Explanation of *Jpn Pharmacol Ther* 42(12): 995-1004 (2014) by authors KK & YK

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Effects of Collagen Peptide Ingestion on Skin Properties:

A Placebo-controlled Double-blind Trial

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ABSTRACT

Objective Effects of fish-derived collagen peptide (FCP) on skin properties were investigated.

Methods A placebo-controlled double-blind trial was conducted with Japanese women (35–65 years of age) with dry and saggy facial skin. They ingested 5 g of placebo or FCP for 8 weeks, and skin properties and subjective feeling of the skin condition were examined before and after ingestion at 4 and 8 weeks.

Results The decrease in the number of wrinkles was significantly greater ($P = 0.034$) in the FCP group than in the placebo group at 8 weeks on sub-group analysis (age < 60). In women with dry facial skin, texture (uniformity of skin color and surface smoothness) improved significantly ($P = 0.010$) in the FCP group compared with the placebo group, and the number of red areas tended to decline ($P = 0.054$) at 8 weeks in the FCP group compared with the placebo group. A significant ($P = 0.008$) improvement in the skin condition (subjective self-evaluation) after FCP ingestion was observed at 8 weeks.

Conclusions Ingestion of FCP improves skin properties and the skin condition.

KEY WORDS Fish-derived collagen peptide; Skin; Wrinkle; Texture; Red area

INTRODUCTION

Collagen is one of the main biological proteins, comprising about one-third of the total protein mass in our body. Heat-denatured collagen extracted from animal hide, bone or fish scales is referred to as gelatin. Collagen peptide (CP) is prepared by limited digestion of gelatin with enzymes, and is widely used as an ingredient in supplements for improving our health. It has been reported that daily ingestion of CP relieves joint pain of the knees[1)] and improves blood flow[2)], nails[3)] and hair[4)]. Ingested CP is digested and absorbed not only as free amino acids but also as oligopeptides composed of 2 or 3 amino acids[5)]. Among those oligopeptides, prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly) are abundant in the blood and show physiological activity *in vitro* such as stimulating the proliferation of skin fibroblasts[5~7)].

Daily ingestion of CP also improves skin

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ABBREVIATIONS
CP: collagen peptide
FCP: fish-derived collagen peptide
TEWL: transepidermal water loss
Pro-Hyp: prolylhydroxyproline
Hyp-Gly: hydroxyprolylglycine

properties; it increases moisture content in the stratum corneum and elasticity of the skin[8~10], and reduces eye wrinkle volume[11]. Skin erythema induced by ultraviolet irradiation was suppressed after ingesting CP[12]. Furthermore, it was reported that participants who ingested CP experienced improvement in their skin condition[13].

Skin properties vary considerably among individuals because the skin, the outermost organ in the body, is influenced by natural ageing factors and environmental conditions. Therefore, it is likely that the efficacy of CP ingestion depends on age and skin condition. The purpose of the present study was to investigate the effect of fish-derived collagen peptide (FCP) ingestion on skin properties. In this clinical trial, the skin properties of participants (35–65 years of age; mainly in their 40s and 50s) were assessed with measuring instruments, a digital image analyzer and sensory evaluation. Sub-group analysis of data was performed with regard to age and skin type.

This study was conducted by SOUKEN Co., Ltd. (Tokyo, Japan) in compliance with the Declaration of Helsinki according to the protocol approved by the ethics committee of Shiba Palace Clinic (protocol no. NP-14375). The study started on January 14 and ended on April 4, 2014.

MATERIALS AND METHODS

1 Participants

A total of 185 participants were recruited by SOUKEN who had met the inclusion criteria but not the exclusion criteria. They were given written documents about the study content, methods and possible adverse effects, and they received an approval to participate the test from the responsible physician (Takashi Koikeda, M.D.). All subjects gave informed consent prior to commencement of the study.

The entry criteria were: Japanese women aged 35–65 years (mainly in their 40s and 50s; the ratio of women aged 35–49 versus those aged 50–65 years was 1:1) who had dry and saggy facial skin.

1) Screening criteria

Ninety subjects were selected by screening using the following criteria: 1) those who were considered to be suitable for the study on visual examination (presence of wrinkles and absence of wounds, pimples, warts or burns); 2) those who were considered to be suitable for the study by SOUKEN through background research; 3) those with a low total score (please see below) after palpation and visual examination by a cosmetic expert who examined the skin texture of the cheek, dryness at the corner of the left eye and mouth, skin age compared with actual age, and elasticity of the cheek upon pressure; 4) those with a low moisture content in the stratum corneum of the inner forearm (Corneometer value); 5) those with a low moisture content in the stratum corneum of the right cheek (Corneometer value); and 6) those with a low moisture content in the stratum corneum of the corner of the left eye (Skicon-200EX value). In order to determine the order of the candidates, scores were given to criterion #3 in multiples of 4 (4, 8, 12, etc.); to criterion #4 in multiples of 3 (3, 6, 9, etc.); to criterion #5 in multiples of 2 (2, 4, 6, etc.); and to criterion #6 in increments of 1 (1, 2, 3, etc.). Subjects with a low total score were selected. When the total score was the same, those with a low score in criterion #3 were selected.

2) Exclusion criteria

The following participants were excluded: 1) those who took medicines that may affect the test result (adrenal cortex hormones, sex hormones, etc.); 2) those who took a health supplement two or more times per week that may affect the test result (collagen, hyaluronic acid, elastin, chondroitin, glucosamine, placenta or beauty drinks); 3) those who may be allergic to collagen, gelatin, hyaluronic acid, elastin, chondroitin, glucosamine, placenta or beauty drinks; 4) those who had allergic dermatitis, eczema or cutaneous hypersensitivity including atopic dermatitis; 5) those who attended dermatology clinics; 6) those who frequently feel queasy; 7) those who ate collagen- or gelatin-rich foods (beef tendon, internal organs, liver, pig trotters, chicken thighs, chicken wings, fish skin,

eel, sea eel, angler fish, gelatinous juice, collagen stew, gelatin, chicken cartilage, gelatin candy, etc.) two or more times per week; 8) those who were under the care of a doctor for treatment of menopausal symptoms or rejuvenation (hormone replacement therapy, placental remedy [injection or internal use], herbal medicine and so on); 9) those who had received cosmetic medical treatment such as injection of collagen, hyaluronic acid or Botox, or laser treatment and so on; 10) those who drank alcohol or smoked excessively; 11) those who might change their lifestyle during the test period including overseas travel; 12) those whose test sites might be sunburned during the test period; 13) those who might apply body lotion to the test sites on the forearm or bath salts during the test period; 14) those who were pregnant, nursing or might become pregnant; 15) those who were participating or going to participate in other clinical trials during the test period; 16) those who were judged to be unsuitable for the test by the doctor responsible for the present study (Takashi Koikeda).

3) Instruction to participants

Participants were instructed to keep a web diary until the end of the test. They were also instructed to comply with the following: 1) refrain from ingesting or using a medicine that may affect the test; 2) refrain from ingesting health supplements (collagen, hyaluronic acid, elastin, chondroitin, glucosamine, placenta, beauty drinks); 3) avoid eating collagen- or gelatin-rich foods (beef tendon, internal organs, liver, pig trotters, chicken thighs, chicken wings, fish skin, eel, sea eel, angler fish, gelatinous juice, collagen stew, gelatin, chicken cartilage, gelatin candy, etc.); 4) avoid changing the skin care products they were using (skin care cosmetics, body lotion, bath salts, hand cream) and the frequency of application, as well as applying or rubbing body lotion and bath salts on the inner forearm; 5) avoid changing their lifestyle; and 6) to avoid sunburn of the test sites.

Furthermore, participants were instructed not to shave or peel for 2 weeks before the first test day (0 week) until the last test day (8 weeks). They were also instructed to comply with the following on the test day of 0, at 4 and 8 weeks including the night before the test day: 1) avoid eating or drinking excessively and to sleep well; 2) refrain from using a facial beauty instrument (facial roller massager, facial steamer, facial ultrasonic wave device, facial ion introducer and so on) or facial care such as facial pack, facial mask, facial massage, facial muscle training, finger pressure therapy and so on; 3) avoid beauty treatment (face or body), massage, reflexology, chiropractic, finger pressure therapy, acupuncture, moxibustion and so on; 4) refrain from taking a bath or shower on the night before the test day; 5) avoid exercise using a training machine, muscle training, aerobics, dance, yoga, stretch exercise, swimming, walking, jogging and so on; 6) avoid running or bicycle pedaling too fast on their way to the test place; 7) avoid ingesting a stimulant such as pepper, ginger or caffeine; and 8) refrain from taking a bath or shower on the morning of the test day.

2 Test materials

FCP (average MW: 4,000–6,000) granules were prepared from fish scales by Nippi Inc. (Tokyo, Japan). Dextrin powder (Nippon Starch Chemical Co. Ltd., Osaka, Japan) was used as the placebo. All subjects in each group ingested 5 g of FCP or placebo, each with 0.2% flavoring (Peach No. 2039F; San-Ei Gen F.F.I., Osaka, Japan), after every dinner with water.

3 Assessment

The study design was a placebo-controlled double-blind trial. The 90 subjects were either assigned to the placebo group (n=45) or the FCP group (n=45) so that 1) age range, moisture value and scores from palpation and visual examination by a cosmetic expert for skin texture of the cheek, dryness at the corner of the left eye and mouth, skin age compared with the actual age, and elasticity of the cheek on palpation did not differ between two groups; and 2) the ratio of participants of 35–49 years of age to those of 50–65 years of age was 1:1.

The evaluation of skin properties was performed before ingestion (0 week) and after ingestion at 4 and 8 weeks. Examination with measuring

Table 1 Age of subjects

Age	Placebo (n)	FCP (n)
35～39	1	2
40～49	21	20
50～59	18	19
60～65	3	4
Total	43	45

instruments was performed to assess transepidermal water loss (TEWL) of the right cheek using a Tewameter TM300 (Integral, Tokyo); moisture content in the stratum corneum of the inner right forearm and the right cheek using the Corneometer CM825 (Integral) and at the corner of the left eye with Skicon-200EX (Yayoi, Tokyo); and viscoelastic properties R_0 (the final distension of the curve), R_1 (the ability to return to the original state) and R_2 (the overall elasticity of the skin) with Cutometer MPA580 (Integral). Digital image analysis was performed with VISIA Complexion Analysis (Integral) for wrinkles, porphyrin, pores, UV spots, texture, surface spots, brown spots and red areas. A smaller VISIA measurement value indicates that the

Table 2 Measured value and change for all subjects

Item		Unit	n	Group	Measured value					
					Before ingestion		4 weeks		8 weeks	
					Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
TEWL		g/hm^2	43	Placebo	11.0 ± 7.4	0.924	13.9 ± 7.0***	0.894	12.9 ± 3.1***	0.354
			45	FCP	10.2 ± 2.6		13.4 ± 3.7***		13.6 ± 3.2***	
Moisture value	Cheek	A.U.	43	Placebo	51.2 ± 8.5	0.720	55.9 ± 7.3***	0.301	56.9 ± 7.6***	0.957
			45	FCP	50.7 ± 8.3		53.8 ± 10.9*		57.1 ± 7.3***	
	Forearm	A.U.	43	Placebo	27.0 ± 5.6	0.717	28.7 ± 5.2*	0.269	27.1 ± 5.7	0.483
			45	FCP	27.1 ± 5.5		29.9 ± 5.7**		28.6 ± 7.2	
Viscoelasticity	R_0	mm	40	Placebo	0.21 ± 0.06	0.367	0.29 ± 0.07***	0.826	0.29 ± 0.07***	0.657
			45	FCP	0.20 ± 0.06		0.29 ± 0.07***		0.28 ± 0.08***	
	R1	mm	40	Placebo	0.06 ± 0.02	0.431	0.08 ± 0.02***	0.512	0.08 ± 0.02***	0.303
			45	FCP	0.06 ± 0.02		0.08 ± 0.02***		0.08 ± 0.03***	
	R_2	%	40	Placebo	71.5 ± 7.0	0.751	72.4 ± 6.5	0.301	72.5 ± 4.4	0.912
			45	FCP	71.7 ± 6.0		73.4 ± 5.6*		72.8 ± 5.2	
VISIA	Wrinkles	No.	43	Placebo	13.6 ± 8.1	0.192	11.9 ± 8.4*	0.259	13.3 ± 8.3	0.667
			45	FCP	15.8 ± 8.4		13.2 ± 7.9**		13.7 ± 8.1#	
	Porphyrin	No.	43	Placebo	389 ± 425	0.993	414 ± 397	0.950	378 ± 356	0.894
			45	FCP	351 ± 383		378 ± 396		375 ± 418	
	Pores	No.	43	Placebo	897 ± 425	0.488	875 ± 449	0.710	857 ± 429	0.723
			45	FCP	972 ± 446		898 ± 416#		889 ± 415#	
	UV spots	No.	43	Placebo	250 ± 30	0.625	251 ± 31	0.761	248 ± 34	0.871
			45	FCP	247 ± 36		249 ± 34		248 ± 35	
	Texture	No.	43	Placebo	996 ± 516	0.236	898 ± 493**	0.475	964 ± 508	0.458
			45	FCP	1056 ± 407		918 ± 399***		982 ± 405*	
	Surface spots	No.	43	Placebo	106 ± 30	0.707	106 ± 28	0.890	108 ± 27	0.947
			45	FCP	107 ± 25		105 ± 26*		107 ± 26	
	Brown spots	No.	43	Placebo	281 ± 32	0.686	279 ± 36	0.692	279 ± 33	0.732
			45	FCP	281 ± 40		280 ± 38		281 ± 38	
	Red areas	No.	43	Placebo	128 ± 51	0.861	126 ± 55	0.987	121 ± 52*	0.867
			45	FCP	128 ± 41		126 ± 46		117 ± 39*	

skin condition is better.

Palpation and visual inspection by a cosmetic expert and a self-administered questionnaire on skin condition were also conducted. In the questionnaire on skin condition, the question "How do you feel about your skin conditions compared to before ingestion?" was rated as "1", "2" or "3" representing "worse", "no change" and "better" compared with that before ingestion, respectively. For the other questions, "1", "2", "3", "4" and "5" indicated "better", "improved to some degree", "no change", "worsened to some degree" and "worse", respectively. Data are shown as mean ± SD in the tables and mean ± SEM in the figures.

Δ Change						
4 weeks				8 weeks		
Mean ± SD			P	Mean ± SD		P
2.9 ± 3.3			0.673	1.9 ± 7.1		0.372
3.3 ± 3.0				3.4 ± 3.4		
4.6 ± 6.4			0.341	5.6 ± 7.3		0.534
3.0 ± 8.1				6.4 ± 7.5		
1.8 ± 5.1			0.401	0.2 ± 5.2		0.619
2.8 ± 5.5				1.5 ± 7.5		
0.08 ± 0.07			0.355	0.09 ± 0.09		0.597
0.09 ± 0.06				0.09 ± 0.10		
0.02 ± 0.03			0.951	0.02 ± 0.03		0.986
0.02 ± 0.02				0.02 ± 0.03		
0.9 ± 5.4			0.454	1.0 ± 6.5		0.888
1.6 ± 5.4				1.1 ± 7.9		
−1.7 ± 3.9			0.319	−0.3 ± 4.0		0.136
−2.7 ± 5.5				−2.1 ± 6.2		
26 ± 297			0.512	−11 ± 233		0.844
28 ± 255				24 ± 198		
−22 ± 177			0.323	−40 ± 176		0.548
−74 ± 206				−83 ± 215		
1 ± 12			0.475	−2 ± 13		0.298
2 ± 14				1 ± 14		
−99 ± 176			0.248	−33 ± 199		0.112
−139 ± 203				−74 ± 188		
0 ± 11			0.069#	2 ± 11		0.308
−3 ± 8				−1 ± 10		
−2 ± 14			0.920	−2 ± 17		0.341
−1 ± 11				0 ± 13		
−2 ± 26			0.599	−7 ± 23		0.576
−2 ± 25				−11 ± 24		

4 Statistics

The Mann–Whitney U test was used to assess the inter-group difference at 0, 4 and 8 weeks for skin parameter values that were determined with measuring instruments, digital image analyzer, self-evaluation of skin condition, and palpation and visual inspection by a cosmetic expert. $P < 0.1$ (#) was considered marginally significant. $P < 0.05$ (∗) was considered significant, and $P < 0.01$ and $P < 0.001$ were represented by (∗∗) and (∗∗∗), respectively. Wilcoxon signed-rank test (multiple comparison with Bonferroni inequality) was used to assess the intra-group difference before and after ingestion at 4 weeks or before and after ingestion at 8 weeks. $P < 0.1/2$ (#) was considered marginally significant. $P < 0.05/2$ was considered significant, and $P < 0.01/2$ and $P < 0.001/2$ were represented by (∗∗) and (∗∗∗), respectively. The regression equation between age and wrinkle number was determined with Excel 2011.

RESULTS

1) Age of subjects

A total of 90 women participated in this trial but two dropped out for personal reasons. Thus, 43 and 45 participants in the placebo and FCP groups, respectively, were subjected to analysis (**Table 1**). The age of subjects was 49.2 ± 7.2 years in the placebo group and 49.7 ± 6.9 years in the FCP group.

2) Evaluation with measuring instruments and a digital analyzer

2-1) Data analysis of all subjects

Skicon-200EX measurements were excluded from the analysis because measurements on the screening day were significantly different from those on the first test day (0 week). Cutometer values of three subjects in the placebo group were excluded from analysis because their elastic values were abnormal and exceeded 100%. Results of the analysis for all subjects are shown in **Table 2**.

There was no significant inter-group difference between the placebo group and the FCP group in the

measurements and change Δ of TEWL, moisture values (cheek and forearms), viscoelasticity (R_0, R_1, R_2), and VISIA parameters (wrinkles, porphyrins, pores, UV spots, texture, surface spots, brown spots and red areas).

Intra-group changes of TEWL, moisture content (cheek and forearm) and viscoelastic properties of R_0 and R_1 were similar in the placebo group and the FCP group. R_2 increased significantly in the FCP group at 4 weeks, which was different from that in the placebo group, but it was not significant at 8 weeks.

On the other hand, the number of wrinkles decreased significantly at 4 weeks in both groups, but a marginally significant decrease was only observed in the FCP group at 8 weeks. Texture, which is affected by variations in color, roughness and unevenness, also changed significantly in both groups at 4 weeks, but the improvement was maintained at 8 weeks only in the FCP group. A marginally significant decrease in the number of pores at 4 and 8 weeks was noted only in the FCP group. A significant decrease in the number of surface spots was seen in the FCP group only at 4 weeks but not at 8 weeks. The number of red areas, which represents conditions such as acne or inflammation, decreased significantly in both groups at 8 weeks.

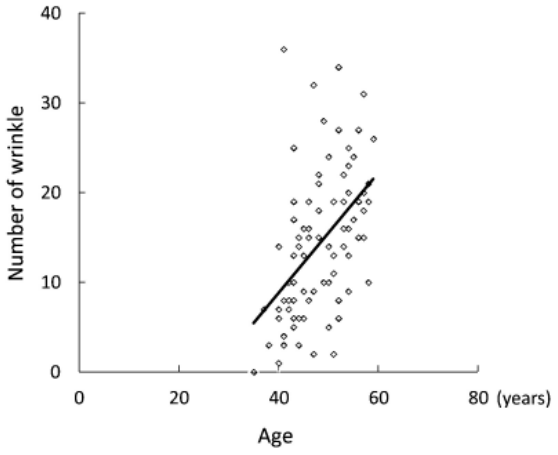

Figure 1 Correlation between age and the number of wrinkles.
Correlation between age and the number of wrinkles of subjects before ingestion (0 week). The correlation equation was y=0.561x−13.049 and contribution (R^2) was 0.227.

Figure 1 shows the relationship between age and number of wrinkles before ingestion of FCP or placebo (0 week). The regression equation was y = 0.561x–13.049 and coefficient of determination (R^2) was 0.227. These results suggest that there was a correlation between age and the number of wrinkles. There was no statistical correlation between age and texture or between age and number of pores.

2-2) Sub-group analysis with regard to age (< 50 and ≥50 years)

The mean age of participants in the group of women less than 50 years of age was 43.3 ± 3.3 years in the placebo group (n = 22; n = 21 for skin viscoelasticity) and 43.9 ± 3.2 years in the FCP group (n = 22). The mean age of participants in the group of women of 50 years of age or older was 55.4 ± 4.2 years in the placebo group (n = 21; n = 19 for

Table 3 Sub-group analysis for subjects (<50 and ≥50 years of age)

<50 years

Item	Unit	n	Group	Measured value							
				Before ingestion		4 weeks		8 weeks			
				Mean ± SD	P	Mean ± SD	P	Mean ± SD	P		
Texture	No.	22	Placebo	934 ± 459	0.348	875 ± 506	0.639	887 ± 435	0.734		
		22	FCP	1030 ± 386		901 ± 401*		923 ± 380*			
Red areas	No.	22	Placebo	109 ± 43	0.313	111 ± 46	0.405	104 ± 44	0.307		
		22	FCP	122 ± 41		120 ± 43		111 ± 41			

≥50 years

Item	Unit	n	Group	Measured value							
				Before ingestion		4 weeks		8 weeks			
				Mean ± SD	P	Mean ± SD	P	Mean ± SD	P		
Texture	No.	21	Placebo	1062 ± 574	0.541	921 ± 490**	0.533	1045 ± 574	0.565		
		23	FCP	1082 ± 434		934 ± 406*		1039 ± 428			
Red areas	No.	21	Placebo	148 ± 53	0.335	143 ± 60	0.481	140 ± 55	0.180		
		23	FCP	134 ± 40		132 ± 49		123 ± 37#			

skin viscoelasticity) and 55.3 ± 4.4 years in the FCP group (n = 23). Image analysis with VISIA showed that texture decreased significantly only in women aged less than 50 years in the FCP group at 4 and 8 weeks, suggesting improvement in their skin condition. A marginally significant decrease in the number of red areas was seen only in women aged 50 or over in the FCP group at 8 weeks (**Table 3**).

2-3) Additional sub-group analysis with regard to age (<60 years)

Additional sub-group analysis of subjects less than 60 years of age revealed that the number of wrinkle decreased significantly in both the placebo (n = 40) and FCP (n = 41) groups at 4 weeks, but this decrease was maintained only in the FCP groups at 8 weeks. Furthermore, the decrease Δ of wrinkle number was significantly larger in the FCP group than in the placebo group at 8 weeks (P = 0.034) (**Figure 2**).

2-4) Sub-group analysis with regard to skin type

When a sub-group analysis was performed for subjects with dry skin as determined by a cosmetic expert at screening, it was found that skin texture decreased significantly in both placebo (n = 25) and FCP (n = 24) groups, but a significant decrease at 8 weeks was noted only in the FCP group. Furthermore, the decrease Δ was significantly larger in the FCP group than in the placebo group (P = 0.010) (**Figure 3**). The number of red areas decreased significantly in the FCP group at 8 weeks, and the decrease Δ in the FCP group was marginally significant (P = 0.054) (**Figure 4**). The moisture content in the forearm was significantly higher in the FCP group at 4 weeks (P = 0.023). Although the mean value was higher at 8 weeks, the difference was not significant.

Δ Change							
4 weeks				8 weeks			
Mean	±	SD	P	Mean	±	SD	P
−59	±	164	0.197	−47	±	213	0.130
−129	±	195		−107	±	205	
2	±	24	0.503	−5	±	14	0.459
−2	±	22		−11	±	27	

Δ Change							
4 weeks				8 weeks			
Mean	±	SD	P	Mean	±	SD	P
−141	±	183	0.733	−18	±	187	0.496
−148	±	215		−42	±	168	
−5	±	29	0.991	−8	±	30	0.916
−2	±	28		−11	±	21	

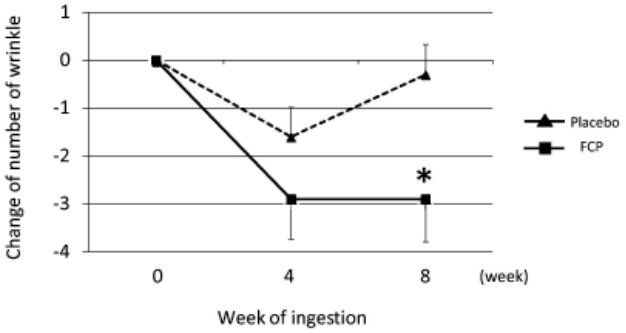

Figure 2 Change in the number of wrinkles.
Change in the number of wrinkles in subjects younger than 60 years of age after ingestion. Mean ± SEM. *P = 0.034

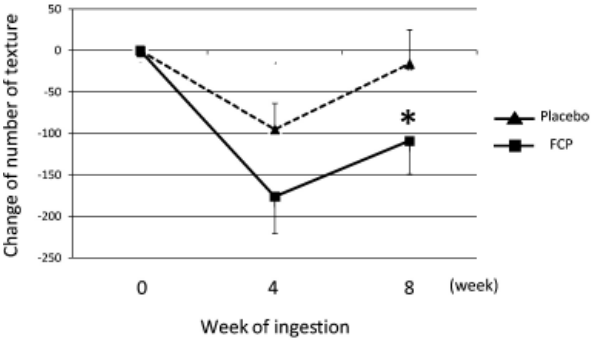

Figure 3 Change in texture.
Change in the value for texture in subjects with dry skin after ingestion. Mean ± SEM. *P = 0.010

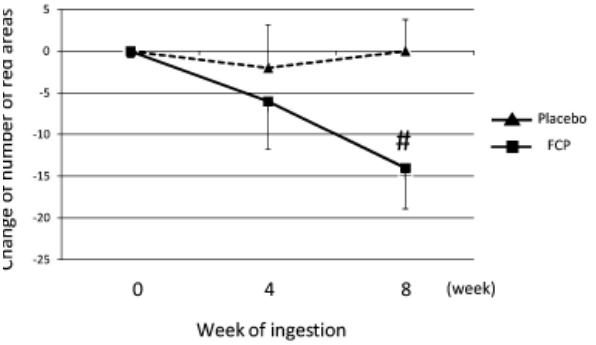

Figure 4 Change in the number of red areas.
Change in the number of red areas in subjects with dry skin after ingestion. Mean ± SEM. #P = 0.054

Table 4 Questionnaire and examination by touch and visual inspection for all participants

Questionnaire on subjective feelings	n	Group	Before ingestion		4 weeks		8 weeks	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
How do you feel about your skin conditions compared to before ingestion?	43	Placebo	2.00 ± 0.00	1.000	2.21 ± 0.41	0.884	2.28 ± 0.50	0.008**
	45	FCP	2.00 ± 0.00		2.22 ± 0.42		2.58 ± 0.50	
How does makeup sit compared to before ingestion?	43	Placebo	3.00 ± 0.00	1.000	2.42 ± 0.73	0.611	2.33 ± 0.71	0.461
	45	FCP	3.00 ± 0.00		2.36 ± 0.71		2.22 ± 0.70	
How is moist feeling compared to before ingestion?	43	Placebo	3.00 ± 0.00	1.000	2.26 ± 0.69	0.044*	2.16 ± 0.69	0.263
	45	FCP	3.00 ± 0.00		2.56 ± 0.50		2.33 ± 0.56	
How is tension and elasticity compared to before ingestion?	43	Placebo	3.00 ± 0.00	1.000	2.65 ± 0.61	0.566	2.63 ± 0.62	0.460
	45	FCP	3.00 ± 0.00		2.62 ± 0.53		2.58 ± 0.54	
How is texture compared to before ingestion?	43	Placebo	3.00 ± 0.00	1.000	2.49 ± 0.70	0.966	2.49 ± 0.63	0.985
	45	FCP	3.00 ± 0.00		2.53 ± 0.59		2.51 ± 0.55	
Examination by touch and visual inspection	**n**	**Group**	**Before ingestion**		**4 weeks**		**8 weeks**	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Visual inspection (texture of cheek)	43	Placebo	3.77 ± 0.61	0.593	3.91 ± 0.57	0.151	3.95 ± 0.58	0.126
	45	FCP	3.76 ± 0.48		3.82 ± 0.44		3.87 ± 0.40	
Visual inspection of dryness at a tail of eye and mouth	43	Placebo	3.26 ± 0.66	0.252	3.44 ± 0.63	0.130	3.40 ± 0.54	0.834
	45	FCP	3.04 ± 0.82		3.24 ± 0.57		3.44 ± 0.62	
Visual inspection (dullness)	43	Placebo	3.49 ± 0.67	0.778	3.60 ± 0.62	0.546	3.56 ± 0.55	0.702
	45	FCP	3.58 ± 0.54		3.56 ± 0.50		3.62 ± 0.53	
Visual inspection (skin age compared to actual age)	43	Placebo	3.65 ± 0.65	0.627	3.77 ± 0.57	0.987	3.74 ± 0.49	0.195
	45	FCP	3.76 ± 0.43		3.78 ± 0.47		3.89 ± 0.44	
Examination by touch (elasticity of cheek)	43	Placebo	3.74 ± 0.66	0.355	3.79 ± 0.64	0.152	3.95 ± 0.49	0.740
	45	FCP	3.84 ± 0.42		3.93 ± 0.25		3.98 ± 0.26	

Table 5 Sub-group analysis of subjective feeling of skin conditions (<50 and ≥50 years of age)

Age	n	Group	Before ingestion		4 weeks		8 weeks	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
<50	21	Placebo	2.00 ± 0.00	1.000	2.18 ± 0.39	0.181	2.14 ± 0.47	<0.001***
	23	FCP	2.00 ± 0.00		2.36 ± 0.49		2.68 ± 0.48	
≥50	22	Placebo	2.00 ± 0.00	1.000	2.24 ± 0.44	0.176	2.43 ± 0.51	0.744
	22	FCP	2.00 ± 0.00		2.09 ± 0.29		2.48 ± 0.51	

3) Sensory evaluation of the skin condition

Skin condition was assessed by self-evaluation and with palpation and visual inspection by a cosmetic expert. The results are summarized in **Table 4**. For the item "How do you feel about your skin conditions compared to before ingestion?" in the subjective assessment, the score was significantly higher in the FCP group than in the placebo group ($P = 0.008$), meaning the percentage of the answer "better" was much higher in the FCP group than in the placebo group. When a sub-group analysis of participants less than 50 years of age was conducted, it was found that the score was significantly higher in the FCP group than in the placebo group ($P < 0.001$). However, in participants aged 50 or over, the score increased also in the placebo group and the inter-group difference was not significant (**Table 5**).

DISCUSSION

In clinical studies on the effects of nutrients, the basal nutrient status must be considered to determine the amount of test material that should be ingested[14]. In this study, the ingestion of 5 g of FCP was employed because 5 g or 10 g of CP resulted in significant changes in skin parameters without adverse effects in Japanese subjects who ingested approximately 1.9 g of collagen daily in

their diet[15, 16]. The effects of FCP ingestion on skin properties were investigated in participants aged 35–65 years (mainly in their 40s and 50s) using measuring instruments, a digital image analyzer, self-evaluation by questionnaire, and palpation and visual inspection by a cosmetic expert.

Digital analysis with VISIA revealed an improvement in the facial skin condition. The number of wrinkles decreased significantly after ingesting FCP for 8 weeks, especially in subjects less than 60 years of age. The decrease of −2.1 on average corresponded to 3.7 years of rejuvenation according to the regression equation ($y = 0.561x - 13.049$). Skin texture that is affected by variation in color, roughness and unevenness was also improved, especially in subjects with dry skin. Furthermore, the number of red areas that represents conditions such as acne or inflammation was decreased marginally in subjects aged 50 years or older with dry skin. We reported previously that daily ingestion of 5 g of FCP suppressed an acute inflammatory response and erythema induced by ultraviolet irradiation to the skin[12]. Therefore, it is possible that FCP ingestion reduces the inflammatory response in the skin. The decrease in the number of pores was marginally significant for subjects of all ages in the FCP group. Taken together, these results indicate that ingestion of FCP reduces wrinkle, improves texture, and reduces red areas and pores, thus making the skin more uniform and smoother. Because facial skin looks more beautiful if wrinkles, uneven color, surface unevenness, pores and spots are less conspicuous, our results support consumer's understanding that the intake of CP is beneficial for beautiful skin.

With regard to the subjective assessment of skin condition, a significant inter-group difference was observed for the score of the question "How do you feel about your skin conditions compared to before ingestion?", meaning that FCP-ingesting subjects felt an improvement in their skin condition. This inter-group difference was also evident in subjects less than 50 years of age but not in subjects aged 50 or over because of the placebo effect. The subjective feeling of improvement of skin condition supports market expansion of CP as a health supplement.

CONCLUSIONS

Effects of fish-derived collagen peptide (FCP) on the skin were investigated. A placebo-controlled double-blind trial was conducted with Japanese women (35–65 years of age) with dry and saggy facial skin. They ingested 5 g of placebo or FCP for 8 weeks, and skin properties and subjective feeling of their skin condition were examined before and after ingestion at 4 and 8 weeks. A significant decrease in wrinkle number and improvement of texture were observed on sub-group analysis of the FCP group. The number of red areas and pores were decreased marginally after FCP ingestion. A significant improvement of the skin condition (subjective feeling) was observed at 8 weeks. Because collagen peptide is a safe and widely used food ingredient, collagen peptide can be used as an ingredient for maintaining skin beauty.

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