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The Effect of Cysteine Peptide Ingestion on Skin Brightness, a Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Human Clinical Trial

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Abstract: Glutathione (GSH) is present in almost all human cells and has a beneficial effect on human skin brightness. Cysteinylglycine (Cys-Gly) and γ-glutamylcysteine (γ-Glu-Cys) are GSH synthesis components. In this study, we defined glutathione (GSH), cysteinylglycine (Cys-Gly), and γ-glutamylcysteine (γ-Glu-Cys) as cysteine peptide and performed a randomized, double-blind, placebo-controlled study to investigate the effects of orally administered cysteine peptide on human skin brightness using a CM-26d portable spectrophotometer in healthy males and females aged between 20 and 65 years old. Eligible participants were randomly allocated into three groups (cysteine peptide 45 mg: $n = 16$, 90 mg: $n = 15$, and placebo: $n = 16$). Each subject ingested six tablets every day for 12 weeks, and skin brightness was measured at 0, 4, 8, and 12 weeks. As a result, the 45 mg group exhibited arm brightening in a time-dependent manner, and a significant difference was observed compared to the placebo at week 12 ($p = 0.028$). Moreover, no serious adverse events and changes related to 270 mg study food were observed in the safety trial. Here, we suggest that cysteine peptide is a promising and safe compound for human skin brightness.

Keywords: cysteine peptide; glutathione; cysteinylglycine; γ-glutamylcysteine; skin brightness; safety

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1. Introduction

Melanin is a principal component of skin pigmentation [1]. Melanogenesis inhibition is a common strategy used to generate a lighter skin tone [2]. Melanogenesis is suppressed through several mechanisms, including inhibition of reactive oxygen species (ROS) production which triggers melanoma initiation, inhibition of a key transcriptional factor of melanin synthesis, direct suppression of tyrosinase, or induction of pheomelanin synthesis rather than darker eumelanin [2–5]. Numerous compounds show promising effects through the activation of these mechanisms and are used as skin-lightening agents in the cosmetic industry [6–8].

Cysteine peptide is used as a generic term for glutathione (GSH), cysteinylglycine (Cys-Gly), and γ-glutamylcysteine (γ-Glu-Cys) in this paper. Glutathione is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine, that naturally occurs in plants, animals, and human cells [9]. It was accorded the status of "Generally Recognized as Safe (GRAS GRN000293)" consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act of the United States Food and Drug Administration (USF-DA) [10]. It is a potent antioxidant and is vital for the detoxification of the human body [11]. Hence, its efficacy is proven against various diseases including liver dysfunction [12] and nervous disorders [13] without side effects. In

addition, GSH demonstrates a significant effect on human skin lightness. The mechanism behind GSH's skin-brightening effect includes prevention of ROS generation, tyrosinase inhibition, and switching of melanin-type production from eumelanin to pheomelanin [14]. A human clinical trial of oral glutathione intake at 250 mg/day (12 weeks) and 500 mg/day (4 weeks) resulted in skin brightening [15,16]. Moreover, Cys-Gly and γ-Glu-Cys are GSH components [17]. Hence, cysteine peptide could significantly affect human skin functions. However, there is no report on the effect of oral cysteine peptide to our knowledge.

Thus, a randomized, double-blind, placebo-controlled study was performed to investigate the effects of oral cysteine peptide on human skin brightness. In addition, we evaluated the safety of cysteine peptide.

2. Materials and Methods

2.1. Study Population (Participants)

2.1.1. Efficacy Trial

The inclusion criteria of this study were as follows:

1. Japanese males and females aged 20–65 years old.
2. Those who have received a full explanation of the purpose and content of the study, and have agreed to participate in the study in writing.

The exclusion criteria of this study were as follows:

1. Subjects currently receiving medication or outpatient treatment for any serious disease.
2. Subjects currently undergoing exercise or diet therapy under the supervision of a physician.
3. Subjects currently undergoing an esthetic salon and beauty treatment (laser treatment, Zeo Skin, and so on) under the supervision of a physician.
4. Subjects that may develop allergies to the test food.
5. Subjects with a current or history of drug dependence or alcohol dependence.
6. Subjects hospitalized for mental disorders (depression and so on) or sleep disorders or having a history of mental disorders.
7. Subjects with an irregular rhythm of life due to night work or shift work.
8. Subjects with extremely irregular eating, sleeping, or other habits.
9. Subjects with an extremely unbalanced diet.
10. Subjects who have or had serious diseases such as brain diseases, malignant tumors, immune diseases, diabetes, liver diseases (hepatitis), kidney diseases, heart diseases, thyroid diseases, adrenal diseases, or other metabolic diseases.
11. Subjects that use health foods, supplements, or medicines (ingredients: glutathione, cysteine, yeast extract, vitamins, placenta, hyaluronic acid, collagen, and so on) that may improve skin quality.
12. Subjects that live in an environment that is prone to sunburn daily (those who often work outdoors or have a habit of exercising outdoors).
13. Subjects that participated in other clinical trials (research) within 3 months from the date of obtaining consent, or those who have plans to participate in other clinical trials (research) during the study period.
14. Subjects that received over 200 mL of blood or donated over 400 mL of blood within 1 month or 3 months prior to the date of obtaining consent.
15. Subjects that are currently pregnant or breastfeeding or are likely to become pregnant during the study period.
16. Subjects who have difficulty complying with the recording of various questionnaires.
17. Subjects who were judged to be unsuitable based on clinical laboratory values and measurements at the time of screening (SCR).
18. Subjects that are judged unsuitable by the principal investigator.

2.1.2. Safety Trial

The inclusion criteria of this study were as follows:

1. Japanese males and females aged 30–64 years old.
2. Subjects who have received a full explanation of the purpose and content of the study and have agreed to participate in the study in writing.
3. Subjects who are not currently receiving treatment or medication.
4. Subjects whose liver function marker values correspond to either of the following A or B (U/L):

   A: $20 \leq \text{ALT} \leq 30$;
   B: $31 \leq \text{ALT} \leq 50$.

5. Subjects who are not expected to meet the exclusion criteria based on the results of background questionnaires in the 1st SCR.

The exclusion criteria of this study were as follows:

1. Subjects who have been diagnosed with serious liver disease (viral hepatitis, drug-induced liver injury, liver cirrhosis, etc.) by a doctor, or those who have a medical history or are suspected (positive hepatitis virus test, etc.).
2. Subjects who are currently undergoing medication or outpatient treatment due to some serious illness.
3. Subjects who are currently undergoing exercise or diet therapy under the supervision of a physician.
4. Subjects that may develop allergies to the test food.
5. Subjects with a current or history of drug dependence or alcohol dependence.
6. Subjects with excessive alcohol consumption.
7. Subjects hospitalized for mental disorders (depression and so on) or sleep disorders or having a history of mental disorders.
8. Subjects with an irregular rhythm of life due to night work or shift work.
9. Subjects with extremely irregular eating, sleeping, or other habits.
10. Subjects with an extremely unbalanced diet.
11. Subjects that have or had serious diseases such as brain diseases, malignant tumors, immune diseases, diabetes, liver diseases (hepatitis), kidney diseases, heart diseases, thyroid diseases, renal diseases, or other metabolic diseases.
12. Subjects that use health foods, supplements, or medicines (ingredients: Ornithine, sulforaphane, turmeric, etc.).
13. Subjects that participated in other clinical trials within 2 months from the date of obtaining consent, or those who have plans to participate in other clinical trials during the study period.
14. Subjects who cannot take the test food as instructed during the intervention period.
15. Subjects that received over 200 mL of blood or donated over 400 mL of blood within 1 month or 3 months prior to the date of obtaining consent.
16. Subjects that are currently pregnant or breastfeeding or are likely to become pregnant during the study period.
17. Subjects who have difficulty complying with the recording of various questionnaires.
18. Subjects were judged to be unsuitable based on clinical laboratory values and measurements at the time of screening.
19. Subjects that are judged unsuitable by the principal investigator.

2.2. Design of the Human Clinical Study

2.2.1. Efficacy Trial

The study protocol was performed according to the guidelines of the Declaration of Helsinki, fixed on 25 October 2021, and approved by the Clinical Research Ethics Review Board of the Medical Corporation Co-creation Association AMC Nishiumeda Clinic (Osaka, Japan) before initiation of the study on 28 October 2021. The clinical study was fully explained to all participants, and all subjects provided signed informed consent. This trial was registered in the UMIN Clinical Trial Registry on 10 November 2021 (UMIN000046030) and was performed from 21 December 2021 to 15 March 2022. This study was designed

to be a randomized, double-blind, placebo-controlled, parallel-group comparative study (Figure 1). Orientation meetings and screening were performed at the hospital by Maruishi Lab Co., Ltd., (Osaka, Japan) on 11, 16, and 18 November 2021. Eligible participants were determined on 11 November 2021 by M&I Science Co., Ltd. (Osaka, Japan). Allocation to 45 mg/day, 90 mg/day, and the placebo group was conducted by an independent organization (Marukatsu Leute Co., Ltd., Hyogo, Japan) using a random number table in Excel software. Registration and allocation codes were then produced by Marukatsu Leute Co., Ltd., for allocation concealment. The allocation factors were age, gender, and lightness of skin. Clinical measurements were performed at 0 w (21 December 2021), 4 w (18 January 2022), 8 w (15 February 2022), and 12 w (15 March 2022). Test samples were sealed in aluminum packs and distributed to each participant by M&I Science Co., Ltd. Confidentiality of items such as the test food allocation table was strictly kept until the key opening, and all the operators, participants, and evaluators were blinded by Marukatsu Leute Co., Ltd. All the results were fixed on 19 April 2022. The key opening was then performed on 20 April 2022.

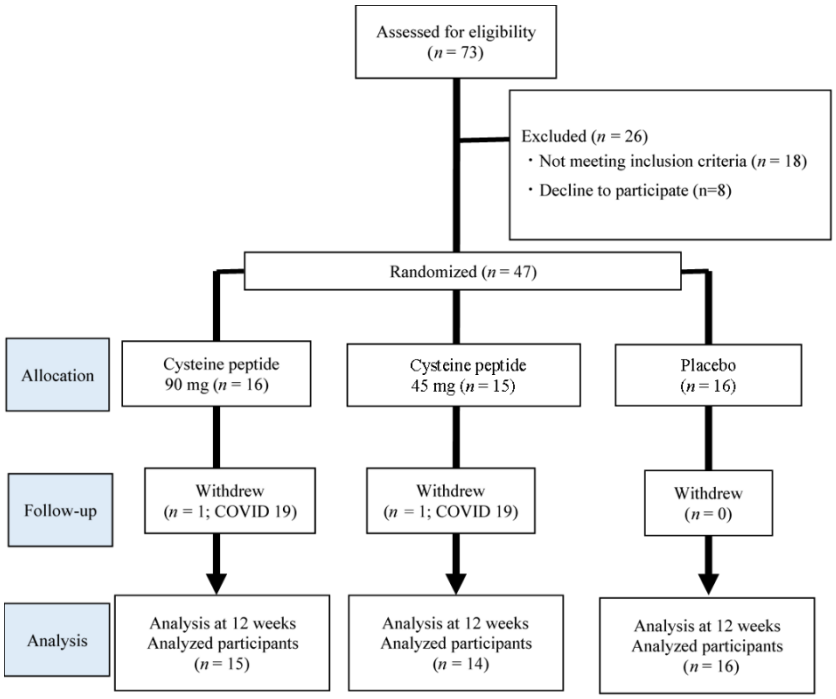

Figure 1. Flow diagram of the study.

2.2.2. Safety Trial

The study protocol was conducted according to the guidelines of the Declaration of Helsinki, fixed on 6 October 2021, and approved by the Ethics Committees of Yoga Allergy Clinic (Japan) on 8 October 2021. The clinical study was fully explained to all participants, and all subjects provided signed informed consent. This trial was registered in the UMIN Clinical Trial Registry on 29 October 2021 (UMIN000045917) and was performed from 28 February 2022 to 24 June 2022. This study was designed to be a randomized, double-blind, placebo-controlled, parallel-group comparative study (Figure S1). In this clinical study, a two-step screening was carried out. Orientation meetings and 1st screening were performed at the hospital by Medical Corporation Diastep Tokyo Skytree Ekimae Internal Medicine on 12 October to 26 November 2021. Eligible participants for 2nd screening were determined on 23 to 28 November 2021 by HUMA R&D CORPORATION (Tokyo, Japan). The 2nd screening was performed on 17 to 25 January 2022, and eligible participants were determined on 17 to 25 January 2022. Allocation to the active or the placebo group was conducted by an independent organization (Clinical Science Institute LLC; Fukuoka, Japan) by stratified randomization using R ver 3.5.2 (R Development Core Team, Vienna, Austria)

software. The allocation factors were gender, age, and liver function markers (ALT, AST, γ-GTP). Clinical measurements were performed at 0 w (28 February to 4 March 2022), 4 w (28 March to 1 April 2022), 8 w (25 to 29 April 2022), 12 w (23 to 27 May 2022) and 16 w (20 to 24 Jun 2022). Test samples were sealed in aluminum packs and distributed to each participant by HUMA R&D CORPORATION. Registration and allocation codes were produced by Clinical Science Institute LLC for allocation concealment. The test food allocation table was strictly kept until the key opening, and all the operators, participants, and evaluators were blinded by Clinical Science Institute LLC. All the results were fixed on 2 August 2022. The key opening was then performed on 3 August 2022.

2.3. Clinical Measurements

2.3.1. Efficacy Trial

This clinical study evaluated the main outcomes, secondary outcomes, and other measurements according to Table 1 for healthy 20–65-year-old men and women.

Table 1. Schedule of the visit.

Measurement		SCR	During Intervention			
		(Visit 1) SCR	(Visit 2) Before Start of Intake	(Visit 3) 4 Weeks after Intake	(Visit 4) 8 Weeks after Intake	(Visit 5) 12 Weeks after intake
Main outcome	L value	○	○	○	○	○
Secondary outcomes	Spot (number)		○	○	○	○
	Wrinkle (number)		○	○	○	○
Physical measurement	Height	○				
	Weight	○	○	○	○	○
	Body fat percentage	○	○	○	○	○
	BMI	○	○	○	○	○
	Vital signs	○	○	○	○	○

SCR: screening. BMI: body mass index.

Main Outcomes

Skin brightness (L value) was measured using a CM-26d portable spectrophotometer (Konica Minolta, Tokyo Japan) at screening, 0 w, 4 w, 8 w, and 12 w. Skin brightness was measured on the cheeks and the outside of the arm.

Secondary Outcomes

The spots (number) and wrinkles (number) were measured using VISIA Evolution (Canfield Scientific, Parsippany, NJ, USA) at 0 w, 4 w, 8 w, and 12 w. The spots and wrinkles were measured on both cheeks.

Physical Measurement

Physical measurements (weight, body fat percentage, BMI, and vital signs) were measured at screening, 0 w, 4 w, 8 w, and 12 w. Height was only measured at screening. These measurements were conducted in Maruishi Lab Co., Ltd., and analysis was performed by M&I Science Co., Ltd.

Adverse Events

All participants recorded their lifestyle questionnaire answers every day. Adverse events were determined by a principal investigator.

2.3.2. Safety Evaluation

This clinical study evaluated the safety measurements of the 270 mg cysteine peptide group or placebo group according to Table S1 for healthy 30–64-year-old men and women. These measurements were conducted by Medical Corporation Diastep Tokyo Skytree Ekimae Internal Medicine, and analysis was performed by Pharma Foods International Co., Ltd. (Kyoto, Japan)

Physical Measurements

Weight, BMI, and vital signs were measured at all visit times. Height was only measured at the 1st screening.

2.3.3. Urinalysis

PH, specific gravity, protein, glucose, urobilinogen, occult blood reaction, bilirubin, and ketone bodies were measured at all visits.

2.3.4. Hematology

White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (Plt) were measured at all visit times.

2.3.5. Blood Biochemistry Test

Triglyceride (TG), total cholesterol (T-cho), blood urea nitrogen (BUN), total bilirubin (T-Bil), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (γ-GTP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), uric acid (UA), low-density lipoprotein cholesterol (LDL-Cho), high-density lipoprotein cholesterol (HDL-Cho), hemoglobin A1c (HbA1c), and blood sugar (BS) were measured at all visits.

2.3.6. Adverse Events

All participants recorded their lifestyle questionnaire answers every day. Adverse events were determined by a doctor.

2.4. Test Sample

2.4.1. Efficacy Trial

Our preliminary study showed the efficacy of 45 mg/day cysteine peptide on human skin (data not shown). Three types of tablets containing yeast extract were prepared. The high-dose active sample (90 mg/day cysteine peptide) contained yeast extract A including cysteine peptide. The placebo sample contained yeast extract B excluding cysteine peptide. The low-dose active sample (45 mg/day cysteine peptide) contained the same percentage of yeast extract A and B. Yeast extracts A and B were derived from the same yeast, and the components were nearly identical except that only A contained cysteine peptide. Participants took the 6 tablets corresponding to their respective group for 12 weeks. Food samples were manufactured and supplied by Mitsubishi Corporation Life Sciences Limited.

The ratio of cysteine peptide in each treatment was as follows: reduced-form glutathione:γ-Glu-Cys:Cys-Gly = 84:11:5.

2.4.2. Safety Trial

Two types of tablets containing yeast extract were prepared. The active sample contained yeast extract A including cysteine peptide. The placebo sample contained yeast extract B excluding cysteine peptide. Yeast extracts A and B were derived from the same yeast, and the components were nearly identical except that only A contained cysteine peptide. Participants took the 18 tablets corresponding to their respective group for 16 weeks. Food samples were manufactured and supplied by Mitsubishi Corporation Life Sciences Limited.

The ratio of cysteine peptide in each treatment was as follows: reduced-form glutathione:γ-Glu-Cys:Cys-Gly = 84:11:5.

2.5. Statistical Analysis

2.5.1. Efficacy Trial

Statistical analysis was performed using EZR version 4.1.2 (Jichi Medical University, Saitama, Japan), and significance was defined as * $p < 0.05$. All data were presented as the mean ± standard error (SE). Comparison between the cysteine peptide groups and placebo groups was performed using repeated-measures two-way analysis of variance (ANOVA) followed by Dunnett's test for the evaluation of significance. Comparison of the data at different time points (4, 8, 12 w) within a group was carried out using a one-way analysis of variance (ANOVA) followed by Dunnett's test.

2.5.2. Safety Trial

Statistical analysis was performed using SPSS Statistics 25.0 (IBM, Illinois, USA) and Excel 2021 (Microsoft, Washington, DC, USA), and significance was defined as * $p < 0.05$. All data were presented as the mean ± standard error (SE). Comparison between the cysteine peptide group and placebo group was performed using repeated-measures two-way analysis of variance (ANOVA) followed by an unpaired t-test for the evaluation of significance. Comparison of the data at different time points (4, 8, 12, 16w) within a group was carried out using a one-way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

3.1. Flow Diagram

The experimental diagram of the efficacy trial is shown in Figure 1. A total of 73 participants were screened and 47 eligible participants were obtained. Based on previous similar studies, each group had a target sample size of 15 [18]. They were randomly allocated to three groups: cysteine peptide 90 mg group (n = 16), cysteine peptide 45 mg group (n = 15), and placebo group (n = 16). Allocation factors were gender, age, and L value (face/arm). The baseline of each group is shown in Table 2. There was no significant difference for any parameter among the 90 mg, 45 mg, and placebo groups at the baseline. Two participants were excluded from the analysis because of coronavirus disease 2019 (COVID 19) infection during the intervention. No significant difference was observed when the dropouts were excluded. The intake rate was over 95% in all groups and no protocol violation occurred. Finally, per protocol set (PPS) analysis was performed.

Table 2. Baseline (0 weeks) characteristics of study participants.

	Cysteine Peptide		**Placebo**
Group	90 mg group	45 mg group	Placebo group
n	16	15	16
Gender (Male = 1, Female = 2)	1.80 ± 0.10	1.80 ± 0.11	1.81 ± 0.10
Age	42.94 ± 3.69	46.6 ± 2.87	44.94 ± 2.83
L value (Face)	62.51 ± 1.03	61.57 ± 0.84	61.83 ± 0.89
L value (Arm)	60.05 ± 1.38	59.06 ± 0.82	59.47 ± 1.56

Data are presented as mean ± SE. None of the characteristics was significantly different.

3.2. Effect of Cysteine Peptide on Human Skin Brightness

L value measurements on the arm and face were performed as the main outcomes to evaluate the effect of oral cysteine peptide intake. All groups showed a significant difference in arm brightness at 12 weeks compared to 0 weeks (Table 3). Moreover, the ΔL value was significantly brighter in the 45 mg group compared to the placebo (p = 0.028) at 12 weeks (Figure 2). The ΔL value of 90 mg was higher than the placebo group at 12 weeks.

However, dose-dependent change was not observed, and the 90 mg group did not show a significant difference compared to the placebo (p = 0.111).

Table 3. Cysteine peptide administration improves the L value in the arm.

Task	Group	*n*	Intervention Period (Week)								
			0	4			8			12	
				Mean ± SE	*p*-value		Mean ± SE	*p*-value		Mean ± SE	*p*-value	
					vs. 0 w	vs. pla.		vs. 0 w	vs. pla.		vs. 0 w	vs. pla.
ΔL value (arm)	Cysteine peptide (90 mg)	15	0	0.98 ± 0.30	0.077	0.922	1.54 ± 0.33	0.002	0.938	2.63 ± 0.44	<0.001	0.111
	Cysteine peptide (45 mg)	14	0	1.08 ± 0.18	0.003	0.996	1.74 ± 0.21	<0.001	0.673	3.02 ± 0.35	<0.001	0.028
	Placebo	16	0	1.11 ± 0.30	0.038		1.42 ± 0.31	0.005		1.52 ± 0.45	0.002	

Data are presented as the mean ± SE. Significant differences between the groups were determined using repeated-measures two-way ANOVA followed by Dunnett's test. Significant differences within the group (vs. 0 w) were determined by one-way ANOVA followed by Dunnett's test. pla.: Placebo.

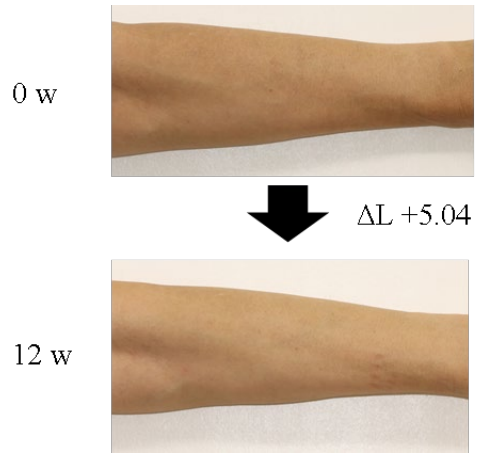

Figure 2. Image of arm in the 45 mg cysteine peptide group at 0 and 12 w. The changed ΔL value was +5.04.

Meanwhile, in within-group comparison, the face brightness of all groups showed significant differences in the ΔL value at 12 w compared to 0 w (Table 4). The L value changes in the cysteine peptide groups were higher than those in the placebo group at 12 weeks; however, there was no significant difference between the cysteine peptide group and the placebo group during this research period.

Table 4. Cysteine peptide administration improves the L value in the face.

Task	Group	N	Intervention Period (Week)								
			0	4			8			12	
				Mean ± SE	*p*-value		Mean ± SE	*p*-value		Mean ± SE	*p*-value	
					vs.0 w	vs. pla.		vs.0 w	vs. pla.		vs.0 w	vs. pla.
ΔL value (face)	Cysteine peptide (90 mg)	15	0	1.99 ± 0.37	<0.001	0.614	2.75 ± 0.45	<0.001	0.452	3.86 ± 0.46	<0.001	0.439
	Cysteine peptide (45 mg)	14	0	2.02 ± 0.50	0.006	0.660	2.99 ± 0.49	<0.001	0.709	3.53 ± 0.55	<0.001	0.761
	Placebo	16	0	2.48 ± 0.40	<0.001		3.44 ± 0.42	<0.001		3.09 ± 0.48	<0.001	

Data are the mean ± SE. Significant differences were determined using repeated-measures two-way ANOVA followed by Dunnett's test. Significant differences within the group (vs. 0 w) were determined using one-way ANOVA followed by Dunnett's test. pla.: placebo.

3.3. Effect of Cysteine Peptide on Human Skin Spots and Wrinkles

The numbers of spots and wrinkles on the left and right cheeks were measured as secondary outcomes. At 4 w, the number of spots on the right cheek in the 45 mg cysteine peptide group significantly decreased compared to the placebo ($p = 0.037$, data not shown). However, those measured at 0 w, 8 w, and 12 w did not show significant differences compared with the placebo. In addition, there was no significant difference in the number of wrinkles in any of the groups compared with the placebo (data not shown).

3.4. Safety Evaluation

In the results of 45 mg or 90 mg cysteine peptide, there were no abnormal changes in the vital signs and body measurements judged by the principal investigator. Although several adverse events occurred during the study period, all the adverse events were unrelated to the study food according to the principal investigator.

Additionally, we evaluated the safety of 270 mg cysteine peptide. Based on previous similar studies, each group had a target sample size of 50 [12]. The flow diagram and baseline characteristics are shown in Figure S1 and Table S2. There were a few significant differences between the 270 mg cysteine peptide and placebo group in blood biochemical tests, hematological tests, and urinalysis safety evaluations. The significant differences between the groups were observed in TP and ALB at 4 w and MCHC at 8 w, and the within-group significant differences were observed in TP and MCHC at 8 w. However, all were determined as within the range of physiological fluctuations (Tables S3–S6) by the principal investigator. In addition, no serious adverse events related to study food were observed. Therefore, the principal investigator judged that 270 mg cysteine peptide intake for 16 weeks was safe.

4. Discussion

This study indicated that oral intake of cysteine peptide was beneficial for human skin brightness based on a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial. Reportedly, 250 and 500 mg/day GSH affects skin brightness [15,16]. However, there is no report of the effect of cysteine peptide on human skin brightness. This study demonstrated that skin brightness was significantly upregulated using 45 mg/day cysteine peptide compared to the placebo. This is the first report showing the effects of cysteine peptide on human skin.

Cysteine peptide consists of GSH, Cys-Gly, and γ-Glu-Cys. GSH is widely present in animals and plants. It is one of the most abundant thiol peptides distributed in high concentrations (1–8 mM), and it plays crucial roles in antioxidant systems [19]. The amount of GSH is regulated by the synthesis in almost all cells [20,21]. It is derived from cysteine, glutamate, and glycine by intracellular enzymes. Glutamate and cysteine are synthesized by γ-glutamyl-cysteine synthetase into γ-Glu-Cys, and GSH is then produced from γ-Glu-Cys and glycine by glutathione synthetase [22]. Cys-Gly is a source of cysteine and glycine. GSH deficiency causes various diseases [23]. Therefore, we hypothesized that cysteine peptide (GSH, Cys-Gly, and γ-Glu-Cys) intake may help to increase the amount of GSH in the human body. Glutathione is absorbed in the intestinal cell without degradation [24]. Moreover, oral intake of GSH enhances its levels in a protein-bound form in human blood [25,26]. These studies suggest that orally administered GSH is absorbed into the blood and oral intake of cysteine peptide might play a beneficial role in the human body.

Oral administration of GSH (500 mg/day) for 4 weeks resulted in lightening of skin color [16]. In addition, GSH intake of 250 mg/day for 12 weeks also influenced skin properties, including the melanin index, compared to the placebo with stratified analysis [15]. The main objective of this study was to elucidate whether cysteine peptide affects skin-lightening efficacy at a dose of 45 or 90 mg/day for 12 weeks. The 45 mg group showed an effect in a time-dependent manner, and the ΔL value was significantly upregulated in the 45 mg group compared to the placebo ($p = 0.028$). This contrasts with a previous report (250 mg/day GSH) showing that there is a significant difference in the melanin index with

only stratified analysis (subjects aged >40 years old) [15]. The differences are probably caused by the physiographic factor of attendance. The present study was performed with male and female Japanese volunteers. Meanwhile, the previous trial was performed with female volunteers from Bangkok, Thailand. The sunshine exposure level affects various skin features. The sun exposure level in Thailand is higher than that in Japan [27]. These facts imply that 45 mg cysteine peptide is favorable for 20–65-year-old men and women living in countries with moderate sun exposure. Additionally, the administration of 500 mg GSH for 4 weeks improves melanin indices compared with placebo [16]. Therefore, higher doses of cysteine peptide (500 mg) may also brighten the skin and shorten time the onset of the effect from 12 weeks; this hypothesis requires further study. Meanwhile, the dose-dependent result on the arm's ΔL value was not observed when comparing the 45 mg and 90 mg groups, and no significant difference was observed in the 90 mg group compared with the placebo. There is only a small difference in the abundance of active compounds between the 45 mg and 90 mg group in this trial, and the actual difference was quite low compared with previous GSH trials (250 or 500 mg) [15,16]. This may explain the lack of dose dependence. Thus, a higher dose such as 250 mg should be set for to comparisone with 45 mg in this study. On the other hand, the higher ΔL value obtained from the arm of the 90 mg group compared with the placebo was insignificant. This motivates us to conduct an additional study with an increased number of participants.

Although GSH is the main ingredient of active compounds in this study, cysteine peptide may have shown a synergy effect through the activation of GSH synthesis signaling. This can be elucidated by investigating the blood GSH abundance in the cysteine peptide intake group to determine whether the intake of Cys-Gly and γ-Glu-Cys is a source of GSH.

All groups (cysteine peptide and placebo) showed a significant difference in the L value of the face when comparing 0 w and 12 w. One of the possible reasons is the season in which the trial was conducted [28]. This study was performed from December to March, which is winter in Japan. Melanin levels generally change depending on the sunshine exposure level; therefore, the brighter skin in all groups might be caused by shortening sunshine exposure time compared to autumn, and the ΔL value could have increased to return to the original skin color of each participant even after 12 weeks. However, a significant difference was observed between the 45 mg group and placebo at 12 weeks. GSH could affect skin turnover [29,30]. From this evidence and these results, we hypothesized that cysteine peptide not only affected the skin color through an anti-melanogenesis effect but also affected turnover in this study. A previous study (250 mg/day GSH) involving stratified analysis only observed a significant difference in the arm melanin index compared to the placebo, but not in the face. This study showed that the L value changes of cysteine peptide groups were higher than the placebo in the arm, and favorable results were observed in the face at 12 weeks. However, there was no significant difference between cysteine peptide groups and the placebo in the face during this research period. These results imply that the face's L value is strongly affected by sunshine and other causes since it is always exposed to the external environment. Meanwhile, the arm is covered with clothes, especially in the winter season when this clinical trial was performed. Therefore, it is supposed that the L value of the arm measurement is a better representation of the effect of cysteine peptide than the face in this clinical study.

Overall, our study demonstrated the results of cysteine peptide on human skin brightness for 12 weeks. The cysteine peptide dosage was much lower than that in previous reports using only GSH. Our strengths include the wide range of age groups for male and female subjects and the fact that it was a randomized, double-blind, placebo-controlled, parallel-group comparative study. Limitations include the low number of attendances and origin. The power from the present result was 0.736. Additionally, the examination of the effect of cysteine peptide was limited to 45 and 90 mg in this trial. Further studies are needed to elucidate the higher dose and lowest available dose of cysteine peptide for brightness. Additionally, the face is constantly exposed to UV rays, which could have influenced the results on the face.

In conclusion, this is the first study reporting that the oral intake of cysteine peptide (45 mg/day) in 20–65-year-old healthy males and females for 12 weeks affects skin brightness compared with the placebo group without any side effects. These results suggest that the use of cysteine peptide is a promising strategy for human skin brightness.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics10030072/s1. Figure S1. Flow diagram of the present study. Table S1. Schedule of the visit. Table S2. Baseline (0 week) characteristics of study participants. Table S3. Physical measurement. Table S4. Urinalysis. Table S5. Blood biochemistry test. Table S6. Blood biochemistry test.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: This clinical study was funded by Mitsubishi Corporation Life Sciences Co., Ltd., and consigned to Pharma Foods International Co., Ltd. Pharma Foods International Co., Ltd., has reconsigned this clinical study with M&I Science CORP or HUMA R&D CORP. Mitsubishi Corporation Life Science Co., Ltd., and Pharma Foods International Co., Ltd., were not involved in the implementation of this clinical study, but Pharma Foods International Co., Ltd., was involved in the analysis. M&I Science CORP. or HUMA R&D CORP concluded a business consignment contract with the testing organization and conducted the test. The remuneration from these business consignment contracts was a legitimate business remuneration for the execution of the test and did not affect the results.

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