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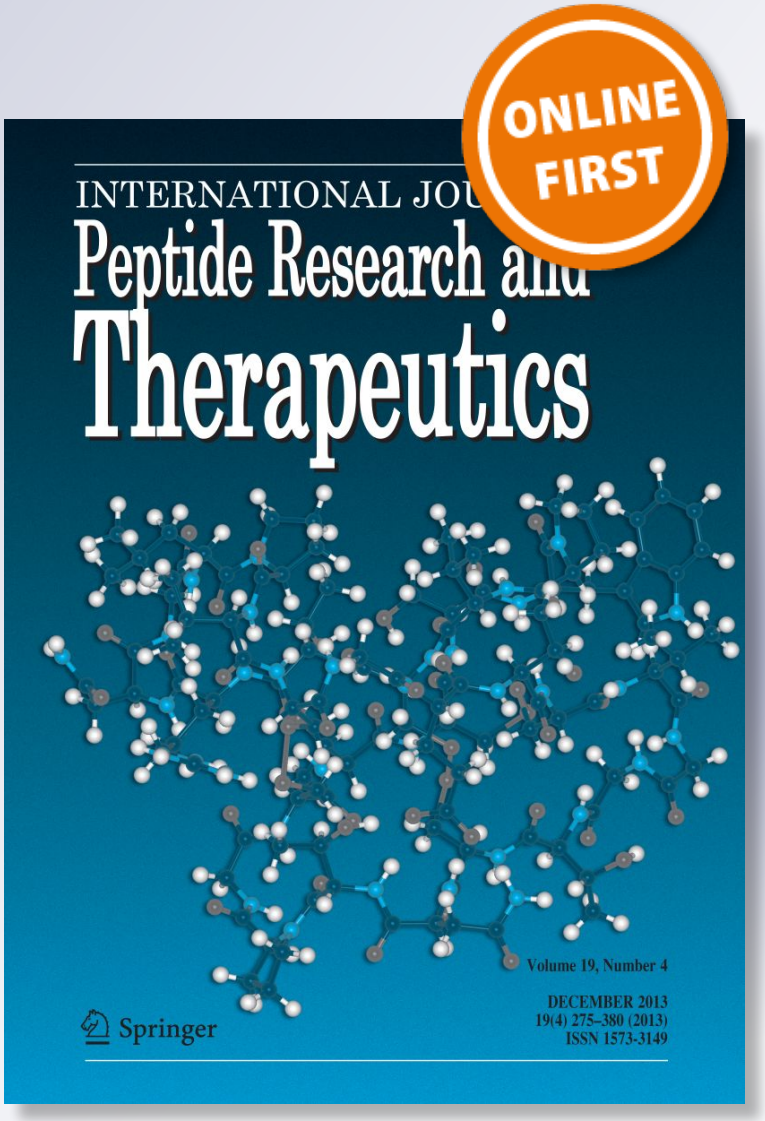

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Effects of Dietary Supplementation with Fish Scales-Derived Collagen Peptides on Skin Parameters and Condition: A Randomized, Placebo-Controlled, Double-Blind Study

Seiko Koizumi[1] · Naoki Inoue[1] · Maiko Shimizu[1] · Chang-ju Kwon[2] · Hwa-young Kim[2] · Kyoung Sik Park[3]

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Abstract Fish scales-derived collagen peptides (CPs) are characterized by their specific amino acid composition with a high concentration of glycine, proline and hydroxyproline. These amino acids have been known to exert beneficial effects on human skin. The aim of the present study was to examine the effects of collagen peptides obtained from fish scales on changes in periorbital wrinkles, facial skin hydration, and skin elasticity in healthy women aged 30–60 years. In the present randomized, placebo-controlled, double-blind trial, 71 subjects consumed a 20 mL beverage containing 3000 mg of CPs or placebo once per day over 12 weeks. Significant decreases in periorbital wrinkles ($p < 0.05$) were observed in the treatment group after 12 weeks of CPs ingestion compared to the control group. This study also demonstrated a consistent trend of enhanced facial skin moisture ($p < 0.001$) and skin elasticity ($p < 0.001$) by dietary intake of CPs without any side effects or adverse events. These findings indicate that fish-derived CPs hold great promise as a natural supplement with cutaneous anti-aging properties.

Keywords Collagen hydrolysate · Periorbital wrinkle · Skin hydration · Skin elasticity

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CPs	Collagen peptides
γ-GTP	γ-Glutamyltransferase
Gly	Glycine
Hyp	Hydroxyproline
IRB	Institutional review board
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
PP	Per-protocol
Pro	Proline
RBC	Red blood cell
TEWL	Transepidermal water loss
WBC	White blood cell

Introduction

Collagen is the main structural protein in the various connective tissues such as skin, tendons, cartilage and bone. It makes up from 25 to 30% of the whole-body protein content in mammals (Squire and Parry 2017). Commercial collagen is extracted from animal sources including porcine skin, bovine bone and fish scales (Blanco et al. 2017). Collagen peptides (CPs) are produced from the process of collagen hydrolysis involving breaking down the molecular bonds between individual collagen strands and peptides using combinations of physical, chemical and biological means (Liu et al. 2015). CPs are widely used as a dietary supplement with intend to aid joint mobility or enhance skin health.

In particular, fish scales-derived CPs have been reported to have specific amino acid composition with a high concentration of hydroxyproline (Hyp), glycine (Gly), and proline

✉ Kyoung Sik Park
parkks@cju.ac.kr

[1] Nitta Gelatin Inc., Yao, Osaka, Japan

[2] Ju Yeong NS Co. LTD., Seoul, Republic of Korea

[3] Department of Biomedical Science, College of Natural Science and Engineering, Cheongju University, Cheongju, 298 Daesung-ro, Chungwon-gu, Cheongju-si, Chungbuk 28503, Republic of Korea

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(Pro) in absorption level (Hu et al. 2017; Kim et al. 2012). Some Hyp-containing peptides including Pro-Hyp and Hyp–Gly are not completely digested into free amino acids and detected in human blood after ingestion of fish-derived CPs (Ohara et al. 2007). Notably, Prolyl-hydroxyproline (Pro-Hyp) stimulates cell proliferation and hyaluronic acid synthesis in dermis (Ohara et al. 2010). Recently, Shin et al. demonstrated that the dietary intake of fish scales-derived CPs significantly improves the wrinkle formation, thickness of the skin, acute erythema and skin hydration (Shin et al. 2016). From our previous studies, we observed a significantly increased viscoelasticity of human skin by dietary supplementation with 2.5–5 g of fish scales-derived CPs (Inoue et al. 2016; Sugihara and Inoue 2012; Sugihara 2015).

This present study was designed to evaluate the effects of fish scales-derived CPs on periorbital wrinkles, facial skin hydration and skin elasticity in healthy women of South Korea.

Materials and Methods

Study Design

This was a randomized, placebo-controlled, double-blind study examining the effects of CPs ingestion over 12 weeks. The protocol documents were submitted to Ellead Institutional Review Board (IRB), and the study was conducted after approval in January 2015 (IRB Number: EL-150113018A002) in compliance with the ethical principles of the Declaration of Helsinki. The research supervisor or researcher explained this study to the subjects signed the informed consent forms. Informed consent was obtained from all individual participants included in this study.

Subjects were randomly assigned to one of the two treatment regimens: CPs or placebo daily. The subjects consumed CPs or placebo product once a day for 12 weeks at the same time points from February to May in 2015. Efficacy assessments were conducted at baseline, and after 6 weeks and 12 weeks of ingestion.

Subjects

Eighty healthy women were enrolled as participants in this study. All participants were aged 30–60 years and confirmed to be "healthy" by blood test. Participants were tested negative for severe acute kidney disease, heart disease and other chronic diseases. They had no allergies or skin sensitivities to cosmetics, medicines, healthy functional foods, or ordinary exposure to sunlight. Selection criteria also included the presence of periorbital wrinkles corresponding to a grade of 2–6 defined by the global photodamage score as diagnosed by a dermatologist. Participants were randomly assigned to one of the two groups in a 1:1 ratio using a computer generated randomization schedule. The subjects were prohibited from taking dietary supplement including vitamins and minerals except for the test product. The subjects were prohibited from using any cosmetics except for the given cosmetics in the test area during the test. Skincare products or activities such as facial masks, massage, skin decortication and laser surgery which may affect the test were prohibited. Excessive exposure to UV was also prohibited during the test.

Study Beverage

The active products contained 3000 mg of CPs (average molecular weight: 3000 Da) derived from the scales of Tilapia fish (*Oreochromis mosambicus*) in the form of a 20 mL beverage. This product was manufactured by Nitta Gelatin Inc. (India), and provided by Ju Yeong NS Co. LTD. (Seoul, South Korea). The placebo product excluded CPs in 20 mL beverage. Color and smell of the placebo product were modified by fragrance and coloring agents to mimic the active products.

Dermatological Measurements

Instrumental measurements on the facial skin were conducted at baseline, and after 6 weeks and 12 weeks of ingestion. The subjects washed out their makeup and then were acclimatized for 30 min in the waiting lounge at a constant temperature of 20–24 °C and humidity of 40–60% RH before facial skin evaluation.

Periorbital Wrinkle

A replica of the periobital wrinkle was made using a silicone kit. To produce the replica, an adhesive plastic frame was pasted around the periorbital wrinkle. Silicon was applied and an impression was made which hardened into a replica of the skin. This replica was analyzed using a visiometer (Skin-Visiometer SV700, Courage & Khazaka, Germany). A visiometer assesses wrinkles by analyzing the intensity of light passing through the replica. Light is measured with a CMOS sensor and analyzed using image analyzer software and calculated by Lambert & Beer's Law. Replicas were analyzed for five criteria: R1: skin roughness; R2: maximum roughness; R3: average roughness; R4: smoothness roughness; R5: arithmetic average roughness. Photographs were also taken of each subject at baseline, after 6 weeks, and after 12 weeks of ingestion.

Skin Moisture Content

The skin moisture content on the cheek was assessed using Corneometer CM825 (Courage & Khazaka, Germany) at baseline, 6 weeks and 12 weeks after taking the product. The test method is based on a capacitance measurement of dielectric constant between electrodes separated on the skin surface. The average values were calculated from three measurements of skin moisture content.

Skin Elasticity

The skin elasticity on the cheek was assessed using Cutometer MPA580 (Courage & Khazaka, Germany) at the baseline, 6, and 12 weeks after taking the product. The test method draws skin with suction into the aperture of the probe and after a defined time releases it again. The method is non-invasive using a 2 mm of probe pressed into the skin. The results were calculated from three measurements with Mode 1, 450 mbar of constant suction for 2 s of suction time and 2 s of relaxation time. R2, R5 and R7 are parameters related to the skin elasticity.

Blood Tests

Blood tests were conducted following items: total protein, albumin, AST (aspartate aminotransferase), ALT (alanine aminotransferase), bilirubin, creatinine, total cholesterol, glucose, hemoglobin, hematocrit, WBC (white blood cell), RBC (red blood cell), platelet, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and γ-GTP (γ-glutamyltransferase).

Statistical Analysis

The study results were analyzed from PP (per-protocol) datasets. PP analysis refers to inclusion in the analysis of only those patients who strictly adhered to the protocol. Statistical comparisons (paired or unpaired *t* tests, Mann–Whitney rank sum tests) were performed by using SIGMASTAT 4.0 (SPSS, Chicago, IL, USA). A p value of less than 0.05 was considered to indicate statistical significance.

Results

Panelist Summary

Three subjects were excluded from participation among 80 subjects because they requested to withdraw. The remaining 77 subjects took either the CPs or placebo (39 subjects in CPs group, 38 subjects in placebo group). Six more subjects dropped out of the study after taking the products because of personal reasons (2 subjects in CPs group, 4 subjects in placebo group). The final 71 subjects (37 subjects in CPs group, 34 subjects in placebo group) completed this study (Fig. 1). Baseline characteristics for the placebo and CPs groups are shown in Table 1. No significant differences were detected between the two groups.

Blood Tests

At baseline and after 12 weeks of treatment, hematological and hemato-biochemical analyses were conducted. The results from the blood tests in the both CPs and placebo groups showed statistically significant differences between before and after taking in total protein, AST, ALT, creatinine, glucose, hematocrit, MCV, MCH and MCHC (Table 1). Furthermore, there were significant differences in platelet and MCV between the CPs and placebo groups after 12 weeks of treatment. However, all of the differences may have no significant meanings due to the changes within the clinically normal ranges.

Adverse Effects

There were no reports of subjects who experienced adverse events. Also, there were no premature terminations caused by a serious adverse event, adverse drug reaction or other adverse event.

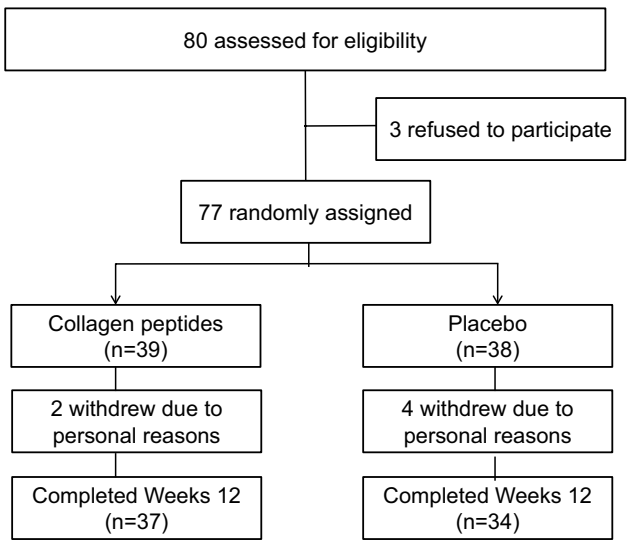

Fig. 1 Trial profile and design

Table 1 Baseline characteristics and changes in hematological and hemato-biochemical parameters after drinking either a CPs beverage or a placebo beverage for 12 weeks

	Placebo (n = 34)		CPs (n = 37)	
	Baseline	Week 12	Baseline	Week 12
Age (years)	47.37 ± 4.36	–	46.49 ± 5.74	–
Body weight (kg)	57.08 ± 6.35	–	56.19 ± 7.16	–
Systolic BP (mmHg)	113.21 ± 9.41	–	115.51 ± 10.24	–
Diastolic BP (mmHg)	66.44 ± 9.01	–	68.23 ± 8.84	–
Total protein (g/dL)	7.58 ± 0.41	7.26 ± 0.39***	7.50 ± 0.34	7.13 ± 0.28***
Albumin (g/dL)	4.57 ± 0.19	4.51 ± 0.22	4.63 ± 0.18	4.56 ± 0.21
AST (U/L)	23.63 ± 4.53	17.78 ± 4.40***	21.38 ± 3.79	17.07 ± 3.93***
ALT (U/L)	19.89 ± 8.54	14.07 ± 6.66***	16.38 ± 5.27	13.41 ± 5.85**
γ-GTP (U/L)	13.39 ± 6.17	14.63 ± 5.77	14.20 ± 9.70	14.56 ± 8.59
BUN(mg/dL)	13.38 ± 3.19	13.11 ± 3.17	12.09 ± 2.73	12.07 ± 2.84
Creatinine (mg/dL)	0.54 ± 0.08	0.65 ± 0.09***	0.56 ± 0.08	0.65 ± 0.08***
Total cholesterol (mg/dL)	186.71 ± 24.92	189.34 ± 24.83	183.46 ± 23.68	182.64 ± 26.10
Glucose (mg/dL)	89.07 ± 6.49	86.86 ± 7.45*	89.10 ± 9.70	85.38 ± 7.44**
Hemoglobin (g/dL)	12.81 ± 0.81	12.85 ± 1.09	13.06 ± 0.99	12.92 ± 1.05
Hematocrit (%)	38.27 ± 2.19	40.25 ± 2.91***	39.06 ± 3.10	40.64 ± 3.13***
WBC (Thous/μL)	5.92 ± 1.30	5.45 ± 1.22**	5.49 ± 1.52	5.51 ± 1.22
RBC (Mil/μL)	4.21 ± 0.23	4.32 ± 0.27**	4.27 ± 0.35	4.30 ± 0.31
Platelet (Thous/μL)	233.76 ± 50.46	233.07 ± 44.25	233.46 ± 54.17	252.51 ± 46.54**,##
MCV (fL)	90.96 ± 4.32	93.02 ± 4.51***	91.49 ± 4.32	94.46 ± 4.62***,#
MCH (pg)	30.47 ± 1.85	29.67 ± 1.73***	30.61 ± 1.72	30.04 ± 1.71**
MCHC (%)	33.48 ± 0.69	31.90 ± 1.01***	33.45 ± 0.63	31.79 ± 0.85***

Data are expressed as mean ± SD

BP blood pressure, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase; *BUN* bilirubin, *WBC* white blood cell, *RBC* red blood cell, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *γ-GTP* gamma-glutamyltransferase

*p < 0.05; **p < 0.01; ***p < 0.001: significantly different from baseline

#p < 0.05; ##p < 0.01: significantly different between CPs group and placebo group

Wrinkle

The wrinkle and roughness parameters, R1 (skin roughness), R2 (maximum roughness), R3 (average roughness), R4 (smoothness roughness) and R5 (arithmetic average roughness) were calculated and statistically analyzed. The magnitude of % decreases at Week 12 in R1, R3, R4 and R5 was significantly greater ($p < 0.05$) in the CPs group than in the placebo group (Table 2). The representative photos showing the effect of CPs treatment on periorbital wrinkles were displayed in Fig. 2.

Facial Skin Moisture Content

The results of the skin moisture content assessment on the cheek from PP analysis showed a significant increase of facial skin hydration in the CPs group over time ($p < 0.05$, Table 2). Also, significant treatment-by-time interactions were found in facial skin moisture content ($p < 0.05$).

Skin Elasticity

The skin elasticity parameters, R2 (gross elasticity), R5 (net elasticity) and R7 (biological elasticity) were calculated and statistically analyzed. Table 2 represents the changes in skin elasticity by treatment for 12 weeks. A statistically significant increase was found in all of the skin elasticity parameters (R2, R5 and R7) in the CPs group after 12 weeks of ingestion compared to baseline ($p < 0.05$). Furthermore, CPs treatment induced a greater increase with a statistical significance at Week 12 in R, R5 and R7 compared to the placebo group ($p < 0.001$).

Discussion

The present study demonstrated that the supplementation with fish scales-derived CPs 3000 mg for 12 weeks was significantly effective in improving periorbital wrinkle, skin moisture content and skin elasticity. These findings confirmed the results of the previous clinical studies showing

Table 2 % Changes in skin parameters after drinking either a CPs beverage or a placebo beverage for 12 weeks

	Placebo (n = 34)			CPs (n = 37)		
		% Change from baseline			% Change from baseline	
	Baseline	Week 6	Week 12	Baseline	Week 6	Week 12
Periorbital wrinkles						
R1 (skin roughness)	0.144 ± 0.004	2.009 ± 1.491	3.698 ± 1.398	0.139 ± 0.003	1.171 ± 1.285	−4.094 ± 1.390$^{\#\#\#}$
R2 (maximum roughness)	0.110 ± 0.002	−1.841 ± 1.158	−0.988 ± 1.275	0.106 ± 0.002	0.102 ± 1.445	−3.022 ± 1.345
R3 (average roughness)	0.083 ± 0.001	−1.597 ± 0.900	−1.496 ± 1.328	0.082 ± 0.001	−2.256 ± 1.492	−5.116 ± 1.286$^{\#}$
R4 (smoothness roughness)	0.063 ± 0.002	6.568 ± 3.052	8.715 ± 3.385	0.062 ± 0.002	0.578 ± 2.779	−4.198 ± 2.649$^{\#\#}$
R5 (arithmetic average roughness)	0.018 ± 0.001	5.882 ± 4.919	11.274 ± 5.528	0.018 ± 0.001	−1.351 ± 3.055	−0.450 ± 5.793$^{\#}$
Skin moisture content						
Skin moisture content	49.585 ± 1.106	1.856 ± 1.047	4.017 ± 1.307	49.812 ± 1.224	6.646 ± 1.446$^{\#}$	10.891 ± 1.315**,$^{\#\#\#}$
Skin elasticity						
R2 (gross elasticity)	0.736 ± 0.008	−0.264 ± 0.458	0.938 ± 0.677	0.730 ± 0.007	2.272 ± 0.493$^{\#\#\#}$	4.320 ± 0.534**,$^{\#\#\#}$
R5 (net elasticity)	0.491 ± 0.011	0.315 ± 0.908	0.574 ± 0.837	0.486 ± 0.010	4.857 ± 0.777$^{\#\#\#}$	6.543 ± 1.089*,$^{\#\#\#}$
R7 (biological elasticity)	0.348 ± 0.008	1.744 ± 0.932	2.034 ± 1.389	0.344 ± 0.007	4.807 ± 0.779$^{\#\#}$	6.814 ± 1.049*,$^{\#\#\#}$

Data are expressed as mean ± SEM

*$p < 0.05$; **$p < 0.01$: significantly different from baseline

$^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$: significantly different between CPs group and placebo group

the daily intake of CPs 3–10 g has beneficial effects on skin health (Inoue et al. 2016; Sugihara and Inoue 2012; Sugihara 2015; Choi et al. 2014; Proksch et al. 2014a, b). There is a strong body of evidences for beneficial effects of fish scales-derived CPs on skin health in several trials. The ingestion of fish scales-derived CPs 3000 mg/day for 4 weeks significantly improved skin moisture content (Choi et al. 2014). In addition, Proksch et al. showed that ingestion of CPs obtained from porcine 2500 mg/day for 8 weeks improved skin wrinkle (Proksch et al. 2014b). Also, they demonstrated that the dietary intake of porcine-derived CPs 2500 and 5000 mg/day for 8 weeks improved skin elasticity without affecting skin moisture content and transepidermal water loss (TEWL) (Proksch et al. 2014a). While each of previous investigations only partially evaluated the beneficial effects of fish scales and porcine-derived CPs on skin parameters including skin moisture content, wrinkles and skin elasticity, this study demonstrated the improvement of all the parameters above by ingestion of them. Furthermore, most of previous human studies have examined the short-term effects of CPs within 8 weeks. To make up the restrictions of intake period in earlier trials, the present study expanded the treatment period up to 12 weeks.

In terms of the underlying mechanism, several human studies have demonstrated high absorption of two major CPs, prolyl-hydroxyproline (Pro–Hyp) and hydroxyprolyl-glycine (Hyp–Gly), into human blood (Kim et al. 2012; Sugihara et al. 2012). Some in vitro studies indicated that Pro–Hyp enhances chemotaxis on dermal fibroblasts (Postlethwaite et al. 1978) and Pro-Hyp and Hyp–Gly promote cell proliferation (Ohara et al. 2010). Additionally, Pro–Hyp enhances the production of hyaluronic acid in dermal fibroblasts (Ohara et al. 2010). Taking into account the bioavailability of these dipeptides, the improvement of skin condition after the intake of fish scales-derived CPs characterized by a high concentration of Hyp, Gly and Pro may be attributed to the high absorption of these bioactive peptides.

Conclusions

This study was conducted to evaluate the clinical efficacy of CPs to improve periorbital wrinkles, skin moisture content, and skin elasticity over 12 weeks. A daily intake of fish scales-derived CPs 3000 mg for 12 weeks was effective in improving periorbital wrinkle, skin moisture content

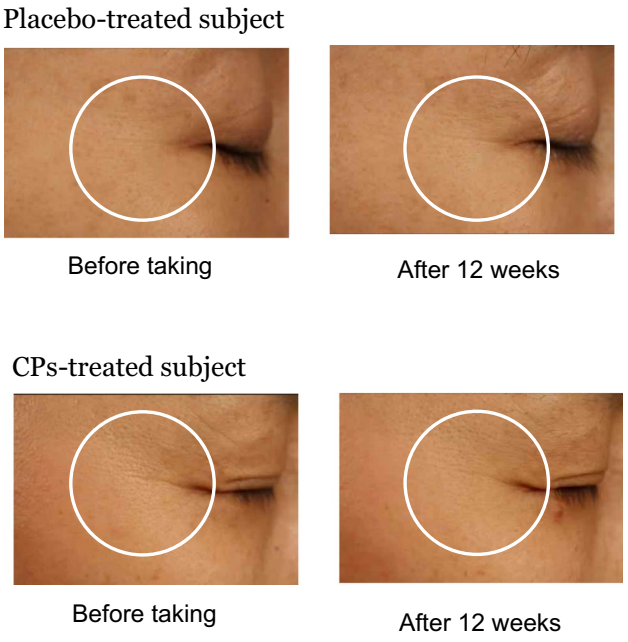

Fig. 2 Representative photographs indicating visual reduction of periorbital wrinkle formation at baseline and after 12 weeks of ingestion of placebo or collagen peptides

and skin elasticity. These beneficial effects of fish scales-derived CPs on skin health may result from their specific amino acid composition with a high concentration of Hyp, Gly and Pro in absorption level.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (IRB Number: EL-150113018A002) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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