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Oral Supplementation with Low-molecular-weight Collagen Peptide Improves Hydration, Facial Lifting, Dermal Density, Skin Desquamation and Nails: A Randomized, Double-blind, Placebo-controlled, and Maintenance of Effect Study

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Abstract Background: Oral low-molecular-weight collagen peptide (LMWCP) hydrolyzed enzymatically is a fish-derived type I collagen hydrolysate with more than 15% of its content made up of tripeptides in the form of Gly-X-Y (X and Y are placed arbitrarily, but are often occupied by proline, hydroxyproline, and alanine) including 3% Gly-Pro-Hyp. LMWCP helps with skin hydration, wrinkles and elasticity via previous findings, has been recognized individually by the Ministry of Food and Drug Safety (MFDS notice No. 2013-30) as a functional food ingredient. In this study, to expand the scope of diversity in the efficacy of LMWCP, we evaluated hydration according to depth of the stratum corneum, facial lifting, dermal density, skin thickness, skin desquamation, and roughness of the nail plate surface. Moreover, the measurement timelines were considered including the early time intake and off-intake periods. Methods and materials: This study was designed as a double-blind, randomized, placebo-controlled for 14 weeks including oral intake for 12 weeks followed by 2 weeks of the off-intake period. Water content (depths of 0.1 mm, 0.5 mm), facial lifting, dermal density, skin thickness and skin desquamation were assessed at baseline, 2 weeks, 4 weeks, 8 weeks, 12 weeks after intake of the oral supplementation and after 2 weeks of off-intake (+2W). The roughness of the fingernail plate was measured at 0W, 8W and 12W. Results: The test group saw significant improvements compared to the placebo group. According to each measurement result, the skin moisture (depth of 0.1 mm) and skin desquamation were improved after 2 weeks of ingestion, and the skin moisture (depth of 0.5 mm), facial lifting, dermal density and skin thickness were improved after 4 weeks. For all measurement items, even after 2 weeks of off-intake, the test group showed a statistically significant improvement compared to the placebo group. In the roughness of the fingernail plate, it was found that the roughness was improved in the test group after 12 weeks compared to before ingestion. Discussion: These results demonstrated that the effects of LMWCP appear in early-intake and are maintained even after off-intake. This study suggests LMWCP as a safe and effective ingredient for anti-skin aging in the nutricosmetic market targeting both internal and external beauty and health.

Keywords: *collagen peptide, dermal density, facial lifting, oral intake, photoaging, skin desquamation, skin hydration*

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1. Introduction

The factors of aging are classified as intrinsic and extrinsic. The former is determined by individual genetic composition, while the latter is affected by environmental influences such as nutrition or life-style. Unlike intrinsic aging, which affects the entire body, including internal organs, extrinsic aging presents restrictively in exposed area such as the face and neck [1,2,3]. Although both intrinsic and extrinsic factors induce age-dependent skin changes, signs of aging are more dominant in photoaged skin which has been proven by studies comparing between skin exposed to UV radiation and protected skin from UV radiation [4]. It is well-known that chronic UV exposure causes the expression of matrix metalloproteinases (MMPs) [3-6]. These degrade skin components, which promotes the breakdown of collagen fibers, resulting in skin dryness, wrinkles and decreased elasticity [5,6]. Interest in collagen intake as a means of preventing skin aging due to UV rays, has increased in recent years [7]. In vitro and In vivo studies have shown that application of collagen peptide or hydrolysate mediate the alleviation of photoaging mechanisms and also improve clinical symptoms of photoaging [8,9]. Hairless mice exposed to ultraviolet radiation were administrated 1000 mg/kg of collagen peptide for nine weeks, and the results indicated that collagen peptide can control various factors related to skin moisturization in a way that increases the water content [10]. Another study in the hairless mouse model showed that oral administration of collagen peptide produced a dose-dependent improvement in UVB-induced wrinkles [11]. In addition to skin, collagen peptide intake improved brittle nails and promoted nail growth [12]. In a previous study with a randomized, double-blind, placebo-controlled study design, we confirmed that oral low-molecular-weight collagen peptide (LMWCP) helps with skin hydration, wrinkles and elasticity without any particular safety issues [13]. LMWCP, via previous findings, has been recognized individually by the Ministry of Food and Drug Safety (MFDS notice No. 2013-30) as a functional food ingredient. However, this study was designed with specific but various assessment items and measurement time points. In this study, to expand the scope of diversity in the efficacy of LMWCP, we evaluated hydration according to depth of the stratum corneum, facial lifting, dermal density, skin thickness, skin desquamation, and roughness of the nail plate surface. To investigate whether there is an initial effect of intake and whether the effects are maintained even after termination of oral intake, measurement timelines were considered including the early time intake and off-intake periods.

2. Material and Method

2.1. Test Product

LMWCP (provided by NEWTREE Co., Ltd., Seoul, Korea) is a collagen hydrolysate obtained from the skin of the sutchi catfish (*Pangasius hypophthalmus*) and it contains more than 15% tripeptide including 3% Gly-Pro-Hyp. It has been individually recognized as a functional food ingredient in notice No.2013-30 of the MFDS. Table 1 was presented the ingredients information per one tablet for the test product and the placebo. One tablet of the test product contains 500 mg of LMWCP and, was taken twice a day (usually in the morning and evening), for a total daily dose of 1000 mg.

2.2. Study Design

This study was designed as a double-blind, randomized, placebo-controlled study according to the Ellead Standard Operating Procedure (EL-P-7400). The total test period was 14 weeks (from October 26, 2020, to February 4, 2021) including oral intake for 12 weeks followed by 2 weeks of the off-intake period. The study schedule was organized as shown in Table 2. This clinical study protocol was approved by the Institutional Review Board of Ellead (approval no. IRB-200806T002).

Table 1. Ingredient information of test and placebo (per 1 tablet)

Ingredients	Test		Placebo	
	Content (mg)	Content (%)	Content (mg)	Content (%)
Low Molecular Weight Collagen peptide[1)]	500.004	83.334	0.000	0.000
Microcrystalline Cellulose	25.992	4.332	411.996	68.666
Lactose mixed preparation[2)]	18.000	3.000	60.000	10.000
Maltodextrin	18.000	3.000	90.000	15.000
Silicon dioxide	9.000	1.500	9.000	1.500
Magnesium Stearate	9.000	1.500	9.000	1.500
HPMC	10.002	1.667	10.002	1.667
Titanium dioxide	6.000	1.000	6.000	1.000
Glycerin	4.002	0.667	4.002	0.667
Total	600.000	100.000	600.000	100.000

[1)] Active ingredient (LMWCP)
[2)] Lactose 95% + Dextrin 5%.

Table 2. Study schedule

	1st visit (screening)	2nd visit (0W)	3rd visit (2W)	4th visit (4W)	5th visit (8W)	6th visit (12W)	7th visit (+2W)
		Oral intake period (12 weeks)					
Enrollment and informed consent of subjects	√						
Blood and urine test	√					√	
Assessment of total daily protein intake		√[3)]				√[3)]	
Assessment of Water contents		√	√	√	√	√	√
Assessment of facial lifting		√	√	√	√	√	√
Assessment of dermal density and skin thickness		√	√	√	√	√	√
Assessment of skin desquamation		√	√	√	√	√	√
Assessment of surface roughness on fingernail plate		√			√	√	
Assessment of adverse reaction			√	√	√	√	√

[3)] dietary recording to three-day of prior week before visit.

2.3. Subjects

The subjects recruited according to the inclusion and exclusion criteria of Table 3, were explained the purpose, method and procedure of this study, as well as the potential adverse reactions or side effects, and they all signed an informed consent form. All subjects were prohibited from using therapeutic products for improving the skin, quasi drugs, functional foods or from undergoing medical treatments or cosmetic procedures such as massage, that could affect the study results from three weeks before the beginning of the study, and they agreed not to modify their daily skincare habits during the study period. In addition, they were guided to receive dietary training and to fill out a meal diary during the designated period so that they could maintain their usual daily protein intake during the study period.

Table 3. Inclusion and exclusion criteria

Inclusion Criteria
Healthy females aged from 30 to 65 years.
Those who have skin sagging on their face and skin water content under 55 A.U.
Those who can maintain their usual daily protein intake for the study period.
Those who fully understand the objective and contents of the study and voluntarily decide to participate.
Those who understand the possible adverse reactions and sign the informed consent form.
Exclusion Criteria
Those who are pregnant, breast feeding or planning pregnancy.
Those who have abnormalities in the general opinion of the specialist as a result of blood and urine tests.
Those who have allergic specific constitution (health functional foods, medicines, cosmetics, food, pollen, ultraviolet rays, etc.) or those with sensitive or hypersensitive skin.
Those who have received nail polish, gel nails, etc. or nail care (including nutritional supplements) on their nails within 1 month before the start of the test.
Those who have prominent nutrition disorder.
Those who have participated in the same clinical evaluation within 6 months.
Those who have otherwise been judged by the research director to be unsuitable for enrollment in this clinical trial.

2.4. Clinical Assessment

For the clinical efficacy tests, the subjects visited at baseline (0W), 2 weeks (2W), 4 weeks (4W), 8 weeks (8W), 12 weeks (12W) after intake of the oral supplementation and after 2 weeks of off-intake (+2W). The subjects washed their face with foam cleanser and stood by in the controlled room conditions (22 ± 2°C and 50 ± 10% relative humidity) for 30 minutes. Water content (depths of 0.1 mm, 0.5 mm), facial lifting, dermal density, skin thickness and skin desquamation were assessed on every measurement day. The roughness of the fingernail plate was measured at visits of 0W, 8W and 12W. The blood test and urine test were conducted at baseline (0W) and the completion of oral intake (12W), respectively.

2.4.1. Assessment of Water Hydration According to Skin Depth

A Corneometer CM825 (Courage+Khazaka electronic, Germany) was used to determine the water content of the stratum corneum down to a depth of about 0.1mm. The principle is based on the capacitance measurement of a dielectric constant through electrode separation on the stratum corneum. A Moisturemeter D (Delfin Technologies, Finland) is a device that measures the dielectric constant of the skin and subcutaneous tissues (depth of 0.5 mm – 5 mm) non-invasively. The water content of the skin up to a depth of 0.5 mm was measured using an XS5 probe. An increase in each value expressed in arbitrary units (A.U.) indicates an increase in the water content according to the skin depth. The average was calculated by measuring three times.

2.4.2. Assessment of Facial Lifting

To evaluate facial lifting, images were captured using a moiré measuring device and high-resolution digital camera under lighting set to maintain identical studio conditions. For the sake of standardization, photography conditions including measurement direction and location were fixed. The corner of the mouth where sagging skin was prominent was selected as the test area. On the facial images with a moiré pattern, the angle (R) between the horizontal line and the contour line drawn at the corner of

the mouth was measured using image analysis software (Image-Pro Plus, USA). A decrease in the R value indicates an improvement in facial lifting.

2.4.3. Assessment of Dermal Density and Skin Thickness

The dermal density and skin thickness were assessed using a Dermascan-C (Cortex Technology, Denmark), which is a high-resolution imaging device that uses 20MHz supersonic waves. The ultrasonic waves (speed of 1.580 m/s) are partially reflected by the skin structure, giving rise to echoes of different amplitudes. The scanned images produced by the reflections of the skin structure were analyzed via built-in software. An increase in the measured values indicates improvement in dermal density and skin thickness.

2.4.4. Assessment of Skin Desquamation

Skin desquamation was evaluated using a Black D-Squame (CuDerm, USA) coupled with image analysis. The samples were gained from defined cheek by tape-stripping, and images of them were captured via iScope (Moritex, Japan) at 700x magnification. these were analyzed using image analysis software (Image Pro Plus, USA) to evaluate the skin desquamation. A decrease in pixels indicates an improvement of skin desquamation.

2.4.5. Assessment of Surface Roughness on the Fingernail Plate

To evaluate the surface roughness of the fingernail plate, the fingernails were measured at 0W, 8 W and 12W using an Antera 3D® CS (Miravex Limited, Ireland), and the measurements were quantified using 3D image conversion and data extraction from various indicators. In order to measure the same test area, the device was fixed and matched to the AOI of the test area. The average Ra values of the index, middle and ring fingers were calculated and evaluated. A decrease in Ra indicates an improvement of nail plate roughness.

2.4.6. Assessment of Safety

To evaluate whether intake of the oral supplementation did not cause any health problems, the blood and urine samples taken from subjects at 0W and 12W were analyzed. To assess adverse skin reactions, observation for erythema, edema, scaling, itching, stinging, burning, tightness, and prickling was performed at 2W, 4W, 8W, 12W and +2W.

Blood test (18 parameters)

; Total Protein, Albumin, ALT, AST, Total Bilirubin, BUN, Hb, Hct, MCH, MCHC, MCV, Platelet, RBC, WBC, Total Cholesterol, Creatinine, Glucose, γ-GTP

Urine test (10 parameters)

; pH, S.G, Bilirubin, Blood, Glucose, Ketone, Leukocyte esterase, Nitrite, Protein, Urobilinogen

2.4.7. Assessment of Total Daily Protein Intake

To monitor the total daily protein intake during the study period, we investigated subjects' meal diary. The subjects recorded -1 week (the prior week of 2nd visit) and after intake 11 weeks (the prior week of 6th visit) the contents of meals for two days of the weekdays and one day on the weekend in the meal log of three-day and submitted. Total daily protein intake was calculated through analysis of the total calorie intake (TCI) using Can Pro program version 3.0 (The Korean Nutrition Society, Korea).

2.5. Statistical Analysis

For efficacy results (PP population), the normality tests were performed by using Kolmogorov-Smirnov test. If the data satisfy the normality, the repeated measure ANOVA with contrast test (†) was used for comparison of before and after intake intra-group. The amount of change from each time point (delta value; formulation shown below) between groups was analyzed using the independent t-test (‡). If the normality requirement was not satisfied, the Wilcoxon signed-rank test (*) was used for the comparison of before and after intake intra-group, and the amount of change from each time point between groups was analyzed using the Mann-Whitney U test (§). Results were expressed as mean and standard deviation (mean ±SD). Safety assessment and total daily protein intake were used ITT population. Statistical analysis of the blood and urine tests used the Wilcoxon signed-rank test (*) and paired t-test (∮) after the normality test for comparison of before and after intake intra-group. A statistically significant difference was set at *P<0.05, **P<0.01 ***P<0.001, §P<0.05, §§P<0.01, §§§P<0.001, †P<0.05, ††P<0.01 †††P<0.001, ‡P<0.05, ‡‡P<0.01 ‡‡‡P<0.001, ∮ P<0.05, ∮ ∮ P<0.01, ∮ ∮ ∮ P<0.001. The SPSS software version 26.0 (IBM Corp., Chicago, IL) was used for the statistical analysis.

Delta value (Δ)= |the measurement value of each time point – the measurement value of 0W|

Improvement rate (%) = Delta value (Δ) / the measurement value of 0W x 100 (%)

3. Results

Eighty-nine healthy females with no abnormal findings in the results of the blood and urine tests and with water content under 55 A.U. were enrolled in this study (ITT population). Nine subjects dropped out during the intake period, and the remaining 80 females completed the study (PP population; test group n=41, placebo group n=39). The age of the subjects who completed the study was from 31 to 65 years. The average age of the test group was 51.88±7.85 years, and of the placebo group was 53.49 ± 6.61 years.

3.1. Water Content According to Skin Depth

Water content values (depth of 0.1 mm) showed a statistically significant increase in the test group at 2W, 4W, 8W, 12W, +2W (Figure 1A). The delta values of water content were significantly increased in the test group compared to the placebo group at 2W, 4W, 8W, 12W, and +2W (Figure 1B). The improvement rate for the water content (depth of 0.1 mm) in the test group was 1.77% (2W), and 3.92% (4W), 4.96% (8W), 3.80% (12W),

3.49% (+2W) higher than in the placebo group. The water content values (depth of 0.5 mm) in the test group showed a significant increase at 2W, 4W, 8W, 12W, and +2W (Figure 2A). The delta values of water content were significantly increased in the test group compared to the placebo group at 4W, 8W, 12W, and +2W (Figure 2B). The improvement rate for water content (depth of 0.5mm) in the test group was 0.99% (2W), 2.40% (4W), 3.23% (8W), 4.26% (12W), and 3.43% (+2W) higher than in the placebo group.

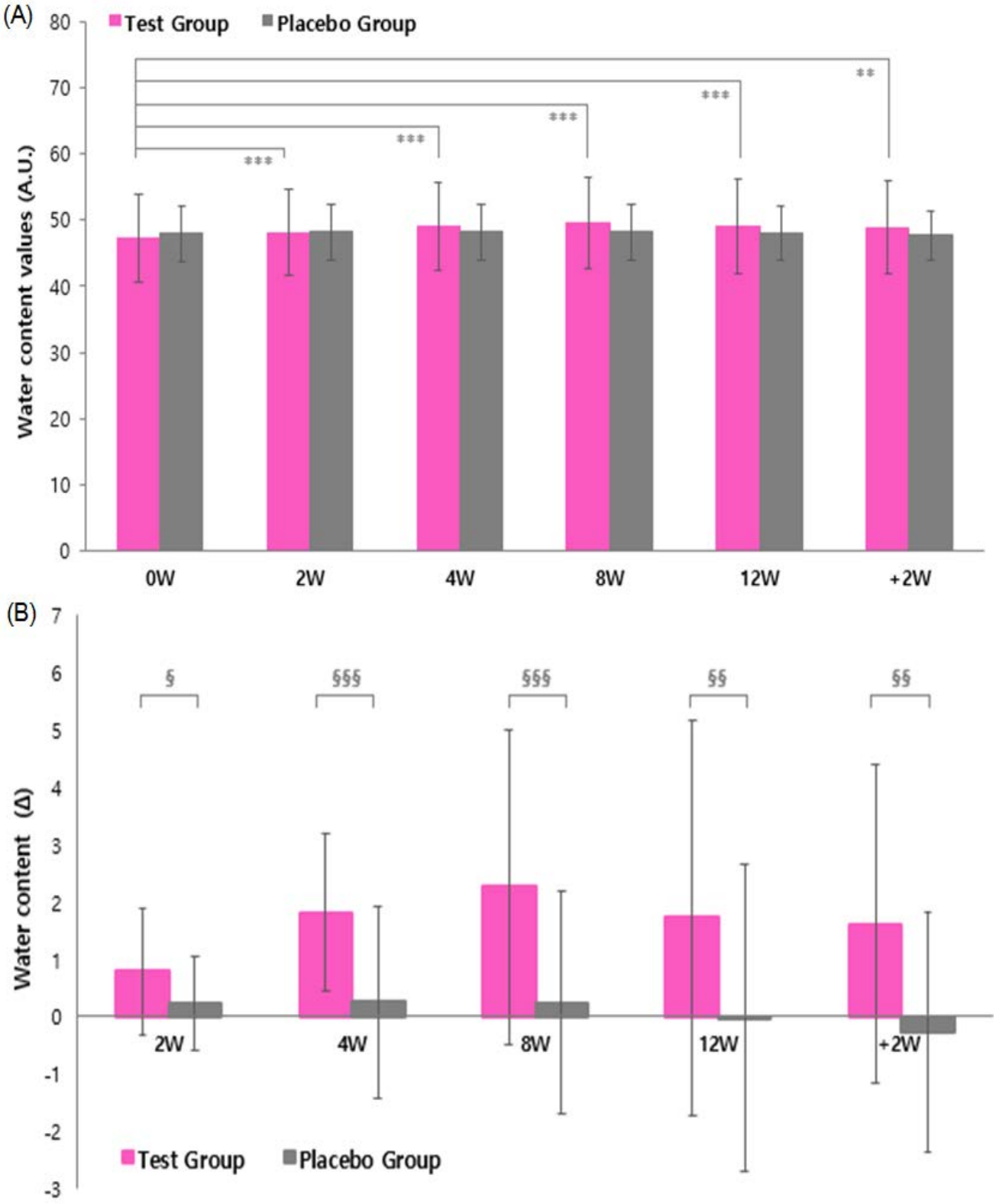

Figure 1. The effect of LMWCP on skin hydration to a depth of 0.1 mm in the stratum corneum using a Corneometer CM825. (A) The water content values (A.U.) in the test group and placebo group are presented as mean ±SD. The statistical analysis was performed using the Wilcoxon signed-rank test with **P<0.01, ***P<0.001. (B) Statistical comparisons between the test group and placebo group were calculated using the Mann-Whitney U test, test using delta values with §§P<0.01, §§§P<0.001

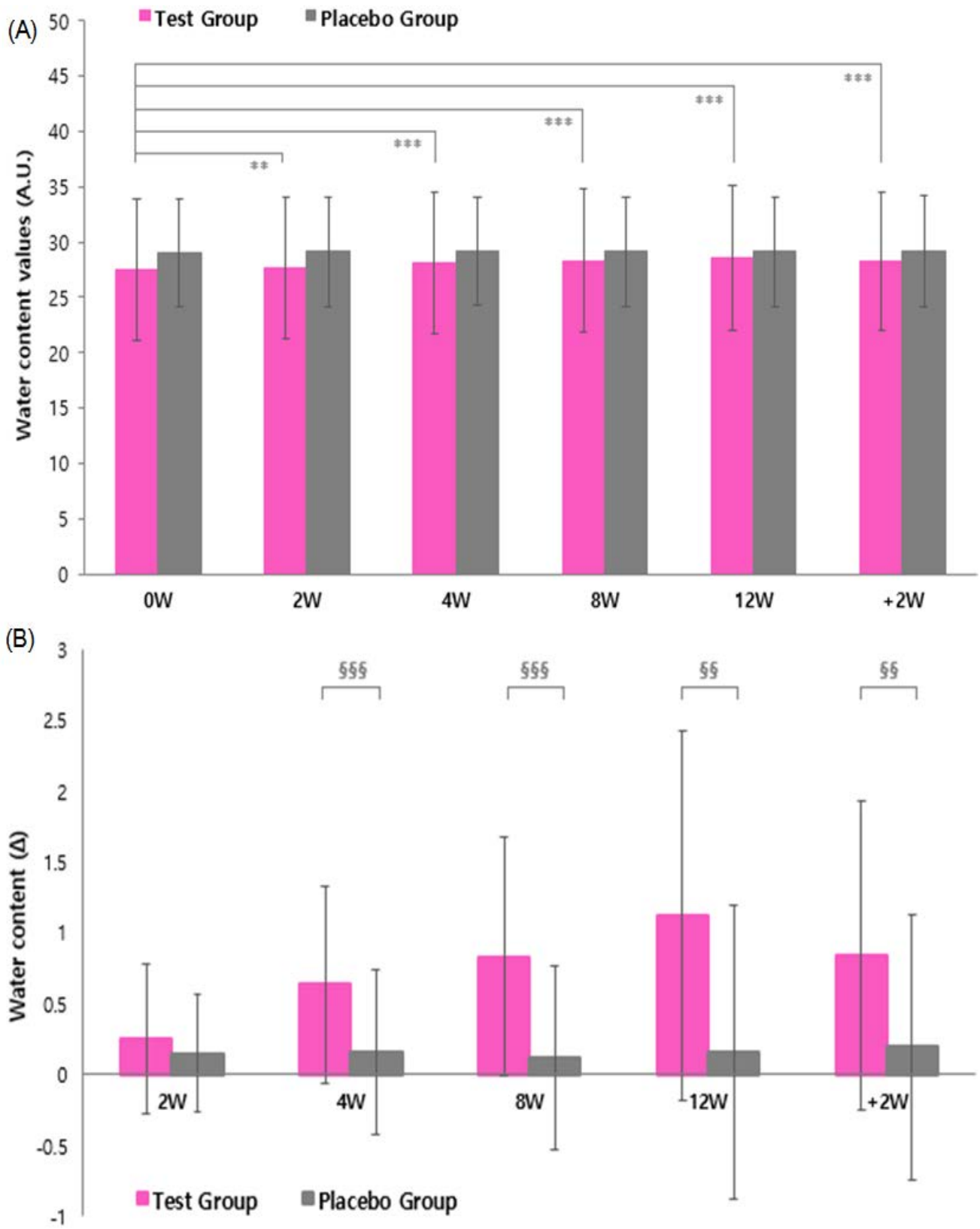

Figure 2. The effect of LMWCP on Skin hydration to depth of 0.5 mm in the stratum corneum using a Moisturemeter D (DXS5). (A) The water content values (A.U.) in the test group and the placebo are presented as mean ±SD. The statistical analysis was performed using the Wilcoxon signed-rank test with **P<0.01, ***P<0.001. (B) Statistical comparisons between the test group and the placebo group were calculated using the Mann-Whitney U test, test using delta values with §§P<0.01, §§§P<0.001

3.2. Facial Lifting

The facial lifting parameter, R values were decreased in the test group at 4W, 8W, 12W, and +2W with significant differences (Figure 3A). Compared to the placebo group, facial lifting showed significant improvement in the test group at 4W, 8W, 12W, and +2W (Figure 3B). The improvement rate for facial lifting in the test group was 0.40% (4W), 0.71% (8W), 0.77% (12W), and 0.55% (+2W) higher than in the placebo group.

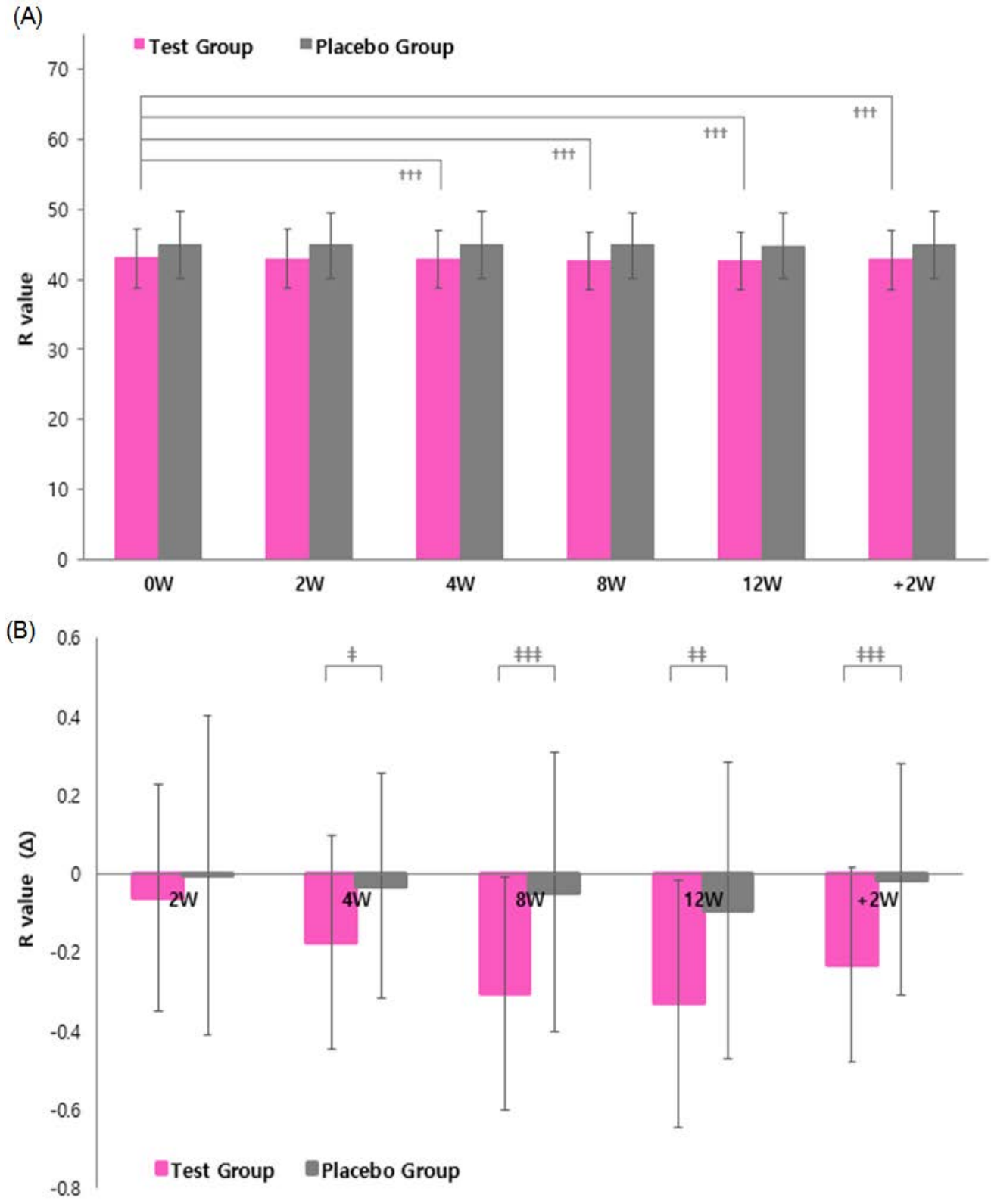

Figure 3. The effect of LMWCP on Facial lifting analyzed using Moiré pattern. (A) R values, which represent the angle between the horizontal line and the contour line drawn at the corner of the mouth, are expressed as mean ±SD. The statistical differences in the test group compared to the placebo were calculated using the repeated measures ANOVA with contrast test with †P<0.05, ††P<0.01, †††P<0.001 (B) The statistical comparisons between the test group and the placebo group were calculated using the independent t-test with ‡P<0.05, ‡‡P<0.01 ‡‡‡P<0.001

3.3. Dermal Density and Skin Thickness

The delta values of dermal density were significantly increased in the test group compared to the placebo group at 4W, 8W, and 12W, and +2W. The dermal density of the test group was increased significantly at 2W, 4W, 8W, 12W, and +2W (Figure 4A, B). The improvement rates in the test group were 1.04% (2W), 1.22% (4W), 1.22% (8W), 1.00% (12W), and 0.93% (+2W) higher than in the placebo group.

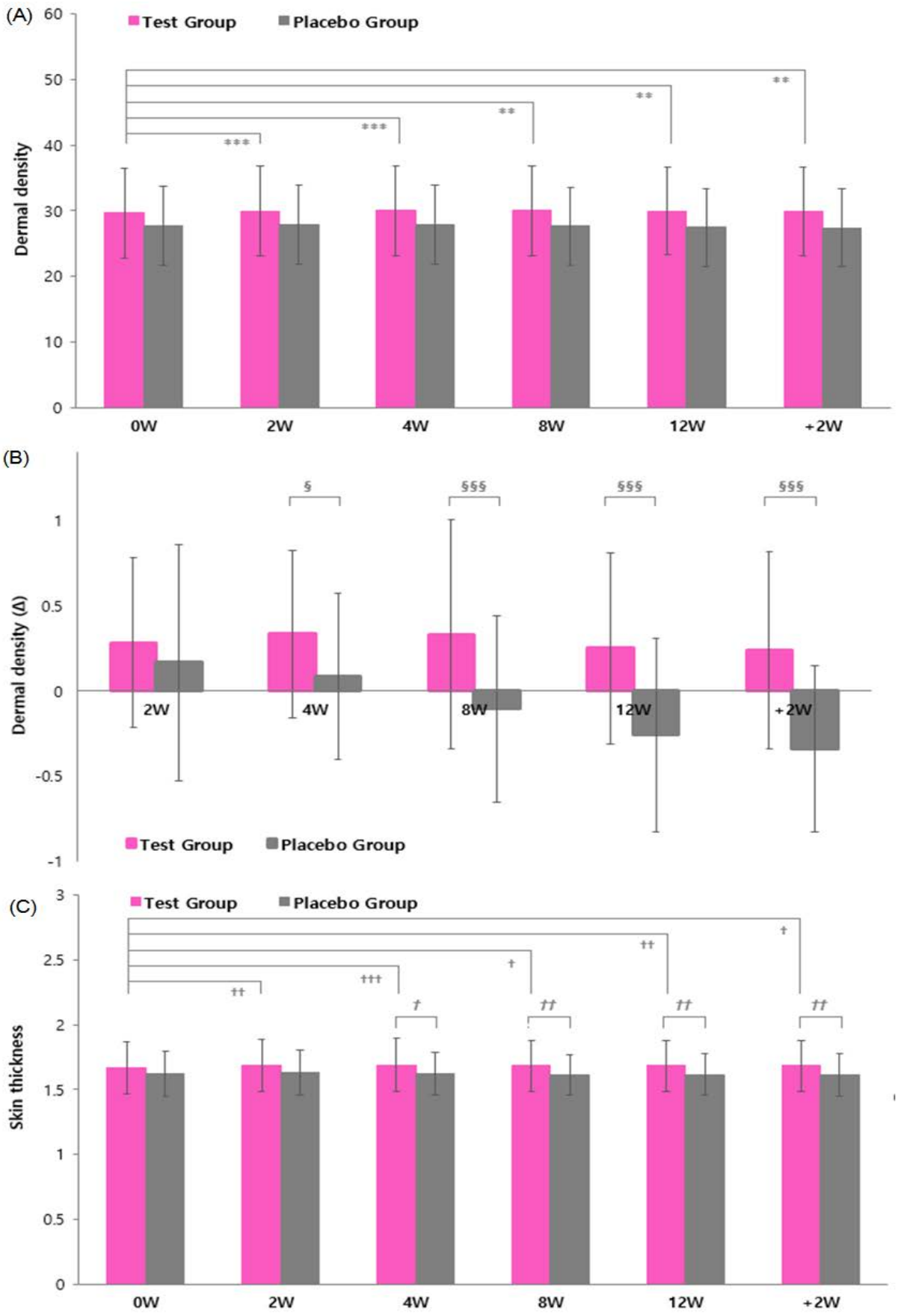

Figure 4. The effect of LMWCP on dermal density and skin thickness using a Dermascan C (a) Dermal density in the test and placebo groups compared to 0W were calculated using the Wilcoxon signed-rank test with **P<0.01, ***P<0.001. (b) § and §§§ indicate significant differences between the test group and the placebo group (§P<0.05, §§§P<0.001, respectively; Mann-Whitney U test using delta values). (c) Statistical analysis of skin thickness in the test group compared to the placebo group were calculated using the repeated measures ANOVA with contrast test with †P<0.05, ††P<0.01, †††P<0.001; †comparison with 0W, ***†*** (***bold italic***) comparison with the placebo group. All values are presented as mean ±SD

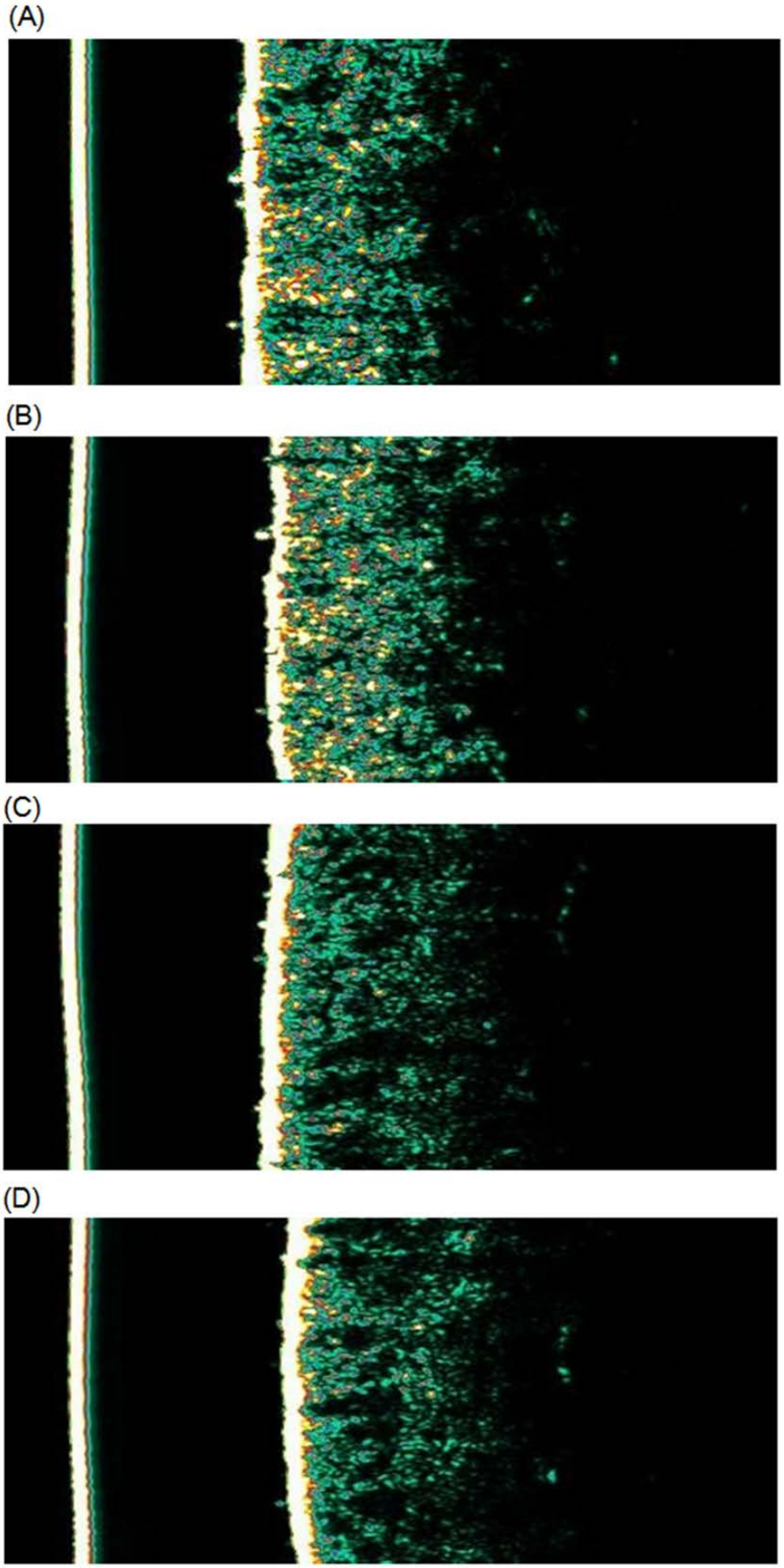

Figure 5. Ultrasound Images of the skin 0W and 12W of LMWCP intake for subject from the test group and from the placebo group. 58-year-old subject of the test group increased the dermal density and thickness for 12 weeks. (a) 0W, (b) 12W. There is no change dermal density and thickness in 52-year-old subject of the placebo group. (c) 0W, (d) 12W**.** Echogenicity color scale: white > yellow > red > green > black

The skin thickness values were significantly increased in the test group at 2W, 4W, 8W, 12W, and +2W and compared with placebo group, were significantly improved in the test group at 4W, 8W, 12W, and +2W (Figure 4C). The improvement rate for skin thickness in the test group was 1.10% (2W), 1.24% (4W), 0.99% (8W), 1.06% (12W), and 1.01% (+2W) higher than in the placebo group. Figure 5 shows that the change of dermal density and skin thickness of the subject from each group. It is shown to increase the level of dermal density in test group comparing with the placebo group.

3.4. Skin Desquamation

The results of skin desquamation in the test group were significantly more decreased at 2W, 4W, 8W, 12W, and +2W ($p<0.05$) than 0W (Figure 6A). Compared with placebo group, the results were significantly improved in the test group at 2W, 4W, 12W, and +2W (Figure 6B). The improvement rate of the test group was 1.99% (2W), 2.07% (4W), 3.22% (12W), and 3.07% (+2W) higher than that of the placebo group.

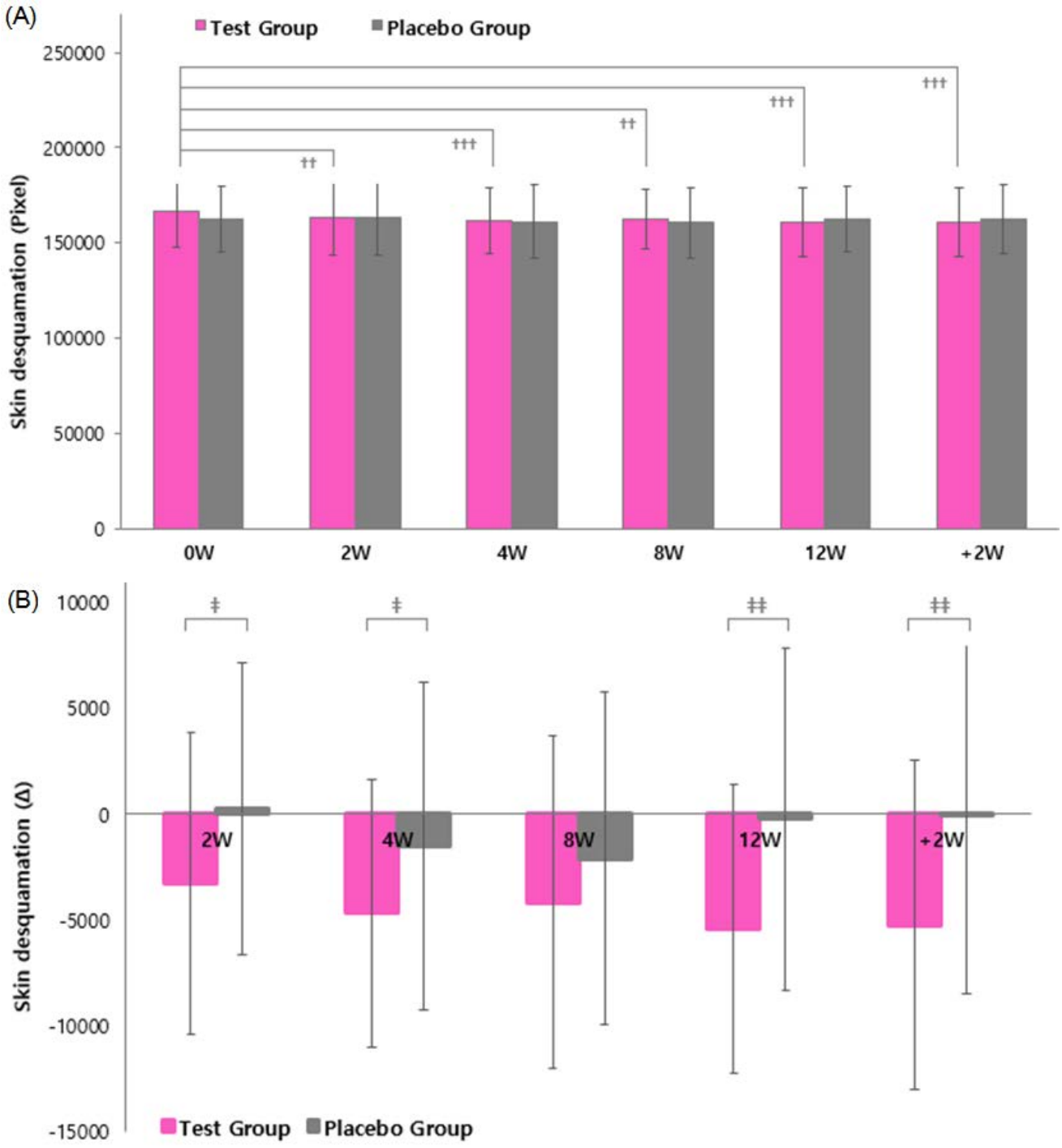

Figure 6. The effect of LMWCP on skin desquamation using a Black D-Squame (A) Pixels representing the amount of skin samples extracted using the tape stripping method are expressed as mean ±SD. The statistical differences in the test group compared to the placebo group were calculated using the repeated measures ANOVA with contrast test with †P<0.05, ††P<0.01, †††P<0.001 (B) The statistical comparisons between the test group and the placebo group were calculated using the independent t-test with ‡P<0.05, ‡‡P<0.01

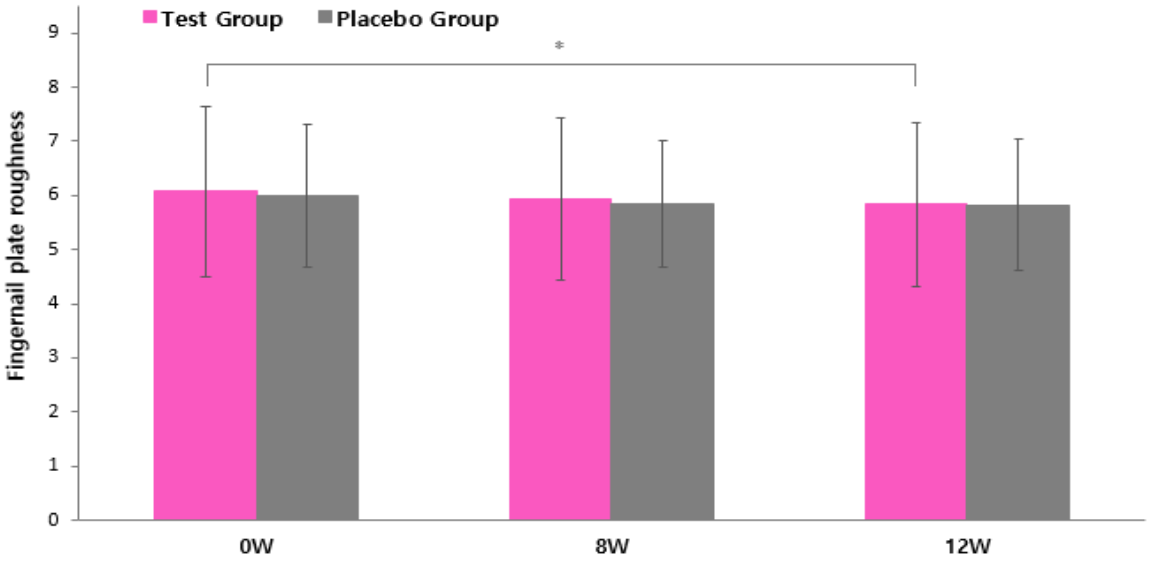

Figure 7. The effect of LMWCP on fingernail plate roughness measured with an Antera 3D. Ra values are represented as mean ± SD. Roughness of the fingernail plate in the test group compared to 0W or the placebo group were calculated using the Wilcoxon signed-rank test (*; P value <0.05) and Mann-Whitney U test using delta values (§; P value, not shown data)

3.5. Surface Roughness of the Fingernail Plate

Although the mean Ra values in the test groups showed no significant difference compared to those in the placebo group at 8W (p=.326) and 12W (p=.821), the mean Ra values in the test group were significantly decreased at 12W (*<0.05) (Figure 7). The improvement rate of the surface roughness of the fingernail plate in the test group was 3.26% at 12W.

Table 4. The results of statistical analysis in safety assessment (ITT papulation)

Blood parameters	Test group[4)]	Placebo group[4)]	Test group vs. Placebo group[5)]
Total protein	***	§§	0.251
Albumin	***	***	0.532
ALT	0.234	0.152	0.836
AST	0.109	0.102	0.830
Total Bilirubin	0.670	0.221	0.406
BUN	0.187	0.062	0.968
Hemoglobin (Hb)	**	§§§	0.258
Hematocrit (Hct)	0.072	0.846	0.193
MCH	**	§	0.723
MCHC	§§§	***	0.957
MCV	***	§§§	0.928
Platelet	§§	§§	0.954
RBC	0.077	**	0.202
WBC	1.000	0.274	0.426
Total Cholesterol	0.184	§	0.808
Creatinine	§§§	§§§	0.487
Glucose	0.118	0.805	0.072
γ-GTP	**	**	0.558
Urine parameters			
pH	0.219	0.776	0.322
S.G.	0.077	§§	0.487
Bilirubin[6)]	> 0.999	> 0.999	1.000
Blood[6)]	> 0.999	> 0.999	0.227
Glucose[6)]	> 0.999	> 0.999	0.227
Ketone[6)]	> 0.999	> 0.999	0.367
Leukocyte esterase[6)]	> 0.999	> 0.999	0.783
Nitrite[6)]	> 0.999	> 0.999	0.616
Protein[6)]	> 0.999	> 0.999	0.709
Urobilinogen[6)]	> 0.999	> 0.999	1.000

4) comparison of before and after intake intra-group Probability P (Wilcoxon signed-rank test, Significant: **p<0.01, ***p<0.001; Paired t-test, Significant: §P<0.05, §§P<0.01, §§§P<0.001)

5) comparison between groups Probability P [the Mann-Whitney U test, test using delta values; Repeated measures ANOVA, sphericity assumption (underscore)]

6) Bilirubin, Blood, Glucose, Ketone, Leukocyte esterase, Nitrite, Protein and Urobilinogen were statistical analyzed using the McNemar test for the comparison of intra-group, and using Fisher's exact test for the comparison between groups.

3.6. Safety Assessment

The results of the blood test identified no significant differences between 0W and 12W in ALT, AST, total bilirubin, BUN, Hct, WBC or glucose of each group, and the RBC, total cholesterol of the test group. However, the total protein, albumin, Hb, MCH, MCHC, MCV, platelet, creatinine, and γ-GTP of each group and the RBC, and total cholesterol of the placebo group showed significant differences ($p<0.05$) between 0W and 12W. The results of the urine test identified no significant difference between 0W and 12W (Table 4). Compared with the parameters of the blood and urine before oral intake, all parameters were within normal ranges at 12W. No adverse events were reported, during the study period.

3.7. Dietary Analysis for Total Daily Protein Intake

As a result of analyzing total daily protein intake using ITT population, the test group and placebo group did not show a statistically significant difference as a result of each between- and within-group effect (group x time) test (Table 5)

Table 5. The amount of total daily protein intake(g) and statistical analysis result (ITT population)

	0W	12W
Test group	69.004 ± 23.721	60.734 ± 22.467
Placebo group	60.552 ± 16.130	58.461 ± 16.288
Probability p (group x time)		0.105

Probability p (Repeated measures ANOVA, sphericity assumption).

4. Discussion

Collagen the major component of the extracellular matrix, is a connective tissue, found in tendons, cartilage, joints, bones, skin, hair, nails, and blood vessels in animals. The structure of collagen is a fibrous protein of a large size and with the shape of a rope. Three chains consisting of amino acids, mainly glycine, proline, hydroxyproline and arginine, are wound around each other and have powerful tensile force and strength due to the collagen triple helix formation. In human skin, collagen is mostly located in the dermis and is associated with other extracellular matrix, functions to retain water and improvements in smoothness and elasticity [14-16]. Contrary to young skin, in aged skin, collagen degradation is accelerated, however, new synthesis is slowed down, resulting in the accumulation of collagen segments and weakened organization. This reduces the dermal density, or skin thickness, in the dermis, which causes sagging and wrinkles, and reduces the ability to trap moisture, resulting in dry skin [17-22]. Collagen has low absorption into the body due to its high molecular weight of approximately 300 kDa and low solubility in water. These characteristics cause difficulties its application to cosmetics and foods [23]. Collagen peptide, which is the hydrolyzed form of collagen with high absorbency, is considered a popular ingredient, with great demand in the biomedical and cosmetic industries due to its benefits on the skin, biocompatibility, bioactivity, and weak immunogenicity [24]. Watanabe-Kamiyama M. et al. reported that in rats administered low-molecular-weight collagen hydrolysate labeled radiation the radioactivity showed maximal activity 3 hours after administration [25]. Yamamoto S. and coworkers demonstrated that collagen hydrolysates were efficiently absorbed when the collagen was ingested in tripeptide form. The functional peptide in this study, LMWCP hydrolyzed enzymatically is a fish-derived type I collagen hydrolysate with more than 15% of its content made up of tripeptides in the form of Gly-X-Y (X and Y are placed arbitrarily, but are often occupied by proline, hydroxyproline, and alanine) including 3% Gly-Pro-Hyp. In previous studies, ingestion of collagen hydrolysate with enrichment of Gly-Pro-Hyp increased the concentration level of collagen peptides in rat plasma and human blood. In particular, as the dipeptide Pro-Hyp was concentrated in the mouse skin, it could be inferred that was derived from the tripeptide Gly-Pro-Hyp [26,27]. Prolyl-hydroxyproline (Pro-Hyp) might not only stimulate the growth of fibroblasts in the skin but also increase their number and migration [28]. Dermal fibroblasts enable the synthesis of collagen, elastic fibers, and hyaluronic acid. Consequently, collagen-derived tripeptides play bioactive role in the skin [29]. These results suggest that oral ingestion of LMWCP containing tripeptides can be efficiently absorbed into the body and can present beneficial effects on the skin. In a previous study with a final 53 healthy females aged 40s-60s, we confirmed that, in the test group taking 1 g of LMWCP for 12 weeks, skin hydration, wrinkle, and elasticity were statistically significantly improved compared to the control group. In this study, we investigated changes in skin moisture, facial lifting, dermal density, skin desquamation, and nail plate surface for 14 weeks after oral intake of LMWCP 1 g for 12 weeks. In terms of study design, the remarkable points compared to previous studies are that in order to clarify the effects of LMWCP, the skin evaluation items were diversified according to skin functional roles and the measurement time points were subdivided and broadened (after off-intake). As an expanded investigation of skin moisturizing, it was designed to check the water content according to the depth of the skin, and the amount of keratinization in it. In addition, dermal density, skin thickness and facial lifting were evaluated in relation to wrinkles and skin elasticity. Especially, assessment of dermal density using high-frequency ultrasonography is a method for measuring the collagen content in skin, noninvasively [30]. The purpose of these designs is to check whether the skin status of the individual encompassing the outer and inner skin, that is, the visible and invisible part, is comprehensively improved. We also designed a pilot study to measure the roughness of the nail plate to confirm that LMWCP affects not only the skin but also the nails. The test group saw significant improvements compared to the placebo group. According to each measurement result, the skin moisture (depth of 0.1 mm), skin desquamation and dermal density were improved after 2 weeks of ingestion, and the skin moisture (depth of 0.5 mm), facial lifting and skin thickness were improved after 4 weeks. For all measurement items, even after 2 weeks of off-intake, the test group showed a statistically significant improvement compared to the

placebo group. These results demonstrated that the effects of LMWCP appear in early-intake and are maintained even after off-intake. In the roughness of the fingernail plate, there was no significant difference between the groups, but it was found that the roughness was improved in the test group after 12 weeks compared to before ingestion. Since the improvement in the roughness of normal nails without any special symptoms was evaluated, it is necessary to perform further research on moisturizing, growth, and strength targeting weak or fragile nails. No adverse reactions were observed, and the results of blood and urine tests were in normal ranges in all of the subjects during the period of the study. In a preference survey after consumption, with no data mentioned, the subjects' answers showed high satisfaction in that it was possible to maintain healthy skin through a simple intake twice a day. Although the beneficial effects of LMWCP ingestion were confirmed in the facial skin in relation to skin photoaging, oral intake may cause systemic reactions, unlike topical application, so it can change the skin on various parts of the body. In recent years, studies have reported that it is beneficial not only to the skin but also the health of various body parts, such as the nails, hair, teeth and gums [31-34]. It is expected that further research will find more roles of LMWCP by observing changes in the body's skin or other body organs due to LMWCP intake. This study suggests LMWCP as a safe and effective ingredient for anti-skin aging in the nutricosmetic market targeting both internal and external beauty and health.

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