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Original Research

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Liquid collagen from freshwater fish skin ameliorates hydration, roughness and elasticity in photo-aged skin: a randomized, controlled, clinical study

Joong Su Lee [1§], **Yeo Cho Yoon** [1], **Ju Myung Kim** [1], **Yun-Ho Kim** [1], **Young-Hee Kang** [2], **and Yong Chul Shin** [3]

[1]Healthcare & Nutrition Laboratory, Amicogen, Inc., Seongnam 13488, Korea
[2]Department of Food and Nutrition and Korean Institute of Nutrition, Hallym University, Chuncheon 24252, Korea
[3]Amicogen, Inc., Jinju 52840, Korea

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[§]Corresponding Author:
Joong Su Lee
Healthcare & Nutrition Laboratory, Amicogen, Inc., C-602, 700 Daewangpangyo-ro, Bundang-gu, Seongnam 13488, Korea.
Tel. +82-070-4266-5098
Fax. +82-31-628-0980
Email. leejs@amicogen.com

ORCID iDs
Joong Su Lee
https://orcid.org/0000-0002-6609-5618
Yeo Cho Yoon
https://orcid.org/0000-0001-5775-5285
Ju Myung Kim
https://orcid.org/0000-0001-6850-1977
Yun-Ho Kim
https://orcid.org/0009-0006-0381-7819
Young-Hee Kang
https://orcid.org/0000-0003-2039-7968
Yong Chul Shin
https://orcid.org/0000-0002-3102-924X

ABSTRACT

BACKGROUND/OBJECTIVES: Collagen is commonly used in diverse forms as a functional component in skincare products. On the other hand, the effects of collagen on human skin are controversial. Dietary collagen hydrolysates from freshwater *Pangasius hypophthalmus* fish skin ameliorated photo-aged skin of hairless mice. This study conducted a randomized, double-blind, placebo-controlled clinical trial to determine if liquid fish collagen (Collagen-Tripep20™, Tripep20) as a drink strengthens skin health and quality.
SUBJECTS/METHODS: In this clinical trial, 85 subjects aged 35–60 yrs were diagnosed with photo-aged skin. Eighty-five subjects were randomized to receive either Tripep20 (n = 44) or placebo (n = 41). Seventy-eight subjects fully participating for a 12-week period consumed 1,000 mg of Tripep20 (n = 41) or placebo (n = 37) in a 50-mL bottle as a daily drink. The intend-to-treat and per-protocol populations were 85 and 78, respectively. Skin hydration, wrinkles, and elasticity were assessed at 0 (baseline), 6, and 12 weeks during the study period.
RESULTS: Skin hydration in the Tripep20 group was significantly higher from 6 weeks ($P < 0.001$) than the baseline. After 12 weeks, the Crow's-feet visual score and skin roughness (R_a, R_q, and R_{max}) were significantly improved in the Tripep20 group than in the placebo group ($P < 0.05$). Consuming liquid collagen Tripep20 greatly enhanced skin elasticity (Gross R2, Net R5, and Biological elasticity R7) in 6 weeks compared to the placebo group. The Tripep20 group showed a significant increase in skin elasticity from the baseline after 6 and 12 weeks ($P < 0.001$). Neither abnormal symptoms nor adverse events were encountered during the study period in subjects ingesting Tripep20 or placebo. The changes in parameters related to hematology and clinical chemistry were within the normal ranges.
CONCLUSION: Oral consumption of liquid collagen Tripep20 was safe and well-tolerated. The results of this study show that freshwater fish-derived liquid collagen Tripep20 can be used as a healthy functional food ingredient to improve skin moisturizing, anti-wrinkling, and elasticity in an aging population.

Keywords: Collagen; fresh water; skin aging; elasticity; clinical trial

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Conflict of Interest
JSL, YCY, JMK, YHK, and YCS were employed by Amicogen Inc.
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Author Contributions
Conceptualization: Yoon YC, Kim JM; Data curation: Lee JS, Shin YC; Formal analysis: Yoon YC, Kim JM, Kim YH; Investigation: Kim JM, Kim YH; Methodology: Yoon YC; Resources: Lee JS; Supervision: Lee JS; Writing - review & editing: Lee JS, Kang YH.

INTRODUCTION

The skin is an intricate structure comprising the largest body area. This organ is a barrier that separates the external and internal environments and is composed of 3 main layers: the epidermis, dermis, and subcutaneous layer [1]. Although age-dependent skin changes are caused by intrinsic and extrinsic processes, these changes are more pronounced in photo-aged skin, such as sun-exposed facial skin, compared to sun-protected buttock skin [2,3]. The photoaging process of the skin is triggered by solar ultraviolet (UV) radiation, leading to the decomposition of collagen and elastic fibers. This process reduces the synthesis of hyaluronic acid (HA) and ultimately leads to wrinkle formation, drying, and elastic loss [4-6].

Collagen forms the main structural framework of the dermal extracellular matrix (ECM), constituting more than 70% of the dermis [7]. Collagen, the most abundant protein in the body, is found in the skin, connective tissues, bones, cartilage, and teeth. This protein imparts strength, elasticity, and structure to these tissues [8]. The unique triple helix structure of collagen is characterized by repeated Gly-X-Y sequences mainly consisting of glycine-proline-hydroxyproline (Gly-Pro-Hyp) triplets. Despite the theoretical possibility of 400 triplets, only a few are observed in collagen, accounting for 50–60% of the sequence. The selective occurrence of these triplets contributes significantly to the stability of the collagen structure [9-11].

The initial hydrolysis of collagen tripeptides is catalyzed by aminopeptidase N bound to the brush border membrane of the intestinal epithelium to form dipeptide proline-hydroxyproline (Pro-Hyp) [10,12]. This dipeptide, an important component of collagen-derived peptides, is absorbed into enterocytes by H^+-coupled oligopeptide transporters [10]. The Gly-Pro-Hyp concentration in plasma is enhanced after consuming low-molecular-weight collagen hydrolysates or tripeptides compared to high-molecular-weight collagen peptides [11]. In addition, Gly-Pro-Hyp and Pro-Hyp are very stable in gastrointestinal fluids and plasma, showing resistance to degradation by gastric acid, digestive enzymes, pancreatin, and plasma peptidases [11]. Gly-Pro-Hyp and Pro-Hyp are remarkably stable in the digestive system and permeate the intestinal barrier efficiently. Hence, dietary collagen may be an effective option for delivering bioactive peptides.

Numerous studies have reported that the supplementation of collagen or its tripeptides improves skin wrinkles, elasticity, and hydration [13-15]. Collagen tripeptides can be a potent anti-aging component, enhancing the synthesis of type 1 collagen and suppressing matrix metalloproteinase (MMP) protein expression [5,16]. A recent study reported hydrolyzed marine collagen ameliorates skin health in an aging population by improving skin wrinkles, elasticity, hydration, radiance, and firmness [17]. The authors' previous study examined whether dietary collagen hydrolysates from *Pangasius hypophthalmus* fish skin ameliorate furrowed and parched skin caused by photo-aging in hairless mice [13]. Gly-Pro-Hyp was the functional component of collagen hydrolysates from the freshwater fish skin. Collagen hydrolysates attenuate deep wrinkles, epidermal thickening, and skin water loss in photo-aged mice by decreasing the expression of aquaporin 3 (AQP3) and hyaluronidase 1 (HYAL1) [13]. The current randomized, double-blind, placebo-controlled clinical trial was performed to determine if the oral consumption of hydrolyzed fish skin collagen (Collagen-Tripep20™, Tripep20), derived from *Pangasius hypophthalmus* fish skin gelatin, improves skin health and quality. In this clinical trial, 1,000 mg of Tripep20 collagen was ingested orally as a daily drink for 12 weeks to 78 female subjects aged 35–60 yrs diagnosed with photo-aged skin.

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Table 1. Formulation of Collagen-Tripep20™ in a 50 mL bottle

Ingredients	Tripep20		Placebo	
	Content (mg)	Content (%)	Content (mg)	Content (%)
Tripep20	1,000	2.000	0	0.000
Vitamin C	100	0.200	100	0.200
Fruit concentrate mix	3,000	6.000	3,000	6.000
Flavor mix	200	0.400	200	0.400
Excipients	400	0.800	400	0.800
Sweetener	1,513	3.025	1,513	3.025
Water	43,788	87.575	44,788	89.575
Total	50,000	100.000	50,000	100.000

Hydrolyzed fish collagen (Collagen-Tripep20™, Tripep20) was prepared from freshwater *Pangasius hypophthalmus* fish skin by collagenase digestion. The average molecular weight of the entire collagen was 500 Da, with a total content of 20% tripeptides containing 3.2% glycine-proline-hydroxyproline.

SUBJECTS AND METHODS

Preparation of test materials and dose determination

Tripep20 was prepared from Vietnam pangasius fish (*Pangasius hypophthalmus*) skin by collagenase digestion. The average molecular weight of the entire Tripep20 collagen was 500 Da, with a total content of 20% tripeptides containing 3.2% Gly-Pro-Hyp. Other *in vivo* studies confirmed the stability and bioavailability of Gly-Pro-Hyp [11,14]. A 50 mL bottle contained 1,000 mg of Tripep20 and vehicle materials (**Table 1**). The placebo was the same formulation in taste and flavor as Tripep20, except for replacing Gly-Pro-Hyp with water.

Based on the guidelines for estimating the maximum safe dose in initial clinical trials of healthy subjects, 1,000 mg of Tripep20 appeared suitable for humans with an average body weight of 60 kg [18]. In addition, several comparable studies using doses from 1.0 to 3.0 g/day have been reported [14,19-21].

Study design and ethical aspects

This study was designed as a randomized, placebo-controlled, double-blind clinical trial. All subjects provided informed consent for inclusion before participation. The current study adhered to the relevant Good Clinical Practice guidelines and the Standard Operating Procedures established by Ellead Skin and Bio Research (Ellead, Seongnam, Korea) from 9 December 2021 to 10 June 2022. This study protocol was reviewed and approved by the Institutional Review Board of Ellead (project number EL-210712320 and IRB No. 211019T001, 10 June 2022).

Study subjects

Healthy women aged 35–60 yrs (n = 121) who volunteered and met the inclusion and exclusion criteria were recruited for this study (**Table 2**). The inclusion criteria stated that the participants must show Crow's-feet scores between 2 and 6, as evaluated by dermatologists using the Global Photodamage Scoring system [22]. Before commencing the clinical study, the subjects were provided with clear and detailed information on the goals, protocols, and potential risks related to participation. One hundred and twenty-one subjects signed an informed consent form, of which 36 individuals withdrew the consent form, and the remaining 85 commenced the study. Seven subjects discontinued the study for personal reasons, leaving 78 who completed the study (**Fig. 1**).

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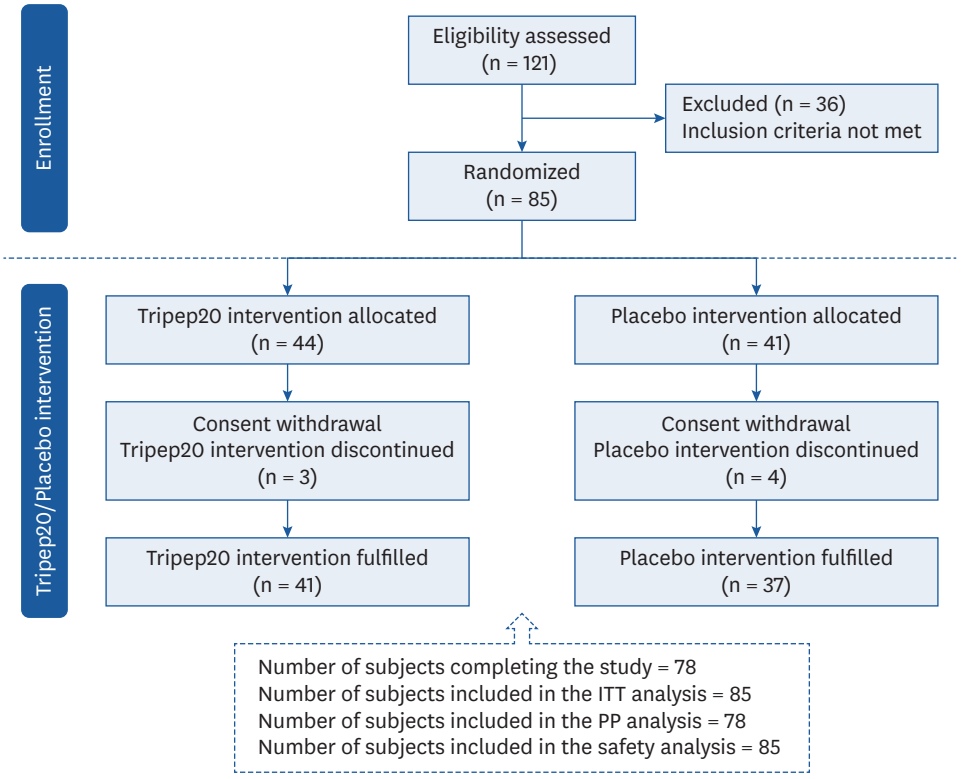

Fig. 1. Subject recruitment and allocation for the randomized clinical trial. Hydrolyzed fish collagen (Collagen-Tripep20™, Tripep20) was prepared from Vietnam pangasius fish (*Pangasius hypophthalmus*) skin by collagenase digestion. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to subjects as a daily drink.

Table 2. Criteria for inclusion and exclusion when recruiting subjects

Criteria	Terms
Inclusion criteria	· Healthy female aged 35–60 yrs · Diagnosed by dermatologists with wrinkle(s) in the Crow's-feet area, with global photo-damage score between 2 and 6 · Subjects who calculate with Corneometer reading of less than 60 A.U. · Informed of the purpose and the protocol of the study and signed a written informed-consent form · Co-operative and available during the study period
Exclusion criteria	· History of allergies to cosmetics, pharmaceutical products, or foods containing ingredients included in the test formulation · Diagnosis of any systemic illness that may be aggravated by participation in the study · Use of oral retinoids or oral steroids in the 6 mon prior to initiation of the study · Use of topical retinoids, anti-wrinkle cosmetic products including retinol and/or AHA, or moisture-rich cosmetic products within the 6 mon prior to initiation of the study · Skincare therapy using lasers or peeling within the 6 mon prior to initiation of the study · Current participation in another clinical test, or participation in any type of wrinkle study within the 6 mon prior to initiation of the study · Abnormal liver function or abnormal renal function · Current smoking habit or history of smoking within the past 1 yr · Excessive alcohol intake · Women who had undergone, or planned to undergo, pregnancy or breastfeeding · Blood pressure > 140/90 mmHg or hypertension with intake of a diuretic · Problems with overall findings in blood-test results as determined by a specialist · History of asthma or allergic disease · History of depression, schizophrenia, alcoholism, drug addiction, or mental illness · Current or previous intake of contraceptives, female hormones, obesity drugs, absorption inhibitors, antidepressants, or appetite suppressants · Any condition judged by the investigator to be unsuitable for participation in the study

A.U., arbitrary units; AHA, alpha hydroxy acid.

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Study schedule

All subjects ingested a daily drink (1,000 mg Tripep20 or placebo in a 50 mL bottle) of the assigned study formulation. All subjects were instructed not to take similar dietary supplements or use any skin care, including face masks, packs, or massages, to minimize interference. Furthermore, to ensure consistent skin conditions throughout the study, the subjects were not allowed to apply topical cosmetics other than those provided by Ellead for a 2-week washout period before starting the study and for the entire 12-week study period. Each subject visited the research center 5 times for pre-consumption evaluation of the study formulation to measure the efficacy: at screening (–2 weeks), dietary survey (–1 week), baseline (0 weeks), at 6 weeks, and 12 weeks after ingesting the study formulation. Finally, the subjects were evaluated for safety 2 days after completing the 12-week study. The subjects were banned from using cosmetics for 12 h before visiting the test facility. Before each evaluation, the subjects cleared the Crow's-feet region, cleansed their entire face with foaming facial cleaners, and rested for 30 min in a room set to a temperature between 20°C and 22°C with a relative humidity between 40 and 60. Each visit used the same techniques on identical facial areas applied to the initial evaluation of the baseline. Before attending the safety evaluation, the subjects were instructed not to consume food or drinks for at least 8 h.

Safety and tolerability

The safety and tolerability were evaluated in subjects receiving the test materials: Tripep20 and placebo [23]. The safety parameters were analyzed using standardized procedures in blood and urine samples. The following analyses were performed: hemoglobin, hematocrit, platelet count, red blood cell count, red blood cell volume, white blood cell count and liver function tests (aspartate aminotransferase, alanine aminotransferase, and total bilirubin), and kidney function tests (urinary pH, creatinine, and urine-specific gravity). The tolerability was assayed through dermatological examinations before, during, and after application [24]. Through interviews and questionnaires, the adverse events were monitored throughout the study [25]. Finally, a questionnaire was completed at the end of the study.

Skin hydration analysis

Skin hydration of the external layer of the epidermis (stratum corneum) was examined on the cheek using a Corneometer (Corneometer CM 825; Courage and Khazaka, Cologne, Germany) [26]. At least 5 measurements were made at various sites in the test area on the cheek. The means of 3 values were used for the analysis, except for the maximum and minimum readings.

Dermatological examinations

For the dermatological examinations using a double-blind method, 2 dermatologists assessed the Crow's-feet area using a global photodamage scoring system. Reaction findings and type before and after the study were documented and scored on a 7-point scale: 0 = no finding, 1 = none/mild skin reaction, 2 = mild skin reaction, 3 = mild/moderate skin reaction, 4 = moderate skin reaction, 5 = moderate/severe skin reaction, 6 = severe skin reaction, and 7 = very severe skin reaction. The eye wrinkles on the Crow's feet area were measured using a PRIMOS optical 3-dimensional *in vivo* skin measurement device (GFMesstechnik GmbH, Berlin, Germany).

Skin roughness analysis

The skin roughness was measured using the PRIMOS 3-dimensional skin imaging system [27]. The measurements were taken on the same site at the baseline, 6-week, and 12-week

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points of use of the study formulation. This study evaluated the values of R_a representing the arithmetic average roughness, R_q of root mean square roughness, and R_{max} denoting maximum roughness depth in the stored images. The R_a, R_q, and R_{max} values indicate the depth of the wrinkles from the skin surface.

Skin elasticity analysis

Skin elasticity of the designated Crow's-foot area was evaluated using a Cutometer MPA 580 device. The measurements were repeated 3 times with 2 s of suction and 2 s of relaxation for each measurement. The curves of skin deformation obtained were analyzed using Win Cutometer MPA software to extract the following skin elasticity: R2 (Overall elasticity of the skin), R5 (Net elasticity), and R7 (Biological elasticity).

Statistical analysis

Statistical analyses were conducted using 2 approaches: an intention-to-treat (ITT) analysis and a per-protocol (PP) analysis, with significance levels of 0.05, 0.01, and 0.001, unless stated otherwise. For ITT analysis, the missing data were handled using the last-observation-carried-forward method. PP analysis was used for efficacy evaluation, while ITT analysis was used for the safety assessment. All the efficacy results of blood, urine, and vital signs were analyzed using descriptive statistics and compared before and after treatment using paired *t*-tests. A significant difference from the baseline for changes in skin hydration and wrinkling was evaluated with the Wilcoxon signed-rank test and repeated measures analysis of variance with contrast test. A Mann-Whitney *U* test using the delta values was used to compare the treatment groups.

RESULTS

Baseline characteristics of the subjects

Eighty-five female subjects aged 35–60 yrs were randomized at the baseline and allocated to either the Tripep20 (n = 44) or the placebo (n = 41) group. Three subjects in the Tripep20 group and 4 in the placebo group withdrew consent for personal reasons. The ITT population and PP population were 85 and 78, respectively. The age, body weight, systolic and diastolic blood pressure, and Crow's-feet visual grade were similar in the Tripep20 and placebo groups at the baseline (**Table 3**).

Evaluation of safety of Tripep20 supplementation

During Tripep20 supplementation, blood-chemical and urine tests were carried out, and the vital signs were measured. All the blood and urine data were within the healthy clinical reference ranges after consuming Tripep20 or the placebo for 12 weeks. The hemoglobin

Table 3. Baseline characteristics of the study subjects

Characteristic	Tripep20 (n = 41)	Placebo (n = 37)	*P*-value
Age (yrs)	49.829 ± 6.107	50.135 ± 5.945	0.824
Weight (kg)	57.515 ± 8.303	56.351 ± 6.346	0.493
Systolic blood pressure (mmHg)	116.829 ± 9.508	115.081 ± 10.196	0.436
Diastolic blood pressure (mmHg)	69.659 ± 8.822	67.946 ± 8.107	0.377
Pulsation (beats/min)	77.068 ± 11.218	75.024 ± 7.023	0.544
Crow's-feet visual grade	2.585 ± 0.706	2.946 ± 0.970	0.115

Hydrolyzed fish collagen (Collagen-Tripep20™, Tripep20) was prepared from *Pangasius hypophthalmus* fish skin by collagenase digestion. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to all subjects as a daily drink. The values are presented as mean ± SD. *P*-value for the Mann-Whitney *U* test comparison between the Tripep20 and placebo groups.

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Table 4. Hematology and clinical chemistry analysis at the baseline and week 12 for the 85 subjects in the safety population

Safety parameters	Time points	CTP20 group		Placebo group	
		Mean ± SD	*P*-value	Mean ± SD	*P*-value
Albumin (g/dL)	Baseline	4.486 ± 0.257	0.438	4.476 ± 0.213	0.893
	At 12 weeks	4.502 ± 0.235		4.485 ± 0.165	
Hemoglobin (g/dL)	Baseline	12.702 ± 1.527	0.049*	13.161 ± 1.024	0.001**
	At 12 weeks	12.577 ± 1.249		12.902 ± 1.061	
Hematocrit (%)	Baseline	39.725 ± 4.024	0.697	40.385 ± 2.700	0.245
	At 12 weeks	39.741 ± 3.312		40.076 ± 2.826	
Platelet (10^3/μL)	Baseline	277.477 ± 65.922	0.347	260.415 ± 75.091	0.023*
	At 12 weeks	282.773 ± 61.753		271.463 ± 73.819	
Red blood cell (10^6/μL)	Baseline	4.346 ± 0.302	0.116	4.275 ± 0.285	0.099
	At 12 weeks	4.288 ± 0.269		4.225 ± 0.290	
White blood cell (10^3/μL)	Baseline	5.625 ± 1.501	0.062	5.041 ± 1.282	0.256
	At 12 weeks	5.275 ± 1.261		5.429 ± 2.477	
Mean cell volume (fL)	Baseline	91.355 ± 6.533	< 0.001***	94.532 ± 3.644	0.137
	At 12 weeks	92.745 ± 6.317		94.946 ± 4.529	
Glucose (mg/dL)	Baseline	88.159 ± 8.944	0.226	84.512 ± 8.964	0.060
	At 12 weeks	89.568 ± 11.182		87.268 ± 9.628	
ALT (U/L)	Baseline	15.114 ± 5.537	0.259	13.610 ± 5.176	0.680
	At 12 weeks	14.182 ± 6.431		14.659 ± 7.647	
AST (U/L)	Baseline	18.955 ± 4.046	0.190	18.927 ± 4.782	0.341
	At 12 weeks	18.182 ± 3.611		20.366 ± 8.587	
Total bilirubin (mg/dL)	Baseline	0.410 ± 0.171	0.522	0.550 ± 0.335	0.545
	At 12 weeks	0.425 ± 0.167		0.515 ± 0.545	
Urinary (pH)	Baseline	6.045 ± 0.933	0.064	6.000 ± 0.859	0.434
	At 12 weeks	6.284 ± 0.167		5.915 ± 0.749	
Creatinine (mg/dL)	Baseline	0.603 ± 0.082	0.538	0.645 ± 0.075	0.959
	At 12 weeks	0.608 ± 1.008		0.646 ± 0.090	
Urine specific gravity	Baseline	1.019 ± 0.006	0.042*	1.021 ± 0.005	0.157
	At 12 weeks	1.021 ± 0.008		1.022 ± 0.007	

Hydrolyzed fish collagen (Collagen-Tripep20™, Tripep20) was prepared from *Pangasius hypophthalmus* fish skin by collagenase digestion. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to all subjects as a daily drink. *P*-value for a paired *t*-test or Wilcoxon signed-rank test compared to the baseline. The normality was assessed using a Kolmogorov-Smirnov test, and the homogeneity was examined using an independent t-test and Mann-Whitney *U* test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase.

data, mean cell volume, and platelets at week 12 were significantly different from those of the baseline ($P < 0.05$) (**Table 4**). On the other hand, the changes in these safety parameters were clinically insignificant. All other hematological and clinical chemistry parameters showed no changes after ingesting Tripep20 or placebo (**Table 4**). No significant differences in the systolic pressure (114.841 ± 9.611 vs. 115.537 ± 9.461 mmHg), diastolic pressure (69.045 ± 7.492 vs. 71.073 ± 8.171 mmHg), and heart rate (76.614 ± 10.098 vs. 74.195 ± 6.615 beats) were noted between the Tripep20 and placebo groups. The subjects completed the protocol without showing any adverse symptoms during the study period.

Effects of Tripep20 on skin hydration

Skin hydration was significantly higher in the Tripep20 group from 6 weeks ($P < 0.001$) than at the baseline (**Fig. 2A**). Although there was a significant increase ($P = 0.004$) at 6 weeks in the placebo group, the hydration declined at 12 weeks to the baseline ($P = 0.977$). Furthermore, the positive changes in skin hydration were significantly higher in the Tripep20 group than in the placebo group at 6 weeks ($P < 0.001$) and 12 ($P < 0.001$; **Fig. 2B**). Accordingly, consuming the liquid collagen Tripep20 improves skin moisturizing.

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(A)

Skin hydration analyzed by corneometer CM825					
	Tripep20		Placebo		Tripep20/Placebo
	A.U.	*P*-value[a]	A.U.	*P*-value[b]	*P*-value[c]
Baseline	46.229 ± 8.012		48.121 ± 6.644		
at 6 weeks	47.428 ± 8.065	< 0.001***	48.432 ± 6.700	0.004##	< 0.001†††
at 12 weeks	48.245 ± 8.250	< 0.001***	48.124 ± 6.531	0.977	< 0.001†††

(B)

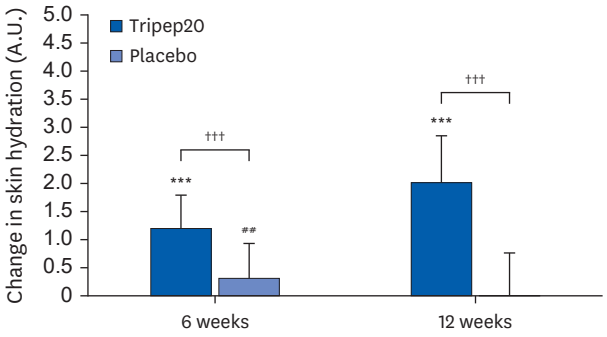

Fig. 2. Effects of hydrolyzed fish skin collagen (Collagen-Tripep20™, Tripep20) on skin hydration (A) and its changes (B) in subjects receiving Tripep20 or placebo. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to the subjects as a daily drink. Skin hydration was analyzed using a Corneometer CM825 device. A.U., arbitrary units.
[a]*P*-values for Wilcoxon signed-rank test, compared to baseline values.
[b]*P*-values for repeated measures analysis of variance with contrast test, compared to baseline values.
[c]*P*-values for the Mann–Whitney *U* test using delta values, compared values of the Tripep20 versus the placebo.
***$P < 0.001$ (Wilcoxon signed-rank test); ##$P < 0.01$ (repeated measures analysis of variance with contrast test); †††$P < 0.001$ (Mann-Whitney *U* test using delta values).

Reduction of skin wrinkles and roughness by Tripep20

The degree of wrinkles in the edges of the cheeks (Crow's-feet) was evaluated photographically by dermatologists using the scores for the wrinkle grades. In the Tripep20 group, the Crow's-feet visual score rating of wrinkles on the cheek was significantly lower after 12 weeks ($P < 0.001$; **Table 5**). In addition, the changes in the Crow's-feet visual score were significantly lower in the Tripep20 group than in the placebo at 12 weeks ($P = 0.004$; **Fig. 3A**).

The current study attempted to quantify skin roughness for wrinkles using an image processing technique from a PRIMOS dermatoscope. The roughness values obtained from the PRIMOS device were presented as R_a, R_q, and R_{max} to evaluate the effectiveness of Tripep20. The photographic roughness values of R_a and R_q decreased gradually but significantly in the Tripep20 group after 6 weeks (**Table 5**). Compared to the placebo group, the decrease in R_a and R_q in the Tripep20 group was much higher at weeks 6 and 12 (**Fig. 3B and C**). Furthermore, ingesting Tripep20 for 12 weeks improved the R_{max} value compared to the placebo ($P = 0.045$; **Table 4**). In addition, the change in the R_{max} value obtained at week 12 was significantly higher in those consuming Tripep20 (**Fig. 3D**). Therefore, Tripep20 can be useful for restoring rough skin and reducing skin wrinkles and furrows.

Qualitative evaluation was performed to show a visible improvement in the consumption of Tripep20 through PRIMOS 3-dimensional pictures contouring eye wrinkles. The Tripep20 skin was smoother and taut at week 12, with fewer wrinkles than the placebo skin (E). This reduction in appearance is a clear positive sign of the efficacy of Tripep20.

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Table 5. Crow's-feet visual scores and skin roughness parameters

Parameters	CTP20 group		Placebo group		CTP20/Placebo
	Mean ± SD	*P*-value[a]	Mean ± SD	*P*-value[a]	*P*-value[b]
Crow's-feet visual grade					
Baseline	2.585 ± 0.706		2.946 ± 0.970		
At 6 weeks	2.500 ± 0.750	0.070	2.919 ± 0.954	0.480	0.312
At 12 weeks	2.293 ± 0.741	< 0.001***	2.919 ± 0.932	0.581	0.004††
Wrinkle by R_a (Arithmetic average roughness, μm)					
Baseline	12.905 ± 2.448		12.845 ± 2.509		
At 6 weeks	12.650 ± 2.471	< 0.001***	12.835 ± 2.501	0.635	0.005††
At 12 weeks	12.404 ± 2.149	< 0.001***	12.789 ± 2.404	0.556	< 0.001†††
Wrinkle by R_q (Root mean square roughness, μm)					
Baseline	15.867 ± 2.971		15.823 ± 3.072		
At 6 weeks	15.592 ± 3.010	< 0.001***	15.829 ± 3.117	0.757	0.012†
At 12 weeks	15.312 ± 2.638	< 0.001***	15.784 ± 2.957	0.803	0.001††
Wrinkle by R_{max} (Maximum roughness depth, μm)					
Baseline	84.204 ± 16.824		84.140 ± 16.181		
At 6 weeks	83.653 ± 17.914	0.499	83.888 ± 17.442	0.582	0.822
At 12 weeks	82.399 ± 16.518	0.046*	84.409 ± 16.341	0.531	0.045†

Hydrolyzed fish collagen (Collagen-Tripep20™, Tripep20) was prepared from *Pangasius hypophthalmus* fish skin by collagenase digestion. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to all subjects as a daily drink. The crow's-feet visual scores were analyzed by dermatologists, and the skin roughness parameters were measured using a PRIMOS High Resolution.

[a]*P*-value for the Wilcoxon signed-rank test, compared to the baseline.

[b]*P*-value for the Mann-Whitney U test using delta values, comparing values between the Tripep20 and the placebo.

*$P < 0.05$, ***$P < 0.001$ (Wilcoxon signed-rank test); †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ (Mann-Whitney *U* test using delta values).

Elevation of skin elasticity by liquid collagen Tripep20

This study attempted to evaluate the efficacy of Tripep20 on skin elasticity using a cosmetics measurement tool. The elasticity values obtained by a Cutometer device were marked as R2, R5, and R7. The R-Parameters of R2, R5, and R7 have been well-documented for over 20 yrs. The gross elasticity R2 expressing resistance versus the ability to return was much higher in the Tripep20 group after 6 weeks than in the placebo group (**Table 6**). In addition, the net elasticity R5 expressing the elastic portion of the suction part *versus* the elastic portion of the relaxation part was enhanced after consuming Tripep20 for 12 weeks (**Table 6**). Furthermore, the R7 value denoting the actual elasticity portion was higher in the subjects receiving Tripep20 than in the placebo ($P < 0.001$). Interestingly, none of the skin elasticity parameters were influenced in the subjects receiving placebo during the study period, indicating that placebo did not improve the skin elasticity (**Table 6**). The changes in skin elasticity increased significantly after 6 weeks of Tripep20 ingestion ($P < 0.001$; **Fig. 4**). Accordingly, Tripep20 consumption can be a dietary option for improving skin quality and being well tolerated by healthy individuals and patients.

DISCUSSION

Collagen has gained popularity as an anti-aging supplement, but its effectiveness in humans is still under debate because of the lack of practical scientific evidence. Several clinical studies on collagen have shown encouraging results regarding the improvement of skin texture after taking collagen tripeptides [14,28-32]. On the other hand, the collagen supplements used in these studies contain additional ingredients, such as antioxidant vitamins or minerals, which promote collagen synthesis or interfere with breakdown. Antioxidants may enhance skin quality by reducing DNA damage from UV light, improving hydration, stimulating collagen and elastin production, reducing the appearance of wrinkles and pigmentation, or reducing inflammation [33-35]. Accordingly, the improvement in skin texture might be due to the

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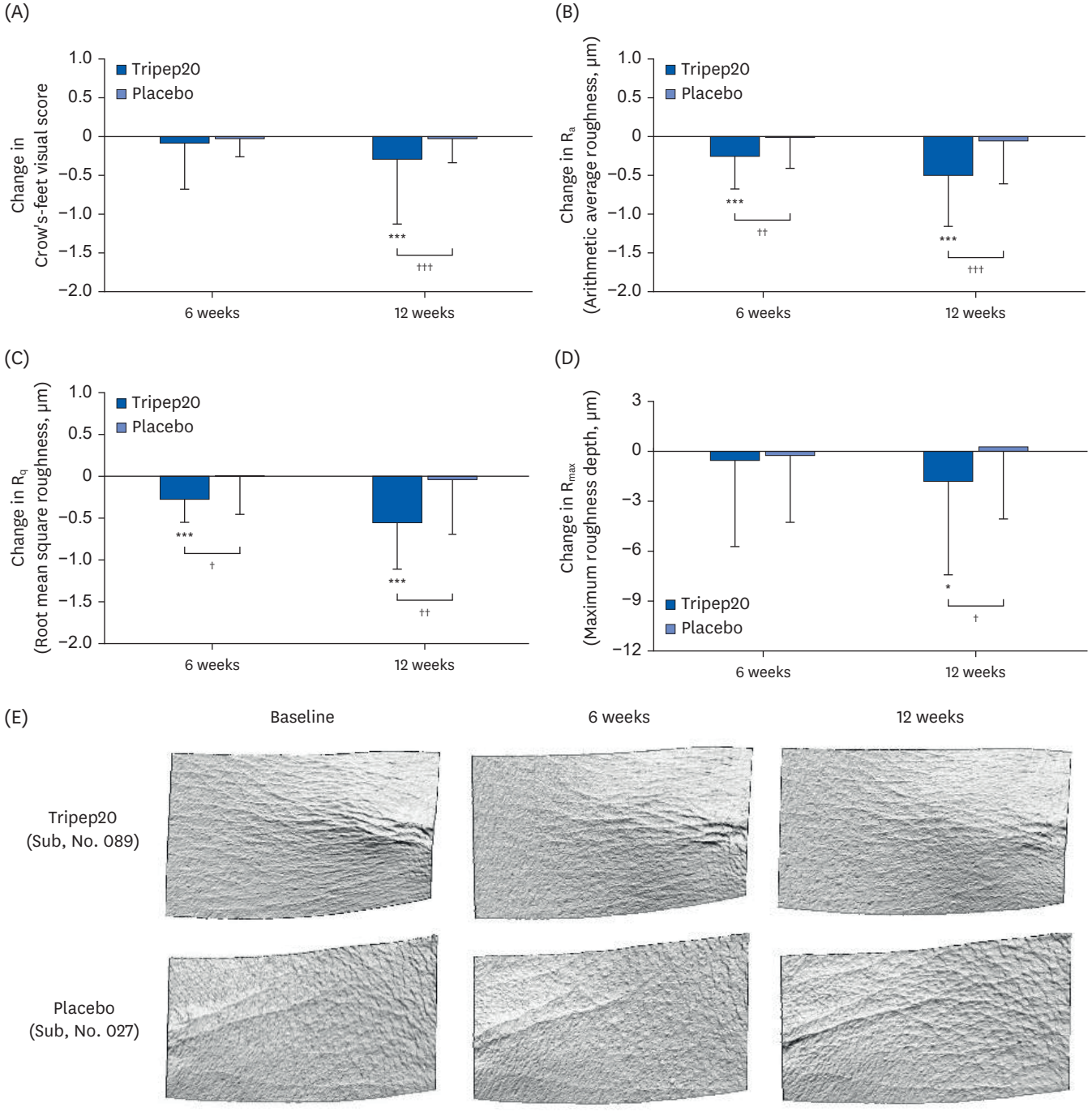

Fig. 3. Changes in Crow's-feet scores and skin roughness parameters in subjects receiving hydrolyzed fish skin collagen (Collagen-Tripep20™, Tripep20) or placebo. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to subjects as a daily drink. (A) Changes in the visual analysis of Crow's-feet scores compared to the baseline values. (B) Changes in the skin wrinkling parameter R_a compared to the baseline values (arithmetic average roughness). (C) Changes in the skin-wrinkling parameter R_q compared to the baseline values (root mean square roughness). (D) Changes in skin-wrinkling parameter R_{max} compared to the baseline values (maximum roughness depth). (E) Representative PRIMOS 3-dimensional pictures of eye wrinkles on Crow's feet area of the baseline, at 6 weeks, and 12 weeks were shown.

The crow's feet visual grade was scored by dermatologists, and the R_a, R_q, and R_{max} values were measured using a PRIMOS High Resolution. The data are expressed as the mean ± SD. A Wilcoxon signed-rank test was used to compare with the baseline. A Mann-Whitney *U* test using delta values was used to compare the values between the Tripep20 and the placebo.

$^{*}P < 0.05$, $^{***}P < 0.001$ (Wilcoxon signed-rank test); $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ (Mann-Whitney *U* test using delta values).

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Table 6. Skin elasticity parameters

Parameters	CTP20 group		Placebo group		CTP20/Placebo
	Mean ± SD	*P*-value[a]	Mean ± SD	*P*-value[a]	*P*-value[a]
Skin Elasticity by R2 (Gross elasticity, A.U.)					
Baseline	0.693 ± 0.055		0.694 ± 0.057		
At 6 weeks	0.710 ± 0.054	< 0.001***	0.694 ± 0.052	0.855	< 0.001***
At 12 weeks	0.726 ± 0.048	< 0.001***	0.693 ± 0.053	0.827	< 0.001***
Skin Elasticity by R5 (Net elasticity, A.U.)					
Baseline	0.596 ± 0.078		0.590 ± 0.076		
At 6 weeks	0.604 ± 0.080	0.002**	0.586 ± 0.073	0.129	< 0.001***
At 12 weeks	0.627 ± 0.080	< 0.001***	0.585 ± 0.072	0.067	< 0.001***
Skin Elasticity by R7 (Biological elasticity, A.U.)					
Baseline	0.457 ± 0.063		0.452 ± 0.065		
At 6 weeks	0.471 ± 0.066	< 0.001***	0.453 ± 0.064	0.665	< 0.001***
At 12 weeks	0.484 ± 0.062	< 0.001***	0.452 ± 0.062	0.862	< 0.001***

Hydrolyzed fish skin collagen (Collagen-Tripep20™, Tripep20) was prepared from *Pangasius hypophthalmus* fish skin by collagenase digestion. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to subjects as a daily drink. The skin elasticity parameters were measured using a Cutometer MPA580 device.

[a]*P*-value for repeated measures analysis of variance with a contrast test, compared to the baseline.

$^{**}P < 0.01$, $^{***}P < 0.001$ (repeated measures analysis of variance with contrast test).

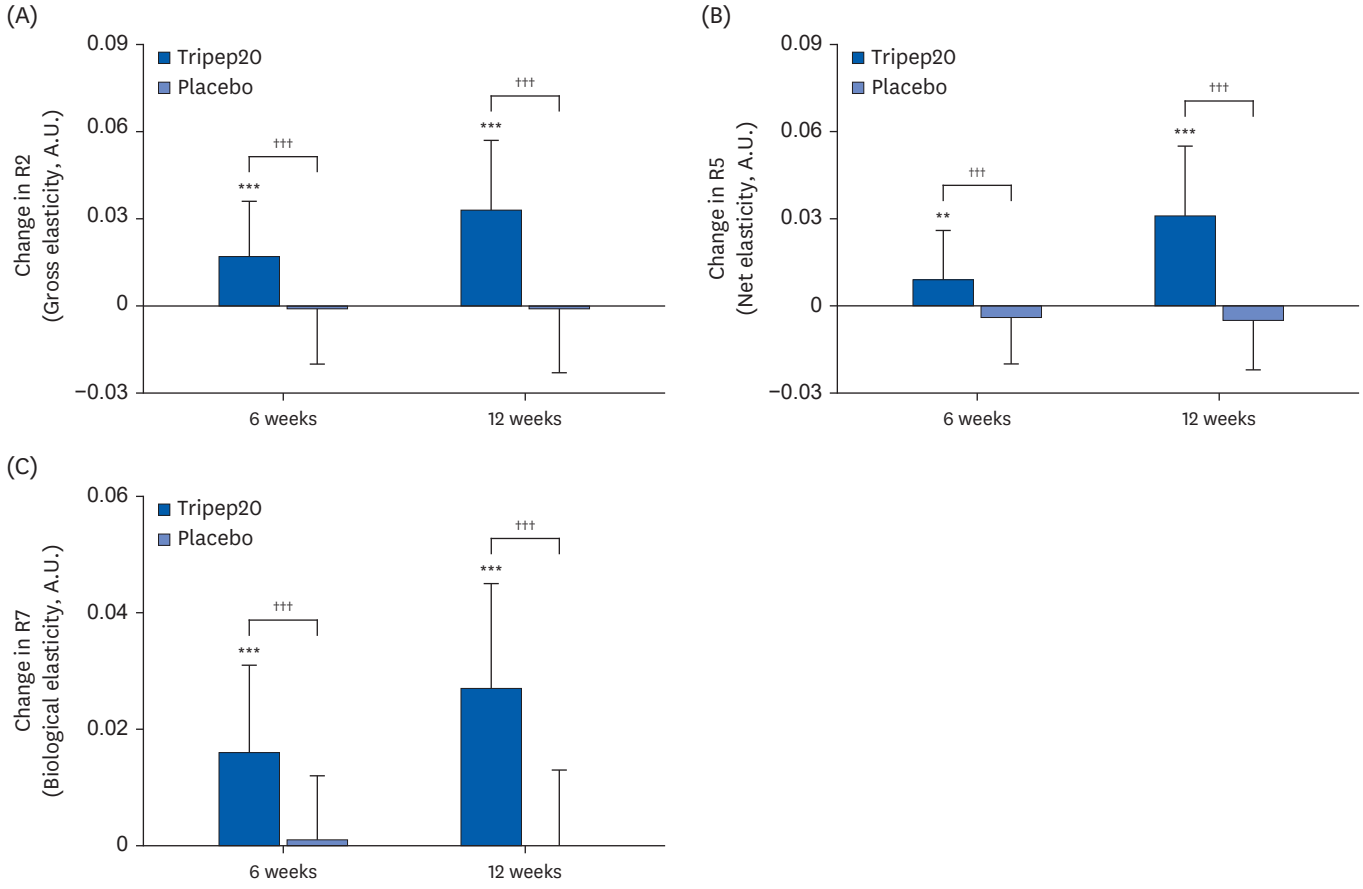

Fig. 4. Changes in the skin elasticity parameters in subjects receiving hydrolyzed fish skin collagen (Collagen-Tripep20™, Tripep20) or placebo. Liquid collagen (1,000 mg Tripep20) or placebo in a 50 mL bottle was administered orally to subjects as a daily drink. (A) Changes in the skin elasticity parameter R2 compared to the baseline values (Gross elasticity). (B) Changes in the skin elasticity parameter R5 compared to the baseline values (Net elasticity). (C) Changes in the skin elasticity parameter R7 compared to the baseline values (Biological elasticity).

The R2, R5, and R7 values were measured using a Cutometer MPA580 device, and changes from baseline values are shown with A.U. The data are expressed as the mean ± SD. A significant difference was evaluated with repeated measures analysis of variance contrast test for the comparison with the baseline and to compare the values between the Tripep20 and placebo.

A.U., arbitrary units.

$^{**}P < 0.01$, $^{***}P < 0.001$; $^{\dagger\dagger\dagger}P < 0.001$.

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presence of bioactive collagen peptides and the combination of antioxidants. The Tripep20 collagen used in this study contains 0.2% vitamin C and 6% fruit concentrate mix. Antioxidant supplementation can affect the incidence of skin cancers differentially in humans [36].

In a previous study [37], the subjects taking 1.0 g collagen tripeptide in capsule form for 12 weeks showed reduced wrinkle formation and skin hydration after adjusting with sunscreen, makeup, sleep, and age (over 45 yrs old). On the other hand, climate factors can influence the positive effects of collagen tripeptides [15]. Unlike previous studies using solid collagen tablets, liquid collagen was introduced in the current study. In this study, 1,000 mg of Tripep20 in a 50 mL bottle was consumed as a daily drink according to the assigned study formula. Collagen pills may maintain identical flavor, color, and taste among intervention groups in randomized controlled clinical trials. Despite this, liquid collagen absorbs better into the bloodstream than solid supplements, enhancing efficiency and shortening the protein digestion time [38].

This clinical trial attempted to substantiate the efficacy, safety, and tolerability of the liquid collagen Tripep20 on skin quality by conducting statistical analyses using valid approaches of ITT and PP. Oral consumption of Tripep20 ameliorated skin health, including skin moisturizing, anti-wrinkling, and elasticity, and was tolerated for 12 weeks among women aged 35–60 yrs diagnosed with photo-aged skin even without any adjusting conditions. The bioavailability of Tripep20 was improved by preparing freshwater catfish skin in liquid form using a digestion method with collagenase from a nonpathogenic Bacillus. The liquid collagen Tripep20 contained 20% tripeptides and 3.2% Gly-Pro-Hyp. A previous report showed that the functional components of collagen tripeptides, Gly-Pro-Hyp and Pro-Hyp, are more efficiently absorbed and reach higher rat plasma levels in the unchanged state compared to high-molecular-weight collagen peptides [11]. The dipeptide Pro-Hyp, which was presumed to be derived from Gly-Pro-Hyp, may enhance the proliferation and migration of dermal fibroblasts from the skin [10,39,40]. Gly-Pro-Hyp, which exists in Triprep20 in significant amounts, can reach the skin easily and efficiently, leading to improved skin function because dermal fibroblasts produce collagen, elastic fibers, and HA [41,42].

Dietary collagen hydrolysates from Vietnam pangasius fish skin restored dorsal skin damaged by photo-aging in mice by reducing the production of dermal proteins, MMP, AQP3, and HYAL1 [13]. Hyaluronan synthase 2 (HAS2) and AQP3, which are expressed by keratinocytes and are involved in hydration, are responsible for transporting moisture from outside the cell into the cell or for preserving moisture within the skin epidermis [43]. These findings can be applied to the results observed in this clinical trial of subjects exposed to photoaging. As an inclusion criterion, this clinical trial included a specific photodamage score for crow's feet, a site susceptible to UV exposure. The mechanical integrity of human skin, manifested as its elasticity, resilience, and toughness, is attributed primarily to the intricate network of collagen and elastin fibers embedded within the dermal ECM [44]. The reduction of MMP proteins by dietary Tripep20 collagen may strengthen the network of collagen and elastic fibers in the skin, ultimately improving skin elasticity. In addition to improving skin wrinkles, the consumption of Tripep20 could have a hydrating effect on the skin by promoting HA production via increased HAS2, HYAL1, and AQP3 expression. Moreover, skin hydration is vital for structural changes in elastin, which leads to changes in skin elasticity [45]. Thus, oral consumption of liquid collagen Tripep20 increased skin moisture, which may have helped improve skin elasticity. These results can be an answer to improving the photo appearance, including direct visual assessment of the periorbital wrinkles and Crow's-feet visual scores.

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In summary, the current clinical trial examined whether the oral consumption of liquid collagen enhanced skin quality. In this clinical trial, 78 women aged 35–60 yrs diagnosed with photo-aged skin consumed 1,000 mg of Tripep20 daily as a drink for 12 weeks. A previous mouse experiment revealed the beneficial effects of Tripep20 on photoaging in the dose range of 206 to 415 mg/kg BW [13]. Based on the previous results, the human application dose was set to 1,000 mg corresponding to 206 mg/kg. The liquid collagen Tripep20, with a Gly-Pro-Hyp content of over 3.2%, improved skin moisturizing, anti-wrinkling, and skin elasticity even in 6 weeks. Consuming Tripep20 was a safe and effective dietary option for restoring photo-aged skin. No adverse events were observed, and the diastolic pressure, systolic pressure, and heart rate were within normal ranges after ingesting Tripep20. The changes in parameters related to hematology and clinical chemistry were within the normal ranges. Accordingly, liquid collagen Tripep20 as a drink could be a valuable strategy for reducing skin aging through various mechanistic pathways. Overall, Tripep20 may be a safe and effective component in functional foods and dietary supplements for skin health.

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