Clinical Effects of Ingesting Collagen Hydrolysate on Facial Skin Properties

—A Randomized, Placebo-controlled, Double-blind Trial—

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ABSTRACT

Objectives The objective of this research was to investigate the effectiveness of daily ingestion of a specific collagen hydrolysate (CH), which contains prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly), on facial skin properties.

Methods In this randomized, placebo-controlled, double-blind trial, 56 women aged 30-55 years were randomized to receive 2.5 g of CH or 5 g of placebo once daily for 8 weeks, with 28 subjects assigned to each group. The hydration, elasticity and roughness properties of facial skin were measured at week 0 (baseline), week 4 and week 8.

Results Levels of skin hydration, elasticity and roughness in subjects who received CH significantly improved between baseline and weeks 4 and 8, while there was no significant improvement in subjects who received placebo. Moreover, the levels of skin elasticity, roughness and the net change of skin hydration improved significantly in the CH group compared to the placebo group by both weeks 4 and 8.

Conclusion The present results suggest that daily ingestion of 2.5 g of CH improves facial skin hydration, elasticity and roughness.

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KEY WORDS Collagen hydrolysate, Clinical study, Skin hydration, Skin elasticity, Skin roughness

INTRODUCTION

Skin properties are important factors in the quality of life. Many skin problems originate from endogenous sources and may have underlying dietary causes[1)]; dietary supplements have demonstrated beneficial effects on skin health. Researchers have studied peptides derived from protein hydrolysates as potential dietary ingredients in the development of functional foods.[2)]

Collagen is well known as a major constituent of connective tissues. Gelatin, a denatured form of collagen, is popularly used in foods. The enzymatic hydrolysate of gelatin is also called collagen hydrolysate (CH) or collagen peptide, and has long been used in pharmaceuticals and food supplements for improving skin and cartilage tissue. Research in mice has demonstrated that after oral administration of radiolabeled CH, the radioactivity was accumulated in skin for up to 96h.[3)] Several peptides have been detected in human peripheral blood following the oral ingestion of CH. Of these collagen-derived peptides, prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly) were identified as major dipeptides.[4-8)] In an animal study, it was demonstrated that Pro-Hyp, with the Pro residue labeled with radioiso-

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tope ^{14}C, was able to reach the skin rapidly after oral administration in mice.[9] In addition, Pro-Hyp stimulated chemotaxis, cell proliferation and expression of hyaluronic acid synthase gene, and Hyp-Gly enhanced cell proliferation more effectively than Pro-Hyp, in cultured dermal fibroblast.[7,10-12] In a clinical study, it was reported that skin hydration in subjects was increased by CH ingestion at doses of 5 and 10 g day^{-1} for 4 weeks.[13] These findings suggested that CH containing Pro-Hyp and Hyp-Gly improves human skin properties.

The present study was conducted to investigate the efficacy of ingesting 2.5 g of CH, which contains these dipeptides, on facial skin properties by a randomized, placebo-controlled, double-blind trial.

MATERIALS AND METHODS

1 Investigational products

The CH used (Wellnex®), which contains dipeptides Pro-Hyp and Hyp-Gly at concentrations greater than 3 g kg^{-1}, was supplied by Nitta Gelatin Inc.(Osaka, Japan), and the placebo (maltodextrin, TK-1) was purchased from Matsutani Chemical Industry Co. Ltd (Itami, Japan). This CH was the enzymatic hydrolysate of fish scale gelatin, and the average molecular weight of the CH was 1000 Da.

2 Study design

This was an 8-week randomized, placebo-controlled, double-blind study examining the effects of CH ingestion on facial skin. Subjects were randomly assigned to one of the two treatment regimens：2.5 g of CH daily or 5.0 g of placebo daily. Subjects were assigned to treatment groups in a 1：1 ratio using a computer-generated randomization schedule. Samples were ingested once a day after dinner for 8 weeks from February to April 2012. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the ethics committee of Shanghai Skin Disease Hospital (Shanghai, China), and written informed consent was obtained from all subjects participating in the study.

3 Subjects

Fifty-six Chinese women diagnosed as healthy by blood test were enrolled in this study. Criteria to select subjects were 30- to 55-year-old women who were conscious of their dry and rough skin, with a Body Mass Index of less than 30. The subjects had not been regularly using other supplements and health foods and had not taken sex hormones the three months prior to the study. None of the subjects had previously been diagnosed with diabetes, nor had been pregnant. The subjects were advised to avoid excessive eating, drinking and exercise, change in regular alcohol intake and use of cosmetic materials, and strong sunburn.

4 Physiological measurements of the skin

All facial skin property measurements were carried out at weeks 0 (baseline), 4 and 8. On each measurement day, the subjects washed off their makeup and then were acclimatized for 30 min in the waiting lounge fixed at a constant temperature of 20±2°C and humidity of 50±5% prior to measurement. Skin hydration and elasticity were measured on one side of the canthus while skin roughness was assessed on one side of the cheek by the same blinded investigator. All measurements were performed in triplicate. To measure skin hydration and skin elasticity as gross elasticity (R2), a Corneometer® 820 (Courage & Khazaka, Cologne, Germany) and Cutometer® SEM 575 (Courage & Khazaka) were used, respectively. Skin roughness was measured using a VisicoFace SSA (Skin Surface Analysis, Courage & Khazaka).

5 Statistics

Statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). When a significant difference was noted between the two groups in baseline, the net change of value between baseline and week 4 or week 8 was calculated to avoid the intergroup difference.

The paired sample *t*-test was applied for comparing data from weeks 4 and *8* to the baseline within a group. The Student's *t*-test was performed for comparison between the study group and the placebo group. The significant level used was the 95% confidence level ($P<0.05$).

RESULTS

1 Panel demographics

There were three dropouts during the study period, mainly due to difficulty in returning to the hospital for personal reasons or moving far away. There was no significant difference in the mean age between the groups at week 8 (**Table 1**). None of the subjects involved in the study showed any problems with their diet.

2 Facial skin hydration

The facial skin hydration of the CH group increased significantly ($P<0.01$) between baseline and weeks 4 and 8, while that of the placebo group decreased (**Table 2**).

Table 1　Panel demographics

Group	Number of subjects			Age at week 8*
	Week 0	Dropouts	Week 8	
CH	28	1	27	43.31±4.70
Placebo	28	2	26	42.31±4.80

*Data are expressed as mean±standard deviation（SD）.
CH＝collagen hydrolysate

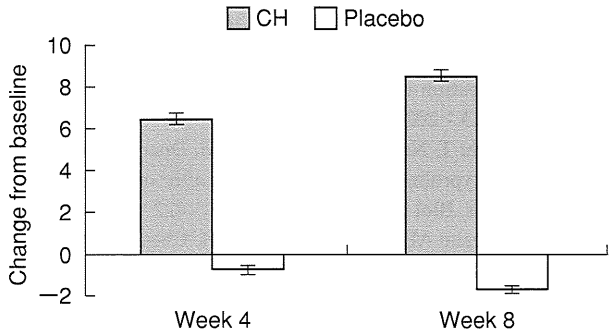

Fig. 1　Change of facial skin hydration from baseline
The changes of skin hydration at weeks 4 and 8 from baseline in the collagen hydrolysate（CH）group were significantly higher than those in the placebo group（$P<0.01$）.
Data are expressed as mean±SEM.

Moreover, there was a significant increase（$P<0.01$）in the net change of skin hydration in the CH group when compared to the placebo by weeks 4 and 8（**Fig. 1**）.

3　Facial skin elasticity

The facial skin elasticity of the CH group increased significantly（$P<0.01$）between baseline and weeks 4 and 8, while that of the placebo group decreased significantly（$P<0.05$）. Moreover, there was a significant increase（$P<0.01$）in the skin elasticity in the CH group by weeks 4 and 8 when compared to the placebo group（**Table 3**）.

4　Facial skin roughness

The facial skin roughness of the CH group decreased significantly（$P<0.01$）between baseline and weeks 4 and 8, while that of the placebo group did not decrease significantly. Moreover, there was a significant reduction in the skin roughness in the CH group by weeks 4（$P<0.05$）and 8（$P<0.01$）when compared to the placebo group（**Table 4**）.

DISCUSSION

The present study demonstrated that the levels of skin hydration, elasticity and roughness in subjects who received 2.5 g of CH significantly improved between baseline and weeks 4 and 8, while there was no significant improvement in subjects who received placebo. Moreover, the levels of skin elasticity, roughness and the net change of skin hydration improved significantly in the CH group compared to the placebo group by both weeks 4 and 8. A recent study by Proksch et al.[14] reported the efficacy of CH intake in skin hydration, transepidermal water loss, elasticity（R5）and roughness of the forearm, not the face. In their randomized, placebo-controlled, double-blind study the subjects were given CH at a dose of 2.5 g day^{-1} or 5 g day^{-1} for 8 weeks. From their results, CH showed superior improvement over placebo on skin hydration and elasticity, but not skin roughness. The discordance between these dif-

Table 2　Facial skin hydration throughout treatment

Group	Baseline	Week 4	Week 8
CH（$n=27$）	65.44±11.20▲	71.91±9.55**	73.98±8.86**
Placebo（$n=26$）	72.49±11.58	71.76±10.73	70.85±10.20

Data are expressed as mean±SD.
**Intra-group comparison（$P<0.01$ vs. baseline）
▲Intergroup comparison（$P<0.05$ vs. placebo in baseline）

Table 3　Facial skin elasticity（R2）throughout treatment

Group	Baseline	Week 4	Week 8
CH（$n=27$）	0.694±0.140	0.760±0.134**△	0.758±0.109**△
Placebo（$n=26$）	0.735±0.121	0.681±0.088*	0.697±0.087*

Data are expressed as mean±SD.
*Intra-group comparison（$P<0.05$ vs. baseline）
**Intra-group comparison（$P<0.01$ vs. baseline）
△Intergroup comparison（$P<0.01$ vs. placebo）

Table 4　Facial skin roughness throughout treatment

Group	Baseline	Week 4	Week 8
CH（$n=27$）	23.15±1.51	22.52±1.53**▲	22.15±1.66**△
Placebo（$n=26$）	23.69±1.74	23.58±1.60	23.42±1.60

Data are expressed as mean±SD.
**Intra-group comparison（$P<0.01$ vs. baseline）
▲Intergroup comparison（$P<0.05$ vs. placebo）
△Intergroup comparison（$P<0.01$ vs. placebo）

ferent studies may be attributed to differences in the CH used, subject race（Chinese vs. Caucasian）and the measurement area（face vs. forearm）.

CH has been demonstrated to have protective effects on chronological skin aging by its influence on collagen matrix homeostasis in rats.[15] The proposed mechanism of action of CH involves reducing the loss of moisture and lipids, promoting anti-oxidative activity, and repairing the endogenous collagen and elastin fibers.[16] It was also reported that CH enhanced hyaluronic acid production in human dermal fibroblasts *in vitro* and in mouse skin *in vivo*.[17] These findings suggest that CH ingestion may contribute to improving skin properties.

We previously reported that Pro-Hyp and Hyp-Gly were absorbed into human peripheral blood after ingestion of CH containing these peptides.[8] Pro-Hyp had a stimulatory activity on the proliferation, migration and hyaluronic acid synthesis of skin fibroblast[10-12], and Hyp-Gly could also enhance the growth of the cell[7], which indicated that CH-derived peptides modulated the cell and extracellular matrix of skin. Thus, it is speculated that the beneficial effects of CH ingestion on skin hydration, elasticity and roughness depend on the physiological activities of CH-derived peptides such as Pro-Hyp and Hyp-Gly.

CONCLUSION

Daily intake of 2.5 g of CH may improve facial skin hydration, elasticity and roughness. Further double-blinded, large-scale studies will be necessary to establish clinical recommendations for CH supplementation.

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