measured by AGE Reader; GLU, fasting blood glucose; GA, glycoalbumin.

Table 4. Skin elasticity (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
R0	His-Thr	12	0.000 ± 0.000	0.008 ± 0.035	0.471	0.477	-0.011 ± 0.025	0.154	0.256
	Orn-Thr	12	0.000 ± 0.000	0.023 ± 0.065	0.249		0.01 ± 0.058	0.557	
R2	His-Thr	12	0.000 ± 0.000	0.015 ± 0.043	0.258	0.830	0.035 ± 0.037	0.008 **	0.592
	Orn-Thr	12	0.000 ± 0.000	0.009 ± 0.076	0.686		0.044 ± 0.048	0.009 **	
R5	His-Thr	12	0.000 ± 0.000	0.036 ± 0.053	0.038 *	0.904	0.048 ± 0.050	0.007 **	0.851
	Orn-Thr	12	0.000 ± 0.000	0.040 ± 0.095	0.173		0.053 ± 0.069	0.021 *	
R6	His-Thr	12	0.000 ± 0.000	0.014 ± 0.050	0.363	0.173	0.005 ± 0.050	0.722	0.831
	Orn-Thr	12	0.000 ± 0.000	0.044 ± 0.057	0.020 *		0.011 ± 0.077	0.632	
R7	His-Thr	12	0.000 ± 0.000	0.024 ± 0.04	0.064 †	0.780	0.036 ± 0.037	0.006 **	0.985
	Orn-Thr	12	0.000 ± 0.000	0.018 ± 0.066	0.380		0.037 ± 0.048	0.023 *	

Results are expressed as mean ± standard deviation. †p < 0.10, *p < 0.05, **p < 0.01, a paired t-test was performed for comparisons with pre-ingestion. SAF, skin autofluorescence.

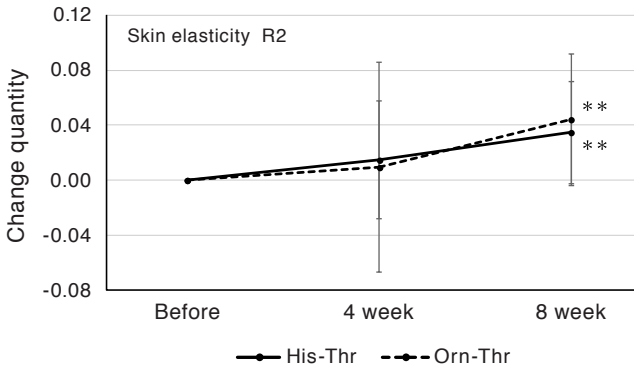

Fig. 3. **Skin elasticity R2 (change quantity).**
Results are expressed as mean ± standard deviation, n = 12. ** p < 0.01, a paired t-test was performed for comparisons with pre-ingestion.

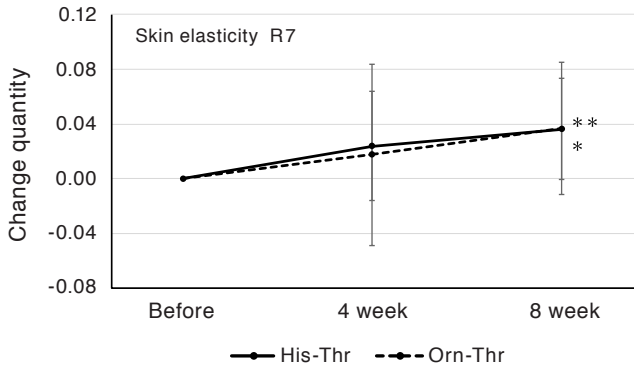

Fig. 4. **Skin elasticity R7 (change quantity).**

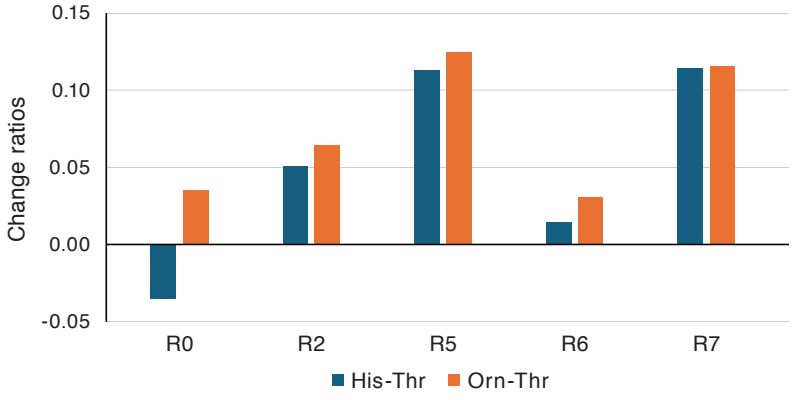

Fig. 5. **Change ratios of skin elasticity at 8 week.**
Results are expressed as mean values of change ratios, n = 12.

Table 5. TEWL (change quantity).

Items	Unit	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
TEWL (Top head of left zygomatic bone)	g/hm^2	His-Thr	12	0.000 ± 0.000	0.768 ± 6.279	0.680	0.267	1.476 ± 6.768	0.466	0.677
		Orn-Thr	12	0.000 ± 0.000	3.094 ± 3.261	0.007 **		2.438 ± 4.089	0.063 †	
TEWL (Left medial upper arm)	g/hm^2	His-Thr	12	0.000 ± 0.000	-0.380 ± 1.735	0.464	0.983	-0.883 ± 1.185	0.025 *	0.739
		Orn-Thr	12	0.000 ± 0.000	-0.367 ± 1.321	0.357		-0.69 ± 1.593	0.162	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, a paired t-test was performed for comparisons with pre-ingestion.
TEWL, trans epidermal water loss.

Table 6. Skin moisture level (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
Skin moisture level (Left medial upper arm)	His-Thr	12	0.00 ± 0.00	-1.12 ± 4.02	0.356	0.356	-3.16 ± 5.17	0.058 †	0.956
	Orn-Thr	12	0.00 ± 0.00	-2.68 ± 4.10	0.045 *		-3.04 ± 6.03	0.109	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, a paired t-test was performed for comparisons with pre-ingestion.

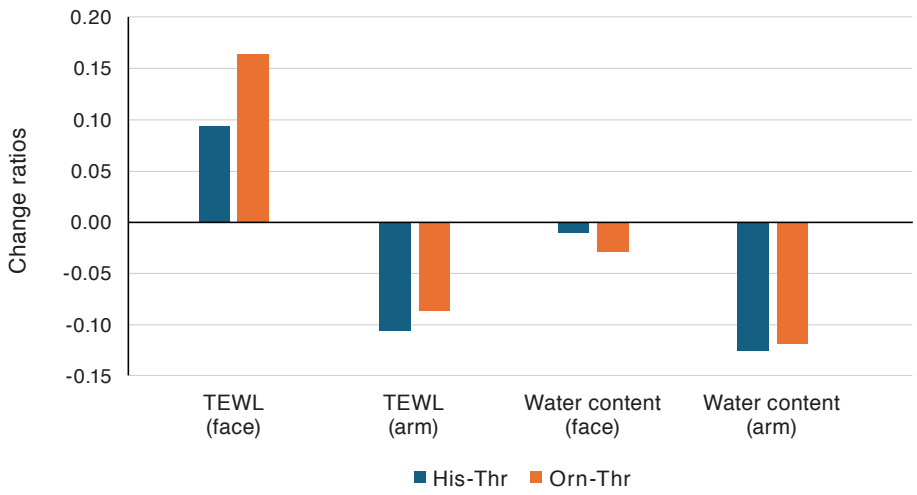

Fig. 6. **Change ratios of skin moisture indices at 8 week.**
Results are expressed as mean values of change ratios, n = 12.

Table 7. VISIA image analysis (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
Age spots (score)	His-Thr	12	0.0 ± 0.0	-2.3 ± 4.8	0.125	0.208	-3.1 ± 4.5	0.037 *	0.143
	Orn-Thr	12	0.0 ± 0.0	0.7 ± 6.5	0.707		-0.3 ± 4.6	0.854	
Wrinkles (score)	His-Thr	12	0.0 ± 0.0	1.4 ± 11.1	0.666	0.393	5.8 ± 13.1	0.152	0.210
	Orn-Thr	12	0.0 ± 0.0	-3.6 ± 16.3	0.467		-0.4 ± 10.4	0.888	
Skin texture (score)	His-Thr	12	0.0 ± 0.0	1.4 ± 3.7	0.206	0.142	1.5 ± 4.2	0.240	0.284
	Orn-Thr	12	0.0 ± 0.0	-0.5 ± 2.5	0.480		-0.2 ± 3.6	0.820	
Pores (score)	His-Thr	12	0.0 ± 0.0	3.0 ± 3.8	0.020 *	0.111	1.7 ± 3.4	0.122	0.342
	Orn-Thr	12	0.0 ± 0.0	0.4 ± 3.7	0.686		3.1 ± 3.6	0.013 *	
UV-related spots (score)	His-Thr	12	0.0 ± 0.0	0.7 ± 1.1	0.068 †	0.725	0.4 ± 1.9	0.533	0.286
	Orn-Thr	12	0.0 ± 0.0	0.5 ± 0.6	0.012 *		1.0 ± 0.7	<0.001 ***	
Brown spots (score)	His-Thr	12	0.0 ± 0.0	0.8 ± 3.5	0.426	0.020 #	1.0 ± 3.0	0.293	0.185
	Orn-Thr	12	0.0 ± 0.0	-2.2 ± 2.3	0.007 **		-0.6 ± 2.6	0.432	
Reddish regions (score)	His-Thr	12	0.0 ± 0.0	2.0 ± 7.0	0.336	0.534	3.8 ± 5.6	0.037 *	0.898
	Orn-Thr	12	0.0 ± 0.0	0.4 ± 5.2	0.773		4.2 ± 6.9	0.060 †	
Porphyrin (score)	His-Thr	12	0.0 ± 0.0	-0.4 ± 2.3	0.596	0.124	-1.7 ± 3.5	0.128	0.242
	Orn-Thr	12	0.0 ± 0.0	0.9 ± 1.6	0.069 †		-0.3 ± 2.0	0.653	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a paired t-test was performed for comparisons with pre-ingestion. # $p < 0.05$, a paired t-test was performed for comparisons at each assessment between groups. UV, ultra violet.

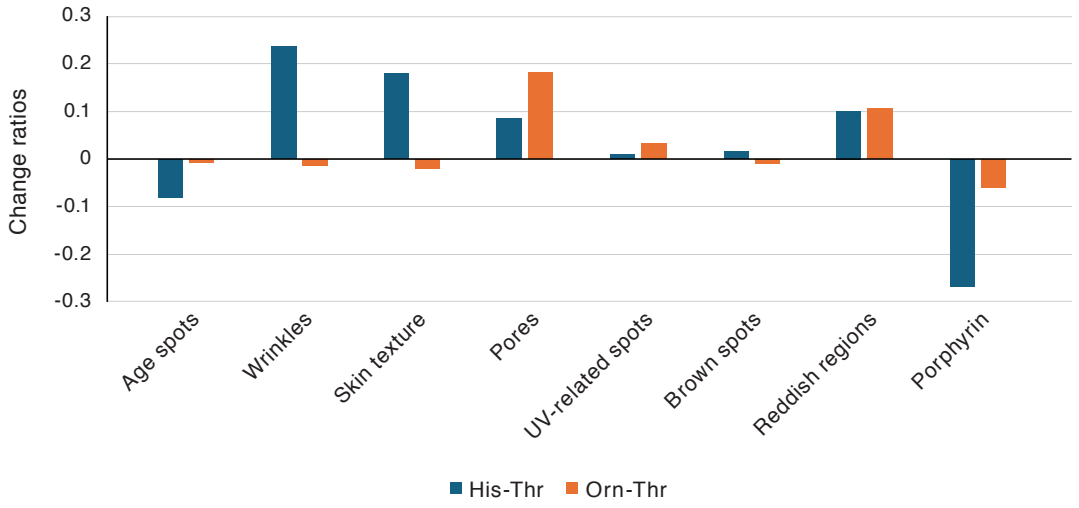

Fig. 7. **Change ratios of VISIA at 8 week.**

Results are expressed as mean values of change ratios, n = 12. UV, ultra violet.

Table 8. Color difference measurements (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
L* (lightness)	His-Thr	12	0.00 ± 0.00	0.78 ± 1.02	0.022 *	0.109	0.53 ± 1.32	0.194	0.022 #
	Orn-Thr	12	0.00 ± 0.00	0.20 ± 0.65	0.307		-0.77 ± 1.24	0.056 †	
a* (redness)	His-Thr	12	0.00 ± 0.00	0.80 ± 0.77	0.004 **	0.026 #	0.84 ± 1.42	0.066 †	0.676
	Orn-Thr	12	0.00 ± 0.00	0.11 ± 0.62	0.547		1.07 ± 1.25	0.013 *	
b* (yellowishness)	His-Thr	12	0.00 ± 0.00	-0.91 ± 1.04	0.012 *	0.041 #	-0.96 ± 0.83	0.002 **	0.700
	Orn-Thr	12	0.00 ± 0.00	0.01 ± 1.03	0.981		-0.78 ± 1.38	0.076 †	
Melanin Index	His-Thr	12	0.00 ± 0.00	-0.06 ± 0.04	0.001 ***	0.060 §	-0.06 ± 0.06	0.005 **	0.138
	Orn-Thr	12	0.00 ± 0.00	-0.02 ± 0.06	0.402		-0.01 ± 0.08	0.535	
Hb Index	His-Thr	12	0.00 ± 0.00	0.15 ± 0.14	0.003 **	0.037 #	0.17 ± 0.25	0.037 *	0.854
	Orn-Thr	12	0.00 ± 0.00	0.03 ± 0.12	0.353		0.19 ± 0.23	0.017 *	
Hb SO_2 Index (blood oxygen saturation level)	His-Thr	12	0.00 ± 0.00	4.33 ± 5.36	0.017 *	0.211	4.10 ± 4.22	0.006 **	0.655
	Orn-Thr	12	0.00 ± 0.00	1.29 ± 6.16	0.482		3.30 ± 4.42	0.025 *	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a paired t-test was performed for comparisons with pre-ingestion. § $p < 0.10$, # $p < 0.05$, a paired t-test was performed for comparisons at each assessment between groups. Hb, hemoglobin.

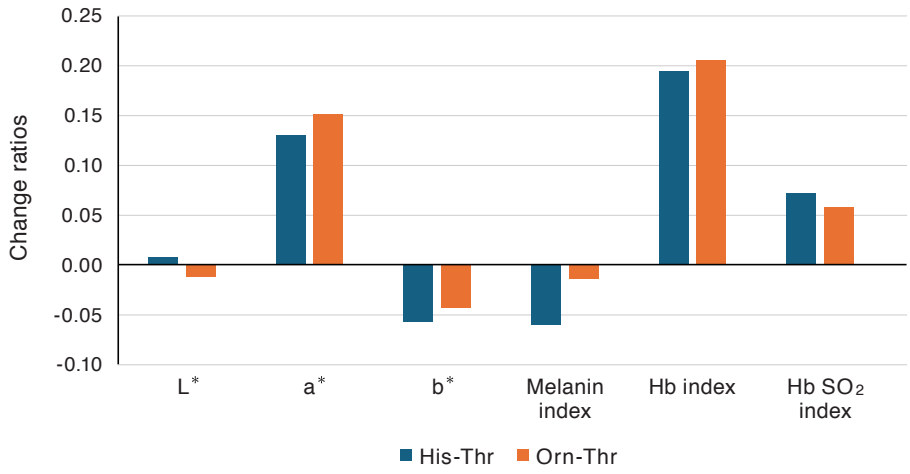

Fig. 8. **Change ratios of color difference meter at 8 week.**
Results are expressed as mean values of change ratios, n = 12. Hb, hemoglobin.

Table 9-a. Visual assessments of skin conditions by dermatologists (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
Crista cutis	His-Thr	12	0.0 ± 0.00	0.4 ± 0.8	0.096 †	0.792	0.8 ± 0.6	0.004 **	0.247
	Orn-Thr	12	0.0 ± 0.00	0.3 ± 0.5	0.046 *		0.5 ± 0.8	0.058 †	
Sulcus cutis	His-Thr	12	0.0 ± 0.00	0.7 ± 0.8	0.023 *	0.156	1.0 ± 1.1	0.024 *	0.475
	Orn-Thr	12	0.0 ± 0.00	0.3 ± 0.5	0.083 †		0.6 ± 0.5	0.008 **	
Comprehensive evaluations	His-Thr	12	0.0 ± 0.00	0.4 ± 0.9	0.129	0.390	0.9 ± 1.1	0.026 *	0.580
	Orn-Thr	12	0.0 ± 0.00	0.6 ± 0.5	0.008 **		1.0 ± 0.6	0.003 **	

Results are expressed as mean ± standard deviation. † $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, a Wilcoxon signed-rank test was performed for comparisons with pre-ingestion.

Table 9-b. Visual assessments of skin conditions by dermatologists (change quantity).

Items	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
Dryness	His-Thr	12	0.0 ± 0.0	-0.1 ± 0.3	0.317	0.061 §	-0.3 ± 0.7	0.103	0.028 #
	Orn-Thr	12	0.0 ± 0.0	-0.5 ± 0.7	0.034 *		-0.9 ± 0.7	0.005 **	
Erythema	His-Thr	12	0.0 ± 0.0	-0.3 ± 0.5	0.046 *	1.000	-0.7 ± 0.8	0.021 *	0.644
	Orn-Thr	12	0.0 ± 0.0	-0.3 ± 0.5	0.046 *		-0.6 ± 0.5	0.008 **	
Scaly skin	His-Thr	12	0.0 ± 0.0	-0.3 ± 0.6	0.180	0.875	0.2 ± 0.8	0.480	1.000
	Orn-Thr	12	0.0 ± 0.0	-0.2 ± 0.8	0.480		0.2 ± 0.8	0.480	
Feeling of stimulation	His-Thr	12	0.0 ± 0.0	0.0 ± 0.0	1.000	0.148	-0.2 ± 0.6	0.317	0.615
	Orn-Thr	12	0.0 ± 0.0	-0.2 ± 0.4	0.157		-0.2 ± 0.4	0.157	
Itching sensation	His-Thr	12	0.0 ± 0.0	-0.3 ± 0.9	0.317	0.547	-0.3 ± 0.9	0.317	0.965
	Orn-Thr	12	0.0 ± 0.0	-0.1 ± 0.7	0.655		-0.2 ± 0.7	0.414	

Results are expressed as mean ± standard deviation. * $p < 0.05$, ** $p < 0.01$, a Wilcoxon signed-rank test was performed for comparisons with pre-ingestion. § $p < 0.10$, # $p < 0.05$, a Mann-Whitney U test was performed for comparisons at each assessment between groups.

Table 10. Blood pentosidine.

Items	Unit	Groups	n	Before ingestion	4 week	p-value temporal change	p-value between groups	8 week	p-value temporal change	p-value between groups
Blood pentosidine	pmol/mL	His-Thr	12	23.72 ± 8.21	30.53 ± 8.52	<0.0001 ***	0.263	26.50 ± 7.76	0.002 **	0.454
		Orn-Thr	12	21.56 ± 4.12	27.28 ± 4.86	<0.0001 ***		24.53 ± 4.44	0.001 ***	

Results are expressed as mean ± standard deviation. ** $p < 0.01$, *** $p < 0.001$, a paired t-test was performed for comparisons with pre-ingestion.

Secondary endpoints

Levels of fasting blood glucose (GLU) and HbA1c showed no significant differences. Glycoalbumin (GA) showed a significant decrease in the His-Thr group at 8 week ($p < 0.01$) in comparison with pre-ingestion. Similarly, the Orn-Thr group showed a significant decrease of GA at 8 week ($p < 0.01$).

Items of AAQOL that significantly changed at 4 week in the His-Thr group included two items, "to easily catch a cold" and "cough and phlegm" (both increase $p < 0.05$) and one item, "not to wanting to talk with others" (increase $p < 0.05$). Among group comparisons, in the His-Thr group "to easily catch a cold" was significantly higher ($p < 0.05$) and "graying hair" was significantly lower ($p < 0.05$). Items of AAQOL which significantly changed at 8 week in the His-Thr group included one item, "poor skin condition" (significantly lowered, $p < 0.05$). In the His-Thr group, there were 3 items, "blurry vision" (increase, $p < 0.05$), "to sweat easily"(decrease, $p < 0.05$) and "frequent micturition" (increase, $p < 0.05$). In group comparisons, in the His-Thr group, two items, "blurry vision" and "graying hair" were significantly lower ($p < 0.05$).

Assessment in safety

No abnormal findings in safety assessments were reported for both the test product group and the control product group regarding safety evaluation items. The following minor symptoms were reported: in the His-Thr group, 3 persons (5 incidents) of eczema on the right cheek, headache and pain on the root of the nose, in the Orn-Thr group, 2 persons (2 incidents) of runny nose/sore throat and stomachache). However, all of these symptoms were minor and there was no factor of a causal relationship with test products. From the above, the safety of the test food product during the 4-week ingestion period was confirmed.

Discussion

Skin covering the human body, whose surface area is 1.6 m^2 (adult), accounts for 16% of total body weight including subcutaneous tissue. Skin is the major organ in the body. The skin plays significant roles including serving as an immune protection system on the skin surface to act against external stimulation, and a sensory system to process input from the environment regarding perspiration, thermoregulation, moisture retention, and transpiration[13]. The face is an important factor for communication and it is vital to maintain the skin in a good condition. Major components of skin are keratin, collagen, elastin[14], and filaggrin, all of which are proteins. Keratin is a strong protein that covers the cells on the skin`s surface. Type-I Collagen and elastin are related to skin elasticity. Filaggrin plays the role of a moisturizing factor.

Skin cell turnover is an active skin generation process taking 4 to 6 weeks, where cell proliferation and protein synthesis occur continuously. Therefore, amino acids need to be supplied in an adequate quantity.

Symptoms of skin aging easily appear to be due to advanced age, such as age spots, wrinkles, sagging, and dry skin. It has been recognized until now that exposure to ultraviolet rays (photoaging) and estrogen deficiency are factors that accelerate skin aging. However, the involvement of GS has drawn attention in recent years[15].

Research in GS has been conducted remarkably and developed so that perceptions of GS are required to be reorganized anew. To understand differences between oxidative stress (OS) and GS, the defense mechanisms that we possess should be explained.

The causative factor of OS is reactive oxygen species (ROS)/free radical. Excessive free radicals damage proteins, lipids and DNA of the body at the molecular level. Its reaction time, which is extremely short, is several μ–m. Therefore, blood ROS concentration cannot be measured. The defensive systems possess antioxidant enzymes such as superoxide dismutase (SOD), peroxidase, and catalase, as well as endogenous antioxidants like glutathione, coenzyme q10 (CoQ10), alpha lipoic acid, and melatonin[16].

The causative factor of GS is aldehydes. Excessive aldehydes damage proteins, lipids and DNA of the body at the molecular level. Its reaction time, depending on the types of aldehydes, concentrations, and temperature, ranges from several minutes to several tens of minutes. Therefore, concentrations can be measured in the blood. Defense systems that act against GS possess ALDH, GAPDH, and GLO (glyoxalase) to metabolize aldehydes[17]. These enzymes, which are largely expressed in the cells of the liver and kidney, play an essential role to defend against GS. Other than that, these enzymes are largely expressed in the stem cells and precursor cells where proliferation is active, to respond to the demands. Aldehyde formation paths in the body are classified mainly into two types: glucose-derived and lipid-derived. Various types of glucose-derived aldehydes are produced through chain reactions, occurring in succession of the increase of ring-open glucose due to postprandial hyperglycemia (a ring-opening glucose formation where aldehyde group (-CHO) is exposed in an open-chain aldehyde form)[18]. The aldehydes are 3DG, GO, MGO, glyceraldehyde, and glycolaldehyde. Multiple studies describe 3DG, GO, MGO as dicarbonyl compounds. However, these compounds are aldehyde type with high reactivities (ketone + aldehyde type) in structure. They must be distinguished from stable forms (ketone + ketone type), (ketone + ester type), and (carboxylic acid + carboxylic acid type). Acetaldehydes, which increase after alcohol ingestion, are glucose-derived aldehydes. Lipid-derived aldehydes, which are formed mainly via oxidation of fatty acids, include acrolein, malonaldehyde, MGO, and 4OH-nonenal[19]. Magnesium oxide (MGO), which is both glucose-derived and lipid-derived, is metabolized by a metabolizing enzyme, GLO (glyoxalase); the body recognizes MGO as a significantly harmful aldehyde.

Amino acids contained in foods are not only vital nutrients but also components in regulating the metabolism of proteins and lipids as well as maintaining the health of muscles etc. Furthermore, amino acids form AGEs via glycation, and are glycated themselves when coexisting with proteins. Therefore, there is a possibility of inhibiting protein glycation. Previous studies[4,5] confirmed and reported that combinations of amino acids enhanced these effects. Based on these basic experimental results, the present study conducted this clinical trial with the mixtures of His, Orn and Thr.

Overview of results

The following were items that showed significant improvements in both the His-Thr and the Orn-Thr groups: GS indices (SAF, 4-week); skin elasticity indices (R2, R5, and R7, every item, 8-week); color difference measurements (Hb Index and Hb SO_2 Index, both items, 8-week); and visual assessments by dermatologists (sulcus cutis, comprehensive evaluation, and erythema, every item, 8-week). These were assumed to be effects of amino acids in general. The evaluations of significant increases of blood pentosidine in both groups are reported below.

Regarding glycative-related indexes, SAF values measured on the upperarm significantly improved in both the His-Thr and the Orn-Thr groups. It had been confirmed that all of these amino acids had inhibitory effects on the AGE formation in the *in vitro* experimental models[4,5]. It was suggested that these effects were exerted equivalently in the human body. The effects were not observed on the forearm. It was assumed that forearms, which were exposed to a large extent, might have been affected by ultraviolet light and dry external air.

Plasma pentosidine concentration significantly increased in both the groups in this trial. The significance of blood AGEs must be reconsidered. In the *in vitro* experimental models of the Glucose/human serum albumin (HSA) system[4], the inhibitory ratios of the pentosidine formation were His 69.5%, Orn 51.3%, and Thr 56.7%. This indicated that amino acids inhibited reactions of [glucose + aldehyde → pentosidine]. Furthermore, in the human body, reactions of [(glucose + aldehyde) + (HSA + amino acid) → pentosidine] were observed. When amino acids were added, naturally, the quantity of the pentosidine formation would increase.

When observing change ratios (large value/small value) regarding skin moisture indices in the His-Thr and the Orn-Thr groups, TEWL (on the cheek) was the largest, 1.76. However, there was no significant difference in group comparisons.

Items which had significant between-groups differences were lightness, L* of color difference assessment and skin dryness in visual assessments by dermatologists; the His-Thr group have excellent effects in comparison with the Orn-Thr group ($p < 0.05$). In general, AGEs exhibit a brown color and the increase of skin deposition induces the decline of L*. Therefore, it was highly possible that His contributed to the decrease of the skin AGE deposition. Dry skin is associated with the glycation of filaggrin and the malfunctions of the skin barrier. Thus, it was highly possible that His exerted preventive activities.

This trial showed that levels of plasma pentosidine concentration significantly increased in both groups. The significance of blood AGEs must be reconsidered. In this trial, blood concentration levels of total amino acid amount increased via oral administration of amino acids. AGEs were formed via reactions between glucose-derived aldehydes and amino acids; these glucose-derived aldehydes were produced due to postprandial hyperglycemia. We postulate that if these reactions had not occurred, or the amount had been small, the aldehydes would have induced more injuries to vascular endothelial cells and/or protein modifications in endothelial cells. That is, it was expected that occurrences of the AGE formation in blood vessels could induce less physical damage. Moreover, AGEs formed in blood are reaction products and have almost no reactivity with other molecules. Via reactions with the receptor for advanced glycation end product (RAGE), which is a specific receptor, as well as intracellular signaling, various activities are exerted. RAGE, which is expressed in vascular endothelial cells and fibroblasts, is related to degradation and cellular uptake. However, it does not induce the formation of proinflammatory cytokine. Furthermore, it is highly possible that AGEs in blood vessels lead to an increase in RAGE expression in vascular endothelial cells, and accelerate anti-glycation due to the soluble RAGE. In particular, RAGE on endothelial cells of the blood-brain barrier, BBB, functions as oxytocin transporters and play an indispensable role for oxytocin transference from the blood to the brain[20,21].

Effectiveness of His on the skin

1) Promotion of wound healing

Skin cell turnover which involves active skin generation requires an amino acid supply as a source of nutrients for cell proliferation and protein synthesis. It is challenging to evaluate which amino acids are effective on metabolism. However, His has been recognized as an important amino acid in wound-healing experimental models.

His, which is one of the essential amino acids, has antioxidative effects. His is converted into histamine in the body and stimulates the sympathetic nervous system, promoting lipolysis. It was suggested in wound-healing models in experimental research employing rat intestine epithelial cells (IEC-6) that His is an important amino acid for wound healing to be equal to arginine (Arg)[22]. It is possible that the delay of wound healing due to His deficiency could be related the decrease of TGF-β1 activity.

2) Homeostasis of filaggrin

Filaggrin, which is a natural moisturizing factor, is abundant in His. Filaggrin plays an important role for the moisture retention in stratum corneum[23]. Its life span is the time required for keratinocytes to move from the granular layer to the stratum corneum. That is, the life span of filaggrin is approximately 4 weeks. The degradation and synthesis of filaggrin is active during this period, and the supplementation of His is essential. Filaggrin is a resource of major component supply for natural moisturizing factors (NMF) such as pyrrolidone carboxylic acid (PCA), urocanic acid (UCA) and others[24].

Other than that, filaggrin is positioned as a skin barrier protein. Deterioration of barrier functions due to deficiency or disorder leads to not only the moisture loss via transpiration but also dry skin without protection from foreign invaders, which are risk factors for the onset of allergic dermatitis and atopic dermatitis (AD)[25]. It has been reported that aged persons with chronic wounds tend to be insufficient in His and TRP (transient receptor potential)[26].

A clinical study has reported on the verification of His effectiveness on AD[25], where adult patients with AD ($n = 24$) ingested placebo or 4 g of oral His every day for 8 weeks. The His group decreased AD 4 weeks after ingestion, which was different from the placebo group ($p = 0.0003$). Infantile patients with AD ($n = 49$, the mean

age: 3.5) ingested 0.8 g of His every day for 12 weeks. As a result, scores of eczema area and severity index decreased to 49 % ($p = 0.02$), and the placebo group showed no effects.

3) Prevention of filaggrin glycation
Filaggrin is a basic protein, which is abundant in His, Arg, Ser (serine), Gly, and glutamic acid (pH > 10). Filaggrin contains almost no nonpolar protein.[23,27]. Arginine (Arg) NH_2-residue in proteins is likely to react with aldehyde groups producing carbonylated proteins and AGEs. It is assumed that the deterioration of moisture retention and skin barrier function is induced due to the glycation of filaggrin.

As mentioned above, it is suggested that His promotes wound healing, the homeostasis of filaggrin, and the prevention of filaggrin glycation.

Research limitation

Formation quantity of aldehydes due to aldehyde spark, which follows blood glucose spikes (postprandial high blood glucose) is different for each individual. First, there are individual differences in blood lipid concentrations. Persons, who have hypertriglyceridemia and/or a high-fat-diet lifestyle, have high levels of lipids in the blood. Aldehydes, which are produced via "oxidation" of fatty acids, are added. Other than "oxidation" of fatty acids, aldehydes are produced via attacks of other aldehydes, and ultraviolet radiation. Second, there are individual differences in blood amino acid concentrations. Amino acids produce AGEs via glycation and are glycated themselves under a condition of coexisting with proteins. There is a possibility of the inhibition of protein glycation. Therefore, it is expected that aldehyde spark would be alleviated via aldehyde-trapping effects of amino acids (the removal of aldehydes), where levels of total blood amino acid concentrations are consistently high, if sufficient quantity of proteins are ingested.

Previous experimental models suggested a reasonable possibility that as an amino acid action mechanism, both "competitive inhibition of aldehydes and proteins in three-dimensional thinking" and "aldehyde-trapping effects" are effective. We will conduct further research to verify the aldehyde-trapping effects of amino acids.

Safey

No adverse event was reported during the trial.

Conclusion

The present trial was conducted with 24 female subjects having low levels of dynamic elasticity measurements (R7) and high levels of a GS marker, SAF, and who were selected from 77 women who were aware of skin deterioration such as loss of elasticity and sagging. Research participants underwent oral ingestion of amino acid mixtures for 8 weeks: 1.65 g of His, 0.8 g of Orn (ornithine) and 1.65 g of Thr (threonine). Effects were evaluated regarding skin elasticity, GS-related indices, and moisture retention. These indices significantly improved in both the His-Thr group and the Orn-Thr group. In the His-Thr group, lightness assessments (L*) in color difference examinations, and assessments of dry skin levels by dermatologists showed significant improvement in comparison with the Orn-Thr group. His is an amino acid, which is indispensable in maintaining the homeostasis of filaggrin with wound healing effects. Therefore, the administrations of His and Orn mixtures were expected to have improvement effects on skin condition, such as firmness, elasticity, sagging, and dryness.

Conflict of interest declaration

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