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The Beneficial Effects of Collagen Tripeptide on Deep Wrinkling and Skin Moisturization: A Randomized Controlled Trial

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Abstract Dietary collagen is now widely considered as an effective supplement to improve skin appearance by improving elasticity and moisturization. However, because the mechanism of absorption in the body is not clear, collagen-peptides are developed as an active ingredient in the health care market for skin. The aim of this study was to assess whether oral administration of collagen tripeptide (CTP) improves wrinkling and skin hydration. This was a double-blind, randomized, placebo-controlled trial. We evaluated skin changes after oral intake of CTP in participants 40-55 years of age who had wrinkles around their eyes and dry skin. They received either a test product with CTP (n=35) or a placebo (n=38) for 12 weeks. At weeks 0, 6, and 12, water contents were measured on the right and left cheek with a Corneometer®, and eye wrinkles were evaluated on both sides around the eyes by Primos CR. In the CTP group, roughness parameters were significantly reduced around the eyes. Additionally, the difference between the CTP and placebo groups was statistically significant. The capacitance for moisture significantly increased after oral supplementation of CTP. Interestingly, the capacitance was significant between the CTP and placebo groups after excluding participants who did not use sunscreen, used colored makeup at least five times a week, or slept less than 5 hours a day. Additionally, more 45 years of age women were significantly increased skin moisture compared to placebo group. Administration of CTP was well-tolerated, and no notable adverse effects were reported in either group. Oral supplementation of CTP is safe and helpful to the skin appearance, improving wrinkle formation and skin hydration.

Keywords: *dietary collagen, collagen tripeptide, tolerance, wrinkling, skin hydration*

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1. Introduction

It is well known that skin undergoes intrinsic or extrinsic aging processes. In aged skin, an overall reduction of collagen amount is seen, due to either decrease in production or increase in degradation. This plays a major role in the alteration of the skin's physical properties, such as wrinkle formation and drying of skin [1]. The aging process also results in loss of hyaluronic acid (HA), causing a reduction in moisture and elasticity of the skin [2]. Collagen and HA are major constituents of human skin, and contribute to the assembly of the extracellular matrix (ECM), which is an important structural component of the dermis. Collagen peptide, often referred to as hydrolyzed collagen, is made through the enzymatic hydrolysis of collagen. Upon digestion, collagen end-products are cleaved into di- and tri-peptides, which are then again used as building blocks for proteins, such as collagen in the body [3,4].

Collagen is formed as a triple helix of polypeptide chains, containing a glycine (Gly) residue at every third position, resulting in (Gly-X-Y)×n repeated structures in all collagenous domains. The X and Y position is frequently occupied by proline (Pro) and hydroxyproline (Hyp), making the Gly-Pro-Hyp (GPH) sequence the most common amino acid triplet in collagen [5]. In the mouse model, the amino acids of collagen were seen to be distributed in the skin as well as plasma, following oral administration of collagen. The Gly-Pro-Hyp (GPH) and its hydrolyzed Pro-Hyp (PH) form were mainly observed [3].

A growing body of evidence demonstrates the beneficial effects of oral collagen administration on skin appearance. Typically, collagen-based dietary supplementation has been shown to reduce wrinkle formation in addition to increasing elasticity and dermal density [6,7]. As previously reported, collagen peptide intake is majorly associated with enhancement of skin moisture. Collagen supplementation has been observed to attenuate ultraviolet B (UVB) ray-induced skin dehydration because of HA synthesis [8,9]. Collagen peptides were also shown to

increase HA generation in dermal fibroblasts [10,11], leading to an improvement of skin barrier function by increasing the water contents of the stratum corneum [10,12,13]. In a double-blind study, 5–10 g collagen peptides were seen to improve moisture content and viscoelasticity of skin within 4 weeks or 8 weeks of supplementation [14,15]. Ohara et al. administered 200 nM of di- or tri-peptide, including PH, in dermal fibroblasts and then measured the synthesis of hyaluronic acid. PH treatment induced a two-fold increase in mRNA expression of the hyaluronan synthase 2 (HAS2) [16]. It was also found that collagen tripeptide (CTP) decreases trans-epidermal water loss by adjusting for climate, humidity, temperature, and UVA rays. The efficacy of CTP was shown to be better than that of placebo after 12 weeks of administration, with tolerance [17]. In this study, we assessed the beneficial effects of CTP derived from the skin of the Nile Tilapia fish on wrinkling and moisture contents, and its safety.

2. Methods

2.1. Ethical Aspects

Eighty one participants received detailed information about all the procedures involved and signed the informed consent form. This study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Institutional Review Board at P&K Skin Research Center (IRB No. P1910-554).

2.2. Study Participants

Healthy Korean female volunteers were enrolled in this study. Eligible participants included those aged 40 to 55 years, if they met the specified inclusion and exclusion criteria. Inclusion criteria included those with eye wrinkles visually evaluated as 3rd grade or higher on both left- and right-sides, as well as those in whom water content measured by the Corneometer® on both left and right cheeks was 49 or less. The main exclusion criteria were as follows: in the treatment of skin disease such as atopic dermatitis or psoriasis, hypersensitivity and allergic reaction to test ingredient, inappropriate appearance for measurement in the test area, a steroid treatment or light treatment within three months, application of cosmetics for wrinkle reduction or high moisturization within 2 weeks. The finalized 76 participants were then randomly assigned to an intervention or CTP group and a placebo group.

2.3. Study Design and Schedule

The study was designed as a randomized, placebo-controlled, double-blind clinical trial with a duration of 12-weeks. The intervention group, that received administration of 1.0 g CTP tablet once daily, was compared with the placebo group, that received a tablet without CTP. Before the first intake of the CTP or placebo, and after six and twelve weeks of intake, participants were dermatologically examined, and tolerability and efficacy data were collected.

Table 1. Demographic information and characteristics of participants prior to supplementation (PPset)

		CTP	Placebo
		n=35	n=38
Age	Mean ± SD	46.11±4.40	45.74±4.69
	Min, Max	40.00,54.00	40.00,54.00
	p-value	0.6696[#]	
Smoking	No	35(100.00)	38(100.00)
	Yes	0(0.00)	0(0.00)
	p-value	-	-
Alcohol drinking	No	22 (62.86)	23 (60.53)
	Stop	1 (2.86)	1 (2.63)
	Less than 1 bottle / week	8 (22.86)	10 (26.32)
	1 ~3 bottles / week	4 (11.43)	4 (10.53)
	More than 4 bottles / week	0 (0.00)	0 (0.00)
	p-value	0.9689‡	
Makeup (Frequency)	0 / week	10 (28.57)	6 (15.79)
	1~2 times / week	10 (28.57)	11 (28.95)
	3 ~4 times / week	9 (25.71)	14 (36.84)
	more than 5 times /week	6 (17.14)	7 (18.42)
	p-value	0.5536†	
Sleeping time (Hours)	Less than 5 hours / day	4 (11.43)	2 (5.26)
	5 ~ 8 hours / day	24 (68.57)	33 (86.84)
	More than 8 hours / day	7 (20.00)	3 (7.89)
	p-value	0.1788‡	
Sunscreen use (Frequency)	0 / week	4 (11.43)	4 (10.53)
	1~2 times / week	7 (20.00)	6 (15.79)
	3 ~4 times / week	13 (37.14)	9 (23.68)
	More than 5 times /week	11 (31.43)	19 (50.00)
	p-value	0.4218‡	

[#]: Compared between CTP and Placebo groups; p-value for Wilcoxon rank-sum test, †: CTP and Placebo; p-value for Chi-square test, ‡: CTP and Placebo; p-value for Fisher's exact test.

2.4. Test Product

The main product (Collagen-Tripep20S; Amicogen Inc., Jinju, Gyeongsangnam-do, South Korea) was prepared from the skin of Nile Tilapia (*Oreochromis niloticus*) through collagenase digestion by non-pathogenic bacteria of the *Bacillus* genus. More detailed information about CTP has been well described in a previous study [17]. Ingredients of the intervention and placebo tablets are listed in Table 2.

Table 2. Test and Placebo products composition

Ingredient	Test (%)	Placebo (%)
Collagen-Tripep20S	31.3	0.0
Cellulose	63.1	94.3
Stearic acid magnesium	2.0	2.0
Silicon dioxide	1.0	1.0
Titanium dioxide	1.2	1.2
HPMC	1.0	1.0
Sucrose fatty acid Ester	0.5	0.5

2.5. Assessment of Safety and Tolerability

Participants who consumed the test product at least once were randomly selected for the clinical test. The types and incidence of adverse reactions, symptom severity, and their relevance to food were evaluated. In addition, clinical pathology tests (hematological, hemochemical tests, urine tests), vital signs (pulse, blood pressure, blood pressure), and physical measurements (bodyweight) were analyzed.

2.6. Measurements of Efficacy

Before the assessment, participants were instructed to rest in a comfortable room with a temperature of 20°C to 25°C and a humidity of 40% to 60% for at least 30 minutes after washing their face. The participants were not allowed to consume water during the rest period. Only one researcher was allowed to assess the participants to ensure that the assessment was done on the exact same treatment area on each visit.

2.6.1. Skin Hydration

Moisture content of the cheek was evaluated using the Corneometer® CM825 (Courage-Khazaka electronic GmbH, Germany) at the baseline and at 6- and 12-weeks of product use. The Corneometer® probe with the sensor was brought in contact with the skin around the cheek, and three measurements were averaged to obtain the moisture content data. The unit of measurement was an arbitrary unit (A.U.), and the value was proportional to actual moisture content.

2.6.2. Wrinkling

Skin roughness was measured using the three dimensional (3D) skin imaging system, Primos CR (Canfield, USA); the measurements were taken at the same site at the baseline, and at 6- and 12-weeks of test product use. The Rmax (maximum peak to valley roughness height), Rp (maximum peak high), and Rv (maximum valley depth) values of the stored images were evaluated. The values of Rmax and Rv denoted the depth of the wrinkles from the skin surface. The value of Rp represented the maximum profile peak height in the area selected.

2.7. Statistical Analysis

The statistical analysis package SAS® (Version 9.4, SAS Institute, Cary, North Carolina, USA) was used to evaluate the efficacy and safety of test product for skin changes. The significance level was set at 5% (i.e., p-value < 0.05 indicates statistical significance). The degree of change was analyzed using a paired t-test, and at each time point, the degree of change between CTP group and the placebo group was evaluated for a statistically significant difference by conducting a two-sample t-test or a Wilcoxon rank sum test, depending on whether normality was satisfied. In addition, the GLM (Generalized Linear Model), which uses the variables age, drinking, smoking, and sleep time as covariates, was used.

After plotting all adverse reactions that occurred after administration of the product, the incidence rate was calculated. The proportion of human subjects who had adverse reactions between each group was calculated and then comparisons were analyzed using the Chi-square test or Fisher's exact test.

3. Results

3.1. Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram of the Controlled Interventional Trial

Seventy-three participants aged 40 to 55 years were statistically analyzed. The mean age in the CTP group (n=35) was 46.11±4.40 years, and in the placebo group (n=38), 45.74 ± 4.69 years. Three participants in the CTP and one in the placebo group withdrew consent for personal reasons that were not associated with the trial. One volunteer withdrew from the study due to an adverse event that was not related to the trial. Additionally, one participant was excluded due to protocol violation, and another was excluded because of less than 70% compliance. Therefore, Intend-To-Treat (ITT) population and Per Protocol (PP) population were 76 and 73, respectively. No statistically significant intergroup differences were observed for age, smoking, skin surgical treatment, alcohol drinking, coffee intake, outdoor activity, makeup frequency, sleep time, and number of sunscreen use. The safety population (SP) also included 81 enrolled participants. The flow of participants through the controlled interventional trial is depicted in a CONSORT conform diagram (Figure 1).

3.2. Tolerability

Most participants completed the protocol without adverse events. In the blood and urine tests, no pathological changes were found. The vital signs and physical measurement were not significantly different after product consumption.

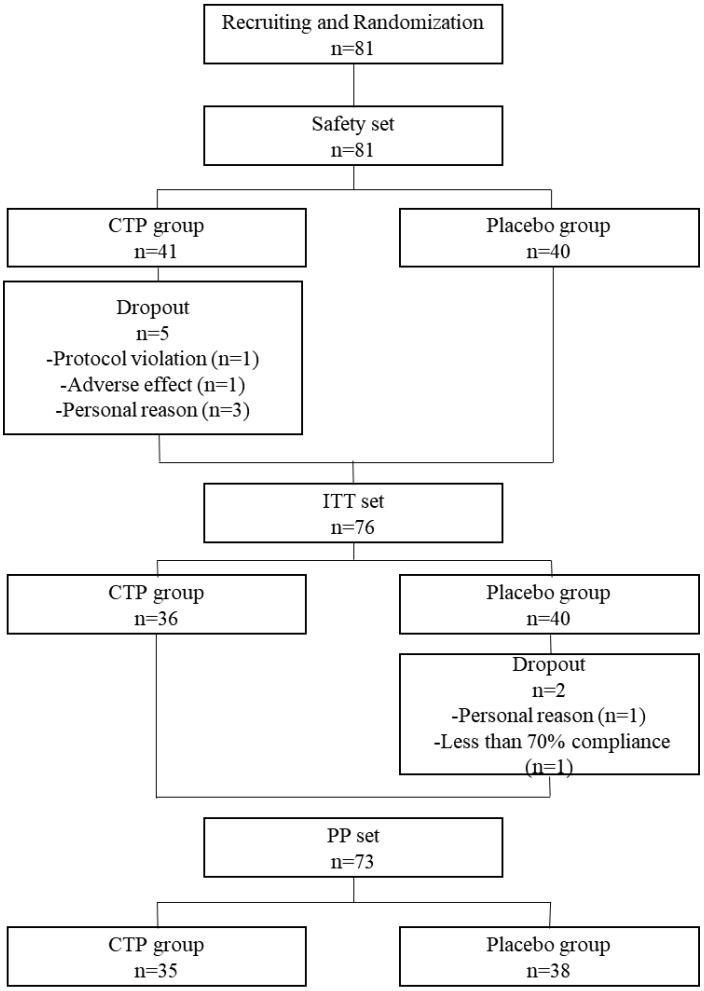

Figure 1. The study flow diagram. Abbreviations: CTP, Collagen tripeptide; ITT, Intention-to-Treat; PP, Per Protocol

Table 3. R-parameters (Primos^{CR}) (PP Set)

		Rmax(μm) Mean±SD		Rp(μm) Mean±SD		Rv(μm) Mean±SD	
		CTP (n=35)	Placebo (n=38)	CTP (n=35)	Placebo (n=38)	CTP (n=35)	Placebo (n=38)
Ave.	Baseline	183.32±29.27	175.96±29.91	107.17±21.15	104.54±21.90	94.03±18.91	88.80±14.41
	Week 6(V3)	177.04±29.61	174.41±34.94	103.12±20.66	104.84±26.10	91.22±19.45	87.05±17.30
	Change from baseline	-6.27±12.94	-1.55±12.45	-4.05±9.64	0.30±9.92	-2.80±7.42$^{\#}$	-1.76±7.47
	p-value**	0.0070	0.4476	0.0181	0.8549	0.0319	0.1560
	Week 12(V4)	172.72±27.90	172.03±34.71	100.45±19.76	102.25±26.04	88.39±18.39	87.41±17.70
	Change from baseline	-10.60±13.34$^{*,\$}$	-3.93±11.77	-6.72±10.95$^{\$}$	-2.29±10.23	-5.64±7.13$^{*,\$}$	-1.39±8.25
	p-value**	<.0001	0.0466	0.0009	0.1767	<.0001	0.3059
Left side	Baseline	182.45±35.15	169.10±32.23	104.36±22.58	98.72±25.96	95.46±24.06	86.54±15.80
	Week 6(V3)	171.54±34.99	167.88±39.20	99.80±22.22	110.79±27.52	90.06±22.72	84.35±18.78
	Change from baseline	-10.92±15.65$^{\#,S}$	-1.22±14.31	-7.17±14.74$^{\#,\$}$	1.11±11.67	-5.41±8.36	-2.18±8.20
	p-value**	0.0002	0.6014	0.0069	0.5601	0.0005	0.1089
	Week 12(V4)	167.30±35.29	165.56±43.27	94.04±23.50	97.17±33.75	88.34±22.51	84.28±20.69
	Change from baseline	-15.15±17.85^{*,S}	-3.54±17.14	-10.31±15.23$^{\#,\$}$	-1.55±14.39	-7.12±7.95*	-2.25±9.82
	p-value**	<.0001	0.2106	0.0003	0.5109	<.0001	0.1658
Right side	Baseline	184.18±39.68	182.82±41.71	109.06±34.30	110.36±30.94	92.60±18.08	91.07±18.42
	Week 6(V3)	182.55±40.75	180.94±44.71	109.06±34.93	109.84±33.16	92.39±21.54	89.75±22.25
	Change from baseline	-1.63±15.66	-1.88±16.14	-0.93±9.62	-0.52±12.60	-0.20±12.11	-1.33±10.48
	p-value**	0.5422	0.4776	0.5723	0.8006	0.9217	0.4401
	Week 12(V4)	178.13±39.19	178.50±42.63	106.86±34.73	107.33±32.44	88.44±18.07	90.54±22.62
	Change from baseline	-6.05±17.61	-4.32±19.51	-3.12±14.41	-3.02±15.91	-4.15±9.87	-0.53±11.63
	p-value**	0.0502	0.1809	0.2088	0.2494	0.0178	0.7815

**: compared within group; p-value for Paired t-test, *: compared between 'CTP' and 'Placebo' groups; p-value for Two sample t-test, $^{\#}$: compared between 'CTP' and 'Placebo' groups; p-value for Wilcoxon rank sum test, $^{\$}$: Compared between 'CTP' and 'Placebo' groups; p-value for GLM adjusted baseline, age, drink, smoke, sleep.

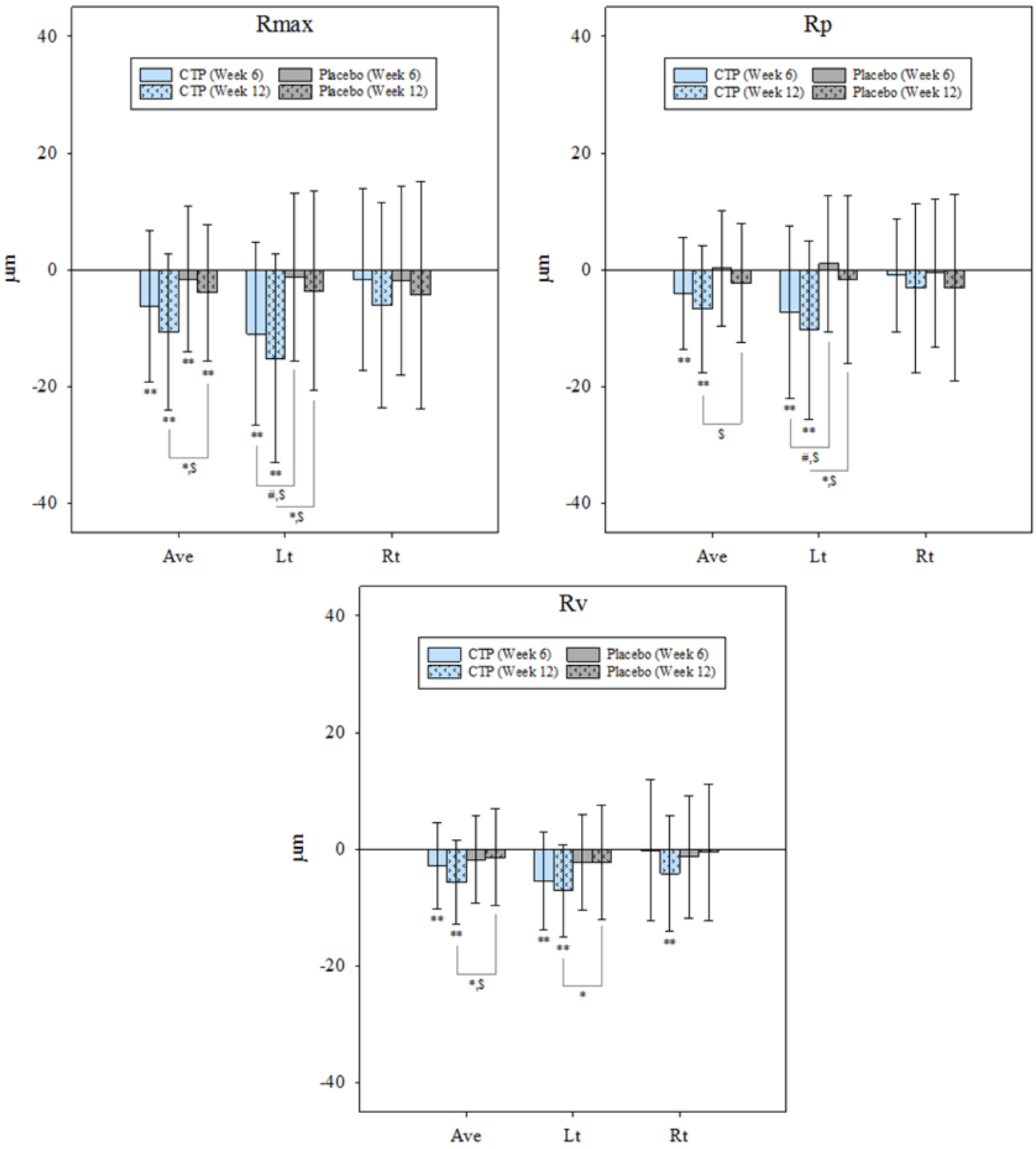

Figure 2. (color online) Changes in Rmax, Rp, and Rv from weeks 6 to weeks 12 (PP Set) (**: compared within group; p-value for Paired t-test, *: compared between 'CTP' and 'Placebo' groups; p-value for Two sample t-test, $^{\#}$: compared between 'CTP' and 'Placebo' groups; p-value for Wilcoxon rank sum test, $^{\$}$: Compared between 'CTP' and 'Placebo' groups; p-value for GLM adjusted baseline, age, drinke, smoke, sleep)

3.3. Effect of CTP on the Wrinkle Depth around the Eyes

The parameters, Rmax, Rp and Rv, were significantly decreased at 6 weeks and 12 weeks after CTP consumption in eyes for both sides (left and right) average. Moreover, the level of Rmax, Rp and Rv in the CTP group was significantly reduced compared with the placebo group at 12 weeks after treatment (Table 3 and Figure 2). Additionally, the Rv was revealed a significant difference between groups at 6 weeks (Table 3 and Figure 2).

3.4. Effect of CTP on Skin Hydration

After 6 and 12 weeks of the trial, both groups showed a trend of increased skin hydration on the right and left cheek, with a greater enhancement in the CTP group (Figure 3). On the right cheek, the levels between two groups was statistically significant at 12 weeks. Furthermore, during the test period, additional analysis was performed on the subgroups, except in participants who did not use sunscreen, used colored makeup at least five times a week, or slept for less than 5 hours per day. In

the results, after 6 and 12 weeks from initial treatment, the cheek skin hydration in both groups had increased, and a higher increase was revealed in the CTP group. Moreover, the difference of skin hydration on cheek between placebo and CTP groups was statistically significant (Figure 4). Additionally, more 45-year-old women had meaningfully increased skin hydration in the CTP group than in the placebo group (Table 4).

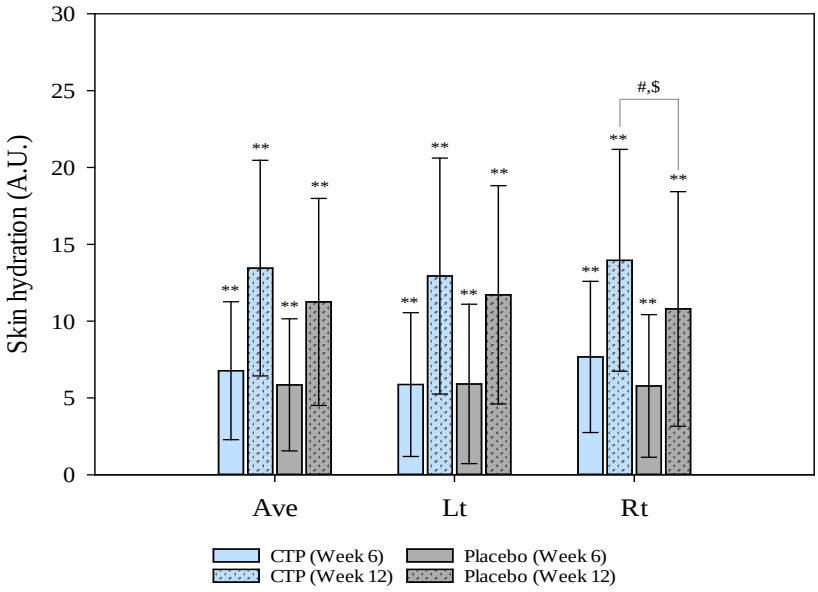

Figure 3. (color online) Changes in capacitance (Corneometer®) from weeks 6 to weeks 12 (PP Set) (**: compared within group; p-value for Paired t-test, #: compared between 'CTP' and 'Placebo' groups; p-value for Wilcoxon rank sum test)

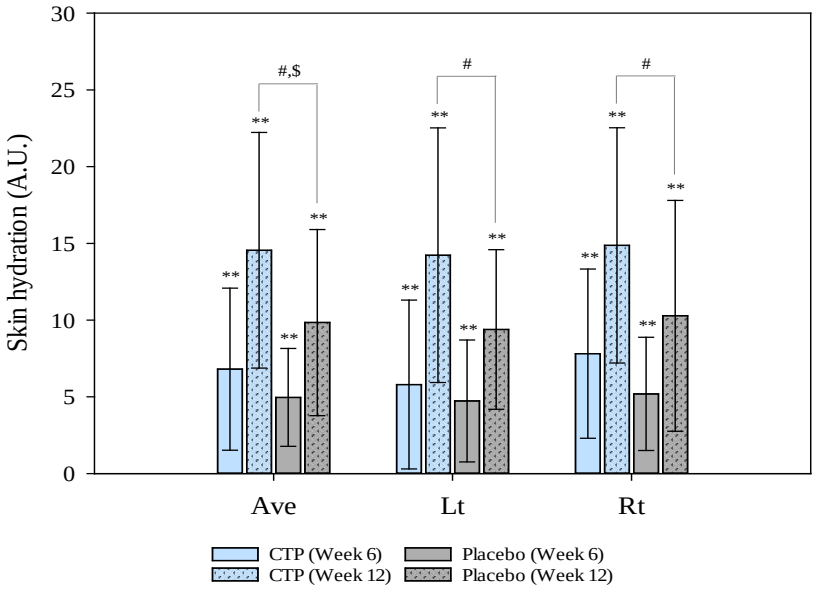

Figure 4. (color online) Changes in capacitance (Corneometer®) from weeks 6 to weeks 12 (Subgroups Set, CTP n=23, Placebo n=27) (**: p<0.05 significant difference from baseline, #: p<0.05 significant difference between CT20S and Placebo at indicated week)

Table 4. Changes in capacitance (Corneometer®) of more 45 years of age women from weeks 6 to weeks 12 (PP Set)

		Skin hydration(A.U) Mean±SD	
		CTP (n=20)	Placebo (n=20)
Ave.	Baseline	41.47±5.50	40.93±5.97
	Week 6(V3)	49.88±7.54	45.75±7.88
	Change from baseline	7.52±4.85#	4.82±3.96
	p-value**	<.0001	<.0001
	Week 12(V4)	55.79±9.57	50.12±8.66
	Change from baseline	14.32±7.83#,$	9.20±5.77
	p-value**	<.0001	<.0001

#: compared between 'CTP' and 'Placebo' groups; p-value for Wilcoxon rank sum test, $: Compared between 'CTP' and 'Placebo' groups; p-value for GLM adjusted baseline, age, drink, smoke, sleep.

4. Discussion

Previously, daily ingestion of 1.0g CTP for 12 weeks has been reported to reduce trans-epidermal water loss, after adjusting for humidity, temperature, and UVA in the region [17]. In this study, we confirm that oral supplementation of CTP for 12 weeks improves wrinkle formation and skin hydration.

At 6- and 12- weeks after CTP intake, the Rmax, Rp and Rv were significantly decreased after 6- and 12-weeks of CTP consumption around both eyes of average, the significant difference between the CTP and placebo groups was evident at 12-weeks. Rv in the CTP group was remarkedly decreased even at 6 weeks and after adjusting the baseline in all participants, except in those who did not use suncreen, used colored makeup at least five times a week, or slept less than 5 hours per day, compared to that in the placebo group. As Rmax, Rp, and Rv indicate maximum length, maximum peak height, and maximum depth from skin surface, respectively. The parameters intend to show large uneveness of the skin surface. In extrinsic ageing, deep wrinkle formation is characterized by skin laxity, caused by chronic sun exposure. Collagen damage plays a major role in wrinkle formation [18]. CTP supplementation may stimulate remodeling of the dermal structure due to collagen peptides rebuilding the ECM [1].

Due to increased skin hydration, the capacitance for moisture contents of skin was increased from baseline in both CTP and placebo groups 6- and 12-weeks. We further analyzed the skin moisturization after adjusting the baseline in all participants except those who did not use sunscreen, used colored makeup at least five times a week, or slept less than 5 hours per day. There was a statistically significant difference between CTP and placebo groups after reforming lifestyle.

Several studies have revealed that age-dependent reduction of collagen synthesis and elevation of collagen degradation could be reversed by oral administration of specific bioactive collagen peptides [1]. We confirmed that in the CTP group, more 45-year-old women had significantly increased skin hydration than in the placebo group. This finding was noted following improved skin condition afer oral CTP. These oligopeptides are obtained by enzymatic hydrolysis of natural collagen. After ingestion, collagens are further metabolized to di- and tri-peptide, and finally accumulate in the skin through the blood stream [3]. In this report, enriched Gly-Pro-Hyp (GPH) was analyzed in the human blood. Moreover, in the mouse model, collagen hydrolysate, an enzymatically hydrolyzed form of collagen, was observed in the plasma and skin, with enrichment of GPH and Pro-Hyp (PH). PH in the skin was found to be derived by GPH hydrolysis, as the administration of pure GPH peptide resulted in similar results [3].

Interestingly, in our study, intake of CTP for 12 weeks resulted in a significant difference in the reduction of wrinkles and increase in moisture amounts. As CTP could be distributed to the body throughout blood circulation, the functional activity of CTP would have possibly contributed to the whole skin after a longer administration time.

With age, the epidermis becomes easily vulnerable to skin barrier impairment due to deficiency of collagen, leading to thinner and drier skin [1]. The moisturization of skin through oral intake of collagen peptide has been revealed by several human studies [11,18]. Ohara et al. showed significant difference in moisture content after 4-weeks of consumption of 5.0 and 10.0 g collagen peptides compared with a placebo in a double-blind study. Jerome et al. demonstrated a significant increase in skin hydration after 8 weeks of collagen peptide intake, as well as induction of collagen and glycosaminoglycan production in an ex-vivo experiment. This efficacy was strongly supported by cellular activation due to stimulation by collagen peptide [19]. Arnold et al. demonstrated that 12.5 mM PH and GPH stimulated the chemotactic activity of fibroblasts in human dermal fibroblast cells.

While skin aging has been observed to be accelerated by lifestyle factors such as smoking, alcohol, stress, lack of sleep as well as long-term exposure to the sun [1], we found an improvement in skin hydration after adjustment of lifestyle issues. This means that the molecular advantage is endogenously induced without the influence of external factors. On the cellular level, collagen-derived PH induces HA synthesis in human dermal fibroblasts, and Hyp increases the mRNA expression of serin palmitoyltransferase-2 and β-glucocerebrosidase, which are involved in ceramide synthesis in the epidermis [15]. Moreover, oral intake of collagen peptide prevented UVB-induced loss of skin hydration in hairless mice. The mRNA expression of hyaluronic synthase 1 and 2 was concurrently increased with hyaluronic acid; conversely, hyaluronidase 1 and 2 decreased after collagen peptide treatment in the mouse skin, leading to an increase in skin hydration [8].

In conclusion, the present study shows that oral intake of CTP is safe and mostly effective in improving eye wrinkles and skin moisturization, although the effectiveness limitedly appeared on the left side. Therefore, further studies are required to assess the efficacy of CTP. However, the beneficial effects were endogenously induced, unaffected by external factors.

Acknowledgements

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Statement of Competing Interests

The authors have no competing interests.

List of Abbrevations

CTP (collagen tripeptide)
HA (hyaluronic acid)
ECM (extracellular matrix)
GPH (Gly-Pro-Hyp)
PH (Pro-Hyp)
HAS2 (hyaluronan synthase 2)
GLM (Generalized Linear Model)
ITT (Intend-To-Treat)
PP (Per Protocol)

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