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Effects of Oral supplementation with eggshell membrane (Ovoderm®-AS) on skin health: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Eggshell membrane (ESM, Ovoderm®-AS) contains bioactive compounds like collagen, elastin, hyaluronic acid, glucosamine, and chondroitin sulfate, which promote cellular activity, enhance collagen production, and reduce UV-induced damage. This double-blind, randomized, placebo-controlled trial evaluated the effects of oral ESM supplementation on skin parameters, including wrinkling, elasticity, dermal density, sagging, hydration, transepidermal water loss (TEWL), desquamation, roughness, and serum MMP-2 and MMP-9 levels. One hundred women aged 25–60 were randomized to receive 600 mg/day of ESM or placebo for 12 weeks, with assessments at baseline, 6 weeks, and 12 weeks. ESM significantly improved wrinkling, elasticity, dermal density, sagging, hydration, TEWL, desquamation, and roughness after 12 weeks ($p < 0.05$). Improvements in elasticity, dermal density, and sagging were observed as early as 6 weeks. No adverse events were reported. In conclusion, ESM is a safe and effective ingredient for improving skin health.

1. Introduction

The skin, as the largest organ in the human body, plays essential roles in protecting against environmental insults and preventing moisture loss. Aging is an unavoidable biological process that affects all organs, but it becomes particularly noticeable in the skin due to its visibility and exposure to environmental factors (Anthony & Benedetto, 1998; Desmond, 2017). Skin aging results from both intrinsic and extrinsic factors. Intrinsic aging, a natural and inevitable process driven by genetics and internal metabolic activities, manifests gradually as a loss of elasticity, fine lines, and changes in texture. Extrinsic aging, on the other hand, arises from environmental influences such as ultraviolet (UV) radiation, pollution, stress, and lifestyle factors (Andrea & Jean, 2012; Desmond, 2017).

UV radiation, particularly UVA (320–400 nm) and UVB (290–320 nm), plays a central role in photoaging by inducing reactive oxygen species (ROS) and upregulating matrix metalloproteinases (MMPs) (Yung Sung et al., 2005). These processes accelerate collagen breakdown and trigger inflammation, resulting in wrinkles, dryness, and sagging (Bae et al., 2008). While intrinsic aging cannot be prevented, extrinsic aging can be mitigated through lifestyle modifications, including the use of functional foods and supplements that possess antioxidant and anti-inflammatory properties. Nutrients such as collagen and hyaluronic acid are particularly beneficial for skin health, promoting hydration and elasticity (Kim et al., 2010; Miranda et al., 2013).

Eggshell membrane (ESM) is gaining recognition as a promising functional ingredient in the field of skin health. It contains bioactive compounds such as collagen, elastin, hyaluronic acid, glucosamine, and chondroitin sulfate, which have demonstrated potential in promoting cellular activity, boosting collagen production, and alleviating UV-induced damage and inflammation (Kalman & Hewlings, 2020; Yoo

Abbreviations: A.U, Arbitrary Units; BMI, Body Mass Index; BUN, Blood Urea Nitrogen; CoR, Coefficient of Restoration; CRIS, Clinical Research Information Service; ELISA, Enzyme-Linked Immunosorbent Assay; ESM, Eggshell Membrane; GCP, Good Clinical Practice; GOT, Glutamate Oxaloacetate Transaminase (AST); GPT, Glutamate Pyruvate Transaminase (ALT); LMM, Linear Mixed Model; MMP, Matrix Metalloproteinase; MFDS, Ministry of Food and Drug Safety (Korea); PP, Per-Protocol; ROS, Reactive Oxygen Species; SC, Stratum Corneum; SOD1, Superoxide Dismutase 1; TEWL, Transepidermal Water Loss; WBC, White Blood Cell.

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Table 1
Ingredients and quantities of test products.

Ingredient	Test		Placebo	
	Content (mg)	Composition (%)	Content (mg)	Composition (%)
Eggshell membrane (ESM)	200.000	30.76920	–	–
Maltodextrin	303.750	46.73080	200.000	30.76920
Microcrystalline cellulose	78.000	12.00000	–	–
Microcrystalline cellulose (101)	–	–	303.750	46.73080
Microcrystalline cellulose (102)	–	–	58.500	9.00000
Caramel color 858	–	–	19.500	3.00000
Milk salts	32.500	5.00000	32.500	5.00000
HPMC	14.235	2.19000	14.365	2.21000
Magnesium stearate	6.500	1.00000	6.500	1.00000
Silicon dioxide	6.500	1.00000	6.500	1.00000
Titanium dioxide	4.615	0.71000	4.875	0.75000
Myvacet	3.120	0.48000	3.120	0.48000
Carmine color	0.780	0.12000	–	–
Lutein colorant	–	–	0.390	0.06000
Total	650.000	100.00000	650.000	100.00000

et al., 2014). Preclinical studies using ESM in SKH-1 hairless mice have shown improved hydration, reduced wrinkling, and downregulation of inflammatory pathways after UVB exposure, highlighting its potential to restore skin function through antioxidant mechanisms like SOD1 activation (Sim et al., 2022)

Despite encouraging results from animal studies, human trials involving ESM have predominantly focused on improvements in hair, nails, and basic hydration. A comprehensive evaluation of ESM's effects on multiple skin parameters remains limited. Therefore, this study aims to bridge this gap by investigating the safety and efficacy of ESM in a randomized, double-blind, placebo-controlled clinical trial. The trial assesses various indicators of skin health, including wrinkling, elasticity, hydration, skin texture, and barrier function. The findings underscore the potential of ESM as a viable "inner beauty" supplement, with promising results for mitigating photoaging and supporting skin health.

2. Material and methods

2.1. Preparation of ESM (Ovoderm®-AS)

The Eggshell Membrane (ESM) used in this study was supplied by Eggnovo S.L., Spain. The production process involved washing the ESM with water to remove residues, followed by drying and sterilization to ensure product safety. The sterilized ESM was finely ground to a uniform particle size of 30 μm for optimal bioavailability. The final product,

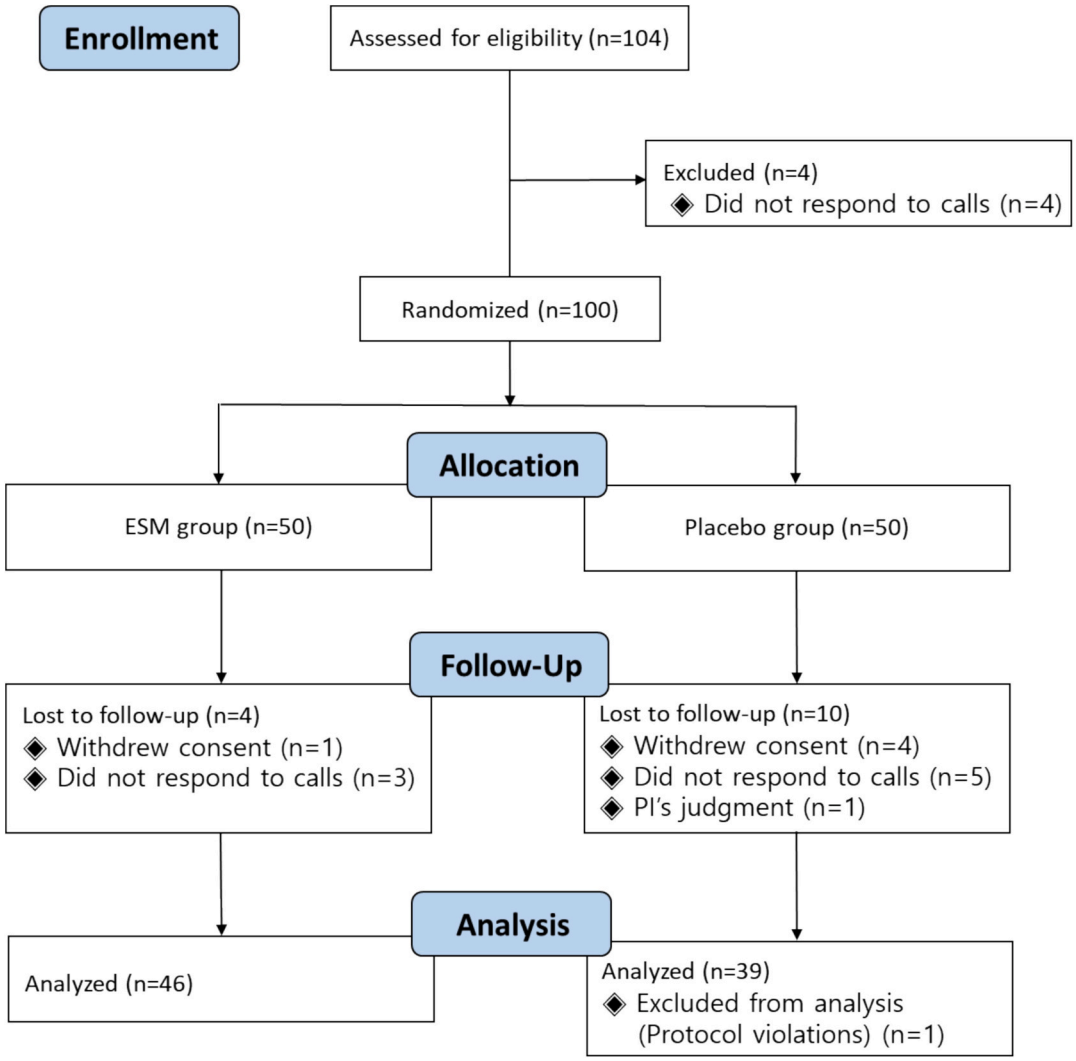

Fig. 1. CONSORT flow diagram.

Table 2
Baseline characteristics of the subjects.

Parameter (unit)	ESM (n = 46)	Placebo (n = 39)	p-value
Weigh (kg)	58.30 ± 9.72	58.81 ± 10.00	0.788
BMI (kg/m^2)	22.33 ± 3.42	22.67 ± 3.48	0.659
Skin wrinkles			
Grade	4.37 ± 1.25	4.72 ± 1.58	0.271
Ra (μm)	13.461 ± 2.475	13.188 ± 1.871	0.574
Rmax (μm)	86.183 ± 14.966	85.188 ± 11.635	0.736
Rz (μm)	63.699 ± 10.586	62.499 ± 8.222	0.566
Rp (μm)	42.340 ± 7.283	41.430 ± 6.200	0.541
Rv (μm)	48.716 ± 9.627	48.665 ± 7.614	0.979
Skin elasticity			
Indent (mm)	0.556 ± 0.088	0.537 ± 0.102	0.368
K (mm)	1.305 ± 0.056	1.301 ± 0.056	0.738
Alpha(Ratio)	0.028 ± 0.008	0.031 ± 0.010	0.165
Mean CoR (Ratio)	0.673 ± 0.056	0.656 ± 0.067	0.197
Area(Ratio)	71.851 ± 17.793	68.780 ± 22.709	0.298
Dermal density			
Distance (μm)	2096.20 ± 65.64	2121.21 ± 60.24	**0.010***
Density (%)	11.51 ± 3.19	12.58 ± 2.62	0.099
Skin sagging (angle)	13.61 ± 3.83	13.87 ± 5.53	0.803
Skin hydration			
Cheek (A.U.)	41.45 ± 4.68	41.44 ± 5.09	0.993
Forearm (A.U.)	31.94 ± 6.93	32.46 ± 6.08	0.718
TEWL			
Cheek (g/m^{2}h)	14.71 ± 3.45	13.38 ± 2.83	0.058
Forearm (g/m^{2}h)	9.54 ± 2.66	8.62 ± 1.69	0.052
Skin Desquamation			
Cheek (%)	16.71 ± 4.10	15.93 ± 2.49	0.483
Forearm (%)	21.80 ± 4.60	21.51 ± 3.94	0.765
Skin roughness			
Ra (μm)	15.225 ± 1.977	14.647 ± 2.519	0.239
Rmax (μm)	94.683 ± 11.740	92.739 ± 15.656	0.526
Rz (μm)	70.113 ± 8.413	68.113 ± 11.170	0.350
Rp (μm)	46.944 ± 5.830	45.210 ± 6.938	0.214
Rv (μm)	53.433 ± 8.188	52.532 ± 10.325	0.661
Serum MMPs			
MMP-2 (ng/mL)	24.313 ± 4.776	26.261 ± 5.389	0.053
MMP-9 (ng/mL)	367.818 ± 258.324	321.801 ± 165.538	0.832

Independent t-test was used to compare the difference between the groups. ESM, Eggshell membrane; n, number; BMI, Body mass index; A.U., Arbitrary Units; TEWL, Transepidermal Water Loss; MMP, Matrix Metalloproteinase.

Ovoderm®-AS, was formulated into tablets. Each tablet contained 650 mg, including 200 mg of ESM combined with excipients. The placebo tablets were identical in appearance, composition, and flavor, except for the absence of ESM (Table 1). Subjects ingested three tablets (600 mg of ESM) or placebo daily.

2.2. Clinical study design

This randomized, double-blind, placebo-controlled study aimed to assess the impact of ESM on skin health parameters, including wrinkling, elasticity, dermal density, sagging, hydration, transepidermal water loss (TEWL), desquamation, and roughness, as well as serum levels of MMP-2 and MMP-9. All research procedures followed Good Clinical Practice (GCP) guidelines and the standard operating procedures of mariedm Co., Ltd.'s Skin Research Center in Seoul, Korea. The protocol received ethical approval from the mariedm Skin Research Center's Institutional Review Board (MDSRC-2200FR-1) and was registered in the Clinical Research Information Service (CRIS) under registration number KCT0009068. Informed consent was obtained from all participants before enrollment. Participants were randomly assigned to either the

Table 3
Effect of ESM on skin parameters.

Parameter (unit)	Time	Group		p-value
		ESM	Placebo	
Skin wrinkle				
Ra (μm)	6 weeks-0 week	−0.206 ± 1.098	0.094 ± 1.048	0.203
	12 weeks-0 week	−0.644 ± 1.140*	0.218 ± 1.279	0.001**
Rmax (μm)	6 weeks-0 week	−0.862 ± 9.046	0.889 ± 7.533	0.340
	12 weeks-0 week	−5.229 ± 7.682*	1.787 ± 9.300	<0.001***
Rz (μm)	6 weeks-0 week	−1.551 ± 5.809	0.338 ± 5.379	0.126
	12 weeks-0 week	−3.788 ± 5.272*	0.776 ± 6.276	<0.001***
Rp (μm)	6 weeks-0 week	−1.232 ± 4.224	0.081 ± 3.478	0.126
	12 weeks-0 week	−2.534 ± 4.128*	0.687 ± 4.657	0.001**
Rv (μm)	6 weeks-0 week	0.284 ± 6.368	0.638 ± 5.615	0.788
	12 weeks-0 week	−2.837 ± 5.166*	1.212 ± 6.570	0.002**
Skin elasticity				
Indent (mm)	6 weeks-0 week	0.053 ± 0.081*	0.013 ± 0.065	0.016*
	12 weeks-0 week	0.151 ± 0.082*	−0.013 ± 0.102	<0.001***
K (mm)	6 weeks-0 week	0.021 ± 0.069*	0.005 ± 0.062	0.285
	12 weeks-0 week	0.052 ± 0.059*	−0.045 ± 0.115*	<0.001***
Alpha (Ratio)	6 weeks-0 week	−0.004 ± 0.005*	−0.002 ± 0.007	0.064
	12 weeks-0 week	−0.009 ± 0.006*	0.001 ± 0.009	<0.001***
Mean CoR (Ratio)	6 weeks-0 week	0.030 ± 0.037*	0.010 ± 0.035	0.016*
	12 weeks-0 week	0.070 ± 0.039*	−0.005 ± 0.048	<0.001***
Area (Ratio)	6 weeks-0 week	13.236 ± 18.125*	2.907 ± 13.306	0.004**
	12 weeks-0 week	37.115 ± 27.966*	−2.136 ± 18.985	<0.001***
Dermal density				
Distance (μm)	6 weeks-0 week	32.37 ± 54.42*	−10.54 ± 28.63*	<0.001***
	12 weeks-0 week	70.02 ± 54.09*	−10.97 ± 56.93	<0.001***
Density (%)	6 weeks-0 week	1.54 ± 1.81*	−0.29 ± 0.82*	<0.001***
	12 weeks-0 week	3.05 ± 2.20*	−0.63 ± 1.45*	<0.001***
Skin sagging (angle)	6 weeks-0 week	0.64 ± 2.14*	−0.35 ± 1.44	0.015*
	12 weeks-0 week	1.41 ± 1.45*	−1.33 ± 1.24*	<0.001***
Skin hydration				
Cheek (A.U.)	6 weeks-0 week	0.09 ± 2.97	0.25 ± 3.65	0.819
	12 weeks-0 week	3.09 ± 3.46*	−2.27 ± 3.90*	<0.001***
Forearm (A.U.)	6 weeks-0 week	−0.17 ± 4.23	−0.51 ± 3.78	0.698
	12 weeks-0 week	3.08 ± 3.81*	−1.57 ± 5.49	<0.001***
TEWL				
Cheek (%)	6 weeks-0 week	−0.40 ± 3.00	0.44 ± 2.87	0.195

(continued on next page)

Table 3 (*continued*)

Parameter (unit)	Time	Group		*p*-value
		ESM	Placebo	
Forearm (%)	12 weeks-0 week	−3.47 ± 3.37*	−0.74 ± 3.00	<0.001***
	6 weeks-0 week	−0.80 ± 2.28*	0.43 ± 2.74	0.027*
	12 weeks-0 week	−2.86 ± 2.61*	−0.18 ± 2.45	<0.001***
Skin desquamation				
Cheek (%)	6 weeks-0 week	0.60 ± 4.51	0.66 ± 2.35	0.944
	12 weeks-0 week	−3.01 ± 4.85*	−0.21 ± 3.46	0.003**
Forearm (%)	6 weeks-0 week	−0.38 ± 4.90	0.17 ± 3.92	0.574
	12 weeks-0 week	−7.27 ± 4.83*	−0.75 ± 2.65	<0.001***
Skin roughness				
Ra (μm)	6 weeks-0 week	−0.002 ± 1.700	0.079 ± 1.265	0.805
	12 weeks-0 week	−0.753 ± 1.692*	0.411 ± 0.915*	<0.001***
Rmax (μm)	6 weeks-0 week	−0.737 ± 10.969	0.141 ± 8.847	0.689
	12 weeks-0 week	−3.839 ± 10.933*	1.720 ± 7.596	0.009**
Rz (μm)	6 weeks-0 week	−0.378 ± 7.868	0.212 ± 5.532	0.696
	12 weeks-0 week	−3.772 ± 8.182*	1.523 ± 4.776	0.001**
Rp (μm)	6 weeks-0 week	−0.093 ± 5.761	−0.286 ± 4.291	0.864
	12 weeks-0 week	−2.530 ± 5.843*	0.499 ± 3.930	0.007**
Rv (μm)	6 weeks-0 week	−0.823 ± 7.260	0.591 ± 5.998	0.336
	12 weeks-0 week	−1.811 ± 6.996	1.508 ± 5.362	0.018*
Serum MMPs				
MMP-2 (ng/mL)	12 weeks-0 week	0.285 ± 4.266	1.193 ± 3.759*	0.566
MMP-9 (ng/mL)	12 weeks-0 week	55.453 ± 235.908*	10.545 ± 156.994	0.134

Values represent changes in parameters after 6 and 12 weeks of supplementation. The changes in values after supplementation were compared within groups (*) using RM-ANOVA (week). The mean difference between the ESM and placebo groups was compared using RM-ANOVA (Group*Week; *$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

ESM or placebo group using block randomization.

2.3. Study subjects

A total of 104 women, aged 25–60 years, with dry facial skin (≤49 a.u.) and crow's feet (grades 3–7) were screened for eligibility. Four participants were excluded due to non-responsiveness. Of the 100 enrolled, 14 participants withdrew for various reasons (e.g., lost to follow-up, protocol violations), leaving 86 participants. One additional subject was excluded for protocol non-compliance, resulting in a final sample of 85 subjects for analysis. Participants underwent comprehensive health assessments, including blood tests (WBC, RBC, hemoglobin, platelet count, albumin, total protein, bilirubin, AST, ALT, glucose, BUN, creatinine, lipids) and urinalysis. Dietary habits were monitored using 24-h dietary recall at baseline, 6 weeks, and 12 weeks. Body weight and BMI were measured before and after the 12-week intervention.

2.4. Study schedule

Participants were instructed to take three tablets (600 mg of ESM or placebo) daily and use only cosmetics provided by the research center during the wash-out and intervention periods to ensure consistent skin conditions. Visits were scheduled at baseline, 6 weeks, and 12 weeks, with a final safety evaluation 2 days after study completion. Skin assessments were conducted under standardized conditions (22 ± 2 °C, 50 ± 5 % humidity).

2.5. Skin assessment measurements

Multiple parameters of skin condition were evaluated following the Ministry of Food and Drug Safety (MFDS) guidelines (Korea) using advanced dermatological instruments and standardized methods:

2.5.1. Wrinkling and roughness

Crow's feet wrinkles and cheek roughness were assessed by a trained dermatologist. A 3D imaging system, PRIMOS® lite (Canfield, USA), measured key roughness parameters: Ra (arithmetic average roughness), Rmax (maximum peak-to-valley distance), and Rz (average maximum height).

2.5.2. Skin elasticity

Skin elasticity on the cheek was measured with the Ballistometer BLS780 (Dia-Stron Ltd., UK), yielding metrics for firmness (K), softness (indentation), and the restoration coefficient (CoR) (Jemec et al., 2001).

2.5.3. Dermal density

Ultrasound imaging with the DUB® SkinScanner (Taberna pro medicum GmbH, Germany) provided dermal thickness and density values, based on signal intensity.

2.5.4. Skin SAGGING

Facial sagging was quantified using an F-ray system (BEYOUNG, Korea) with moiré topography. Contour angles were analyzed using Image-Pro® plus software (Media Cybernetics, USA) to monitor structural improvements (Saito et al., 2008).

2.5.5. Skin hydration and barrier function

Cheek and forearm hydration were assessed with a Corneometer® CM 825 (C + K, Germany), measuring stratum corneum capacitance (Constantin et al., 2014). Transepidermal water loss (TEWL) was evaluated using a Tewameter® TM 300 (C + K, Germany) based on the open chamber principle, to determine the skin's moisture barrier function (Du Plessis et al., 2013).

2.5.6. Skin desquamation

Desquamation levels were measured using D-squame® discs (Cuderm, USA) under controlled pressure. Total protein content in corneocytes was analyzed with the SquameScan® 850 (Heiland electronic GmbH, Germany).

2.6. Serum MMPs measurement

Serum MMP-2 and MMP-9 levels were measured at baseline and 12 weeks using samples processed at the Global Clinical Central Lab (GCCL, Korea). Serum was separated by centrifugation, stored at −80 °C, and analyzed following GCCL protocols.

2.7. Statistical analysis

Data were analyzed using SPSS® version 26 (IBM, USA). Per-protocol (PP) population was used for efficacy analyses, and intention-to-treat (ITT) population was used for safety assessments. Variables were tested for normality using the Shapiro-Wilk test. Time-dependent

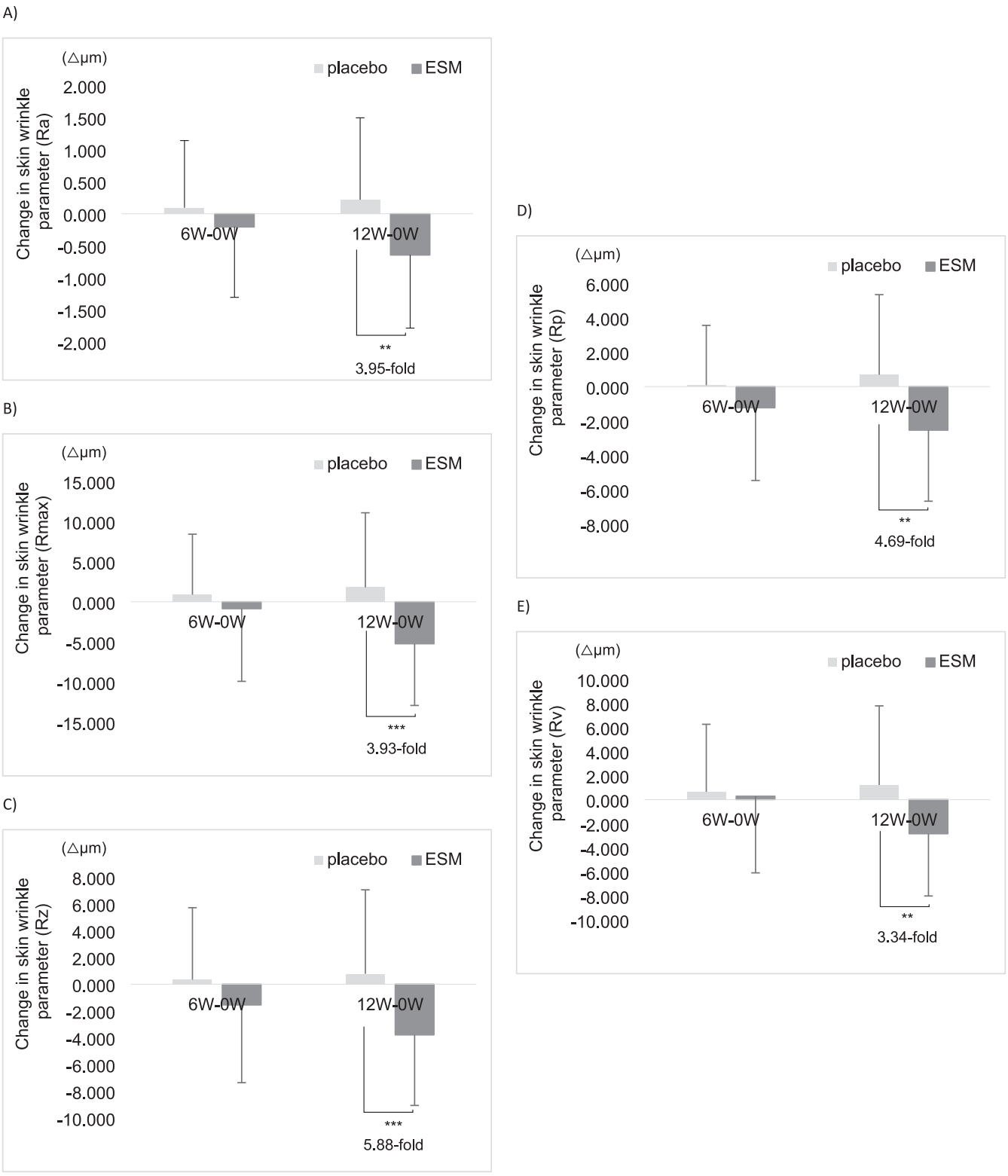

Fig. 2. Changes in skin wrinkle parameters after intake of ESM or placebo product. Wrinkles on crow's feet were measured with PRIMOS® lite, and changes from baseline values are shown. (A) Ra, (B) Rmax, (C) Rz, (D) Rp, (E) Rv; Data are expressed as the mean ± S.D. * indicates significant differences between the ESM group and the placebo group (*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; RM-ANOVA (Group*Week)).

comparisons were analyzed using linear mixed models (LMM), paired *t*-tests, Wilcoxon signed-rank tests, and repeated measures ANOVA. Between-group comparisons used LMM, independent t -tests, and ANCOVA when there was a baseline difference. Results are reported as mean ± standard deviation, and significance is $p < 0.05$. The following formula was used to express the magnitude of improvement in outcome as a multiple:

$$(\text{Change in test group} - \text{Change in control group}) / \text{Change in control group (absolute value)}$$

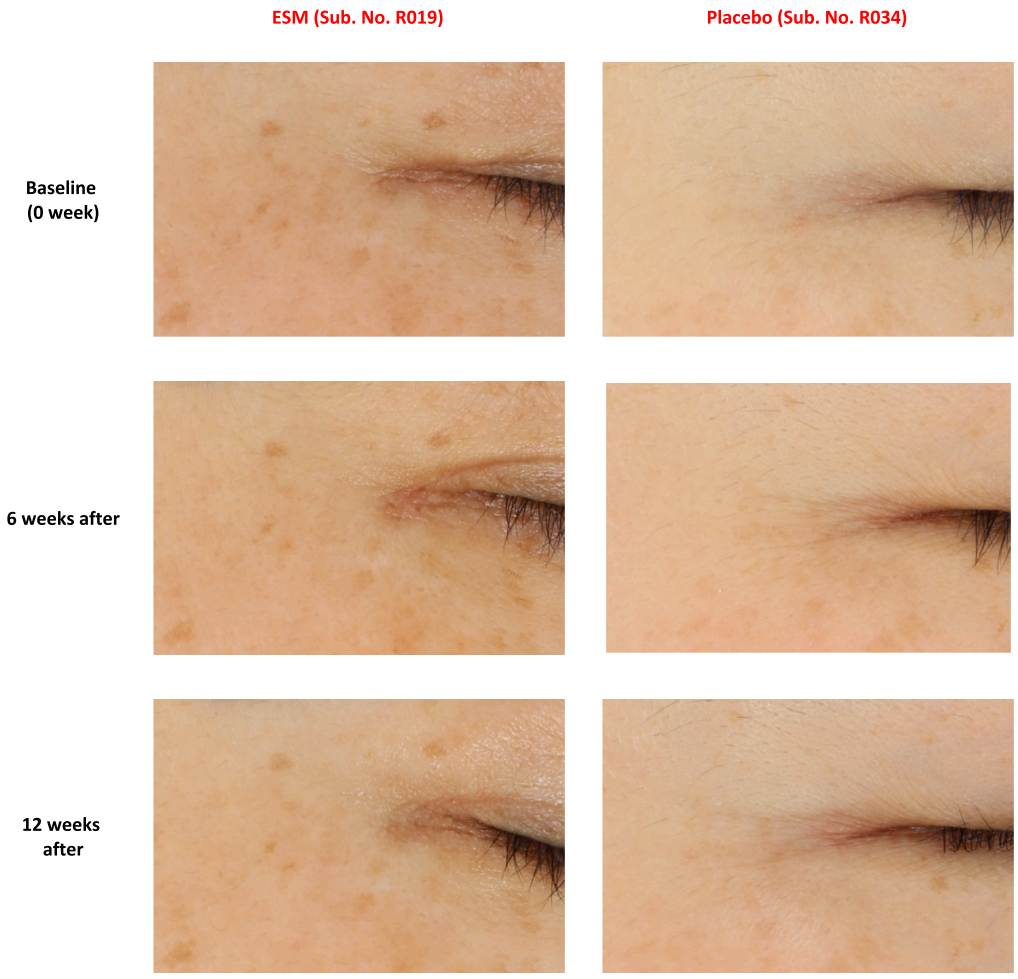

Fig. 3. Photograph of crow's feet wrinkles changes following product consumption.

All comparisons were made against the control value, and the calculated multiple for each parameter is reported in the Results section.

3. Results

3.1. Subject baseline characteristics

A total of 85 participants (ESM group = 46; placebo group = 39) completed the study (Fig. 1). Baseline comparisons showed no significant differences between the groups in most parameters, except for the dermal density distance (Table 2). Compliance with product intake was consistently high, exceeding 95 % in both groups at 6 and 12 weeks (data not shown).

Dietary intake analysis confirmed that there were no significant differences between the groups at baseline or throughout the study period, with participants maintaining consistent dietary habits. However, a significant decrease in vitamin intake was observed in the ESM group at 12 weeks, compared to baseline. Both groups adhered to standardized dietary restrictions, including avoiding health functional foods or foods that could influence study outcomes, maintaining usual eating habits, and limiting excessive intake of vitamin C-rich foods. Additionally, participants in both groups fasted after 10 p.m. before medical examinations, limited their daily water intake to no more than 2 l, and used the same cleansing foam to ensure uniformity in external conditions (data not shown).

These findings confirm that dietary and external variables were well-controlled across groups, minimizing confounding factors and ensuring the reliability of the study outcomes.

3.2. Effect of ESM on skin wrinkles

Visual assessments of crow's feet showed no significant difference between the groups (data not shown). However, instrumental measurements indicated significant improvements in wrinkle parameters (Ra, Rmax, Rz, Rp, and Rv) in the ESM group compared to the placebo after 12 weeks ($p \leq 0.001$, Table 3, Fig. 2). The improvements in wrinkle parameters were quantified as "fold improvements" to highlight the magnitude of differences between the groups. Specifically, the variance in wrinkle reduction (baseline to 12 weeks) for Ra, Rmax, Rz, Rp, and Rv was 3.95-fold, 3.93-fold, 5.88-fold, 4.69-fold, and 3.34-fold greater, respectively, in the ESM group, compared to the placebo group. These ranges reflect the variability across the analyzed parameters.

Clinical photographs (Fig. 3) and 3D images (Fig. 4) illustrate the improvements in periorbital wrinkle over time. The updated data ensure that the comparison includes participants with similar grades of crow's feet at baseline to provide consistent and reliable visual evidence of the observed improvements.

3.3. Effect of ESM on skin elasticity

The ESM group exhibited significant improvements in elasticity parameters (Indent, Mean CoR, and Area) compared to baseline and the

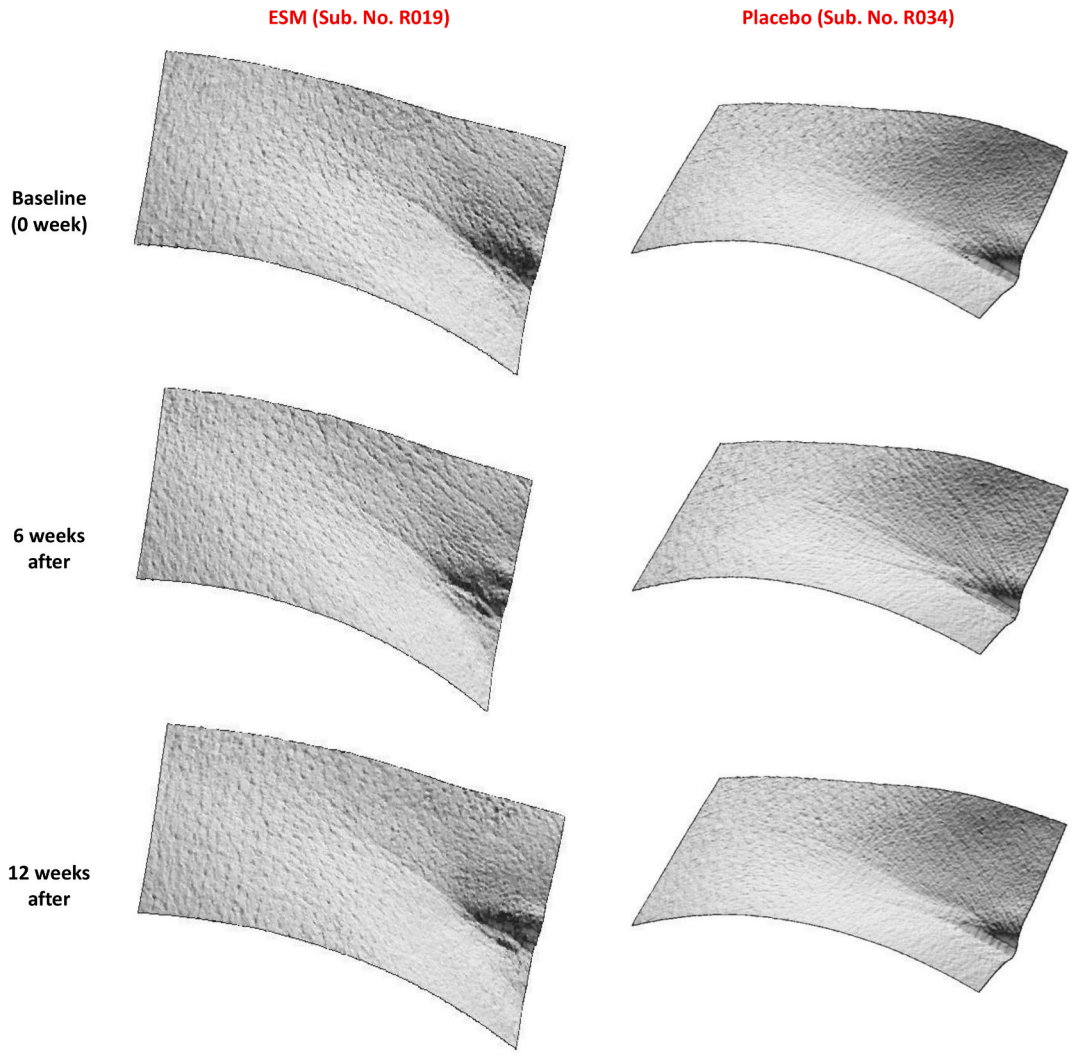

Fig. 4. The 3D image of crow's feet wrinkles changes following product consumption.

placebo group at 6 and 12 weeks ($p < 0.001$; Table 3). At 6 weeks, the ESM group showed mprovements of 3.08-fold for Indent, 2.00-fold for Mean CoR, and 3.55-fold for Area compared to the placebo group. At 12 weeks, these improvements were even greater, with Indent showing a 12.62-fold increase, Mean CoR a 15.00-fold increase, and Area an 18.38-fold increase compared to the placebo group (Fig. 5). The placebo group showed no significant improvements during the study.

3.4. Effect of ESM on dermal density and skin sagging

The ESM group demonstrated significant improvements in dermal density parameters (Distance and Density) at both 6 and 12 weeks compared to baseline ($p < 0.001$; Table 3 and Fig. 6). The placebo group did not show significant changes throughout the study. Improvements in contour angle (skin sagging) were also observed in the ESM group ($p < 0.05$), but not in the placebo group (Fig. 7). Fig. 8 illustrates the reduction in sagging over the 12-week period.

3.5. Effect of ESM on skin hydration and TEWL

Skin hydration on both the cheek and forearm significantly improved in the ESM group at 12 weeks ($p < 0.001$). In contrast, the placebo group showed a significant decrease in cheek moisture ($p < 0.001$). Hydration improvements were 2.36-fold (cheek) and 2.96-fold (forearm) higher in the ESM group compared to the placebo (Table 3, Fig. 9). Similarly, TEWL improved significantly in the ESM group, with a 3.69-fold improvement on the cheek and a 14.89-fold improvement on the forearm compared to the placebo group ($p < 0.001$).

3.6. Effect of ESM on skin desquamation

The ESM group exhibited a significant reduction in skin desquamation on the cheek and forearm at 12 weeks ($p < 0.001$), while no significant changes were noted in the placebo group. The ESM group achieved a 13.33-fold reduction in desquamation on the cheek and 8.69-fold on the forearm compared to the placebo group ($p < 0.01$; Table 3, Fig. 10).

3.7. Effect of ESM on skin roughness

All skin roughness parameters (Ra, Rmax, Rz, Rp, and Rv) improved significantly in the ESM group compared to the placebo after 12 weeks ($p \leq 0.018$; Table 3). The ESM group demonstrated improvements of 2.83-fold for Ra, 3.23-fold for Rmax, 3.48-fold for Rz, 6.07-fold for Rp, and 2.20-fold for Rv compared to the placebo group. Among these, the most pronounced effect was observed in Rp.

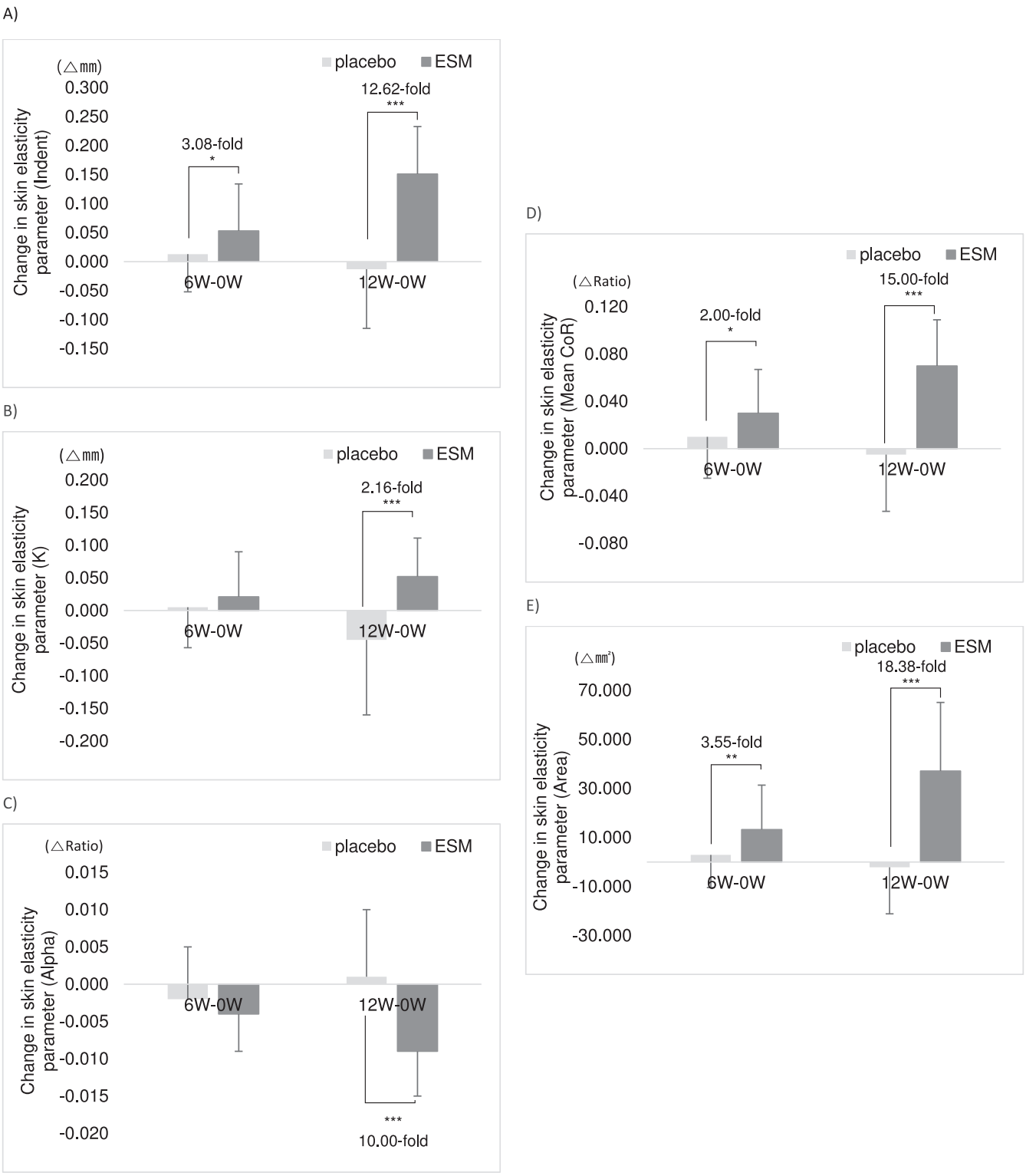

Fig. 5. Change in skin elasticity parameters after intake of ESM or placebo product. Elasticity on cheek was measured with ballistometery, and changes from baseline values are shown. A) Indent, B) K, C) Alpha, D) CoR, E) Area; Data are expressed as the mean ± S.D. * indicates significant differences between the ESM group and the placebo group (*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; RM-ANOVA (Group*Week)).

3.8. Effect of ESM on serum MMPs (MMP-2, MMP-9)

Serum MMP-2 levels increased significantly in the placebo group ($p = 0.042$), while MMP-9 levels increased in the ESM group at 12 weeks ($p = 0.025$). However, no significant differences were detected between the two groups for either MMP (Table 3).

3.9. Safety evaluation

Safety assessments, based on blood and urine analyses, showed no significant changes in any parameter for either group from baseline to 12 weeks. No significant inter-group differences were observed (data not shown). The analyses confirmed that the product was well-tolerated,

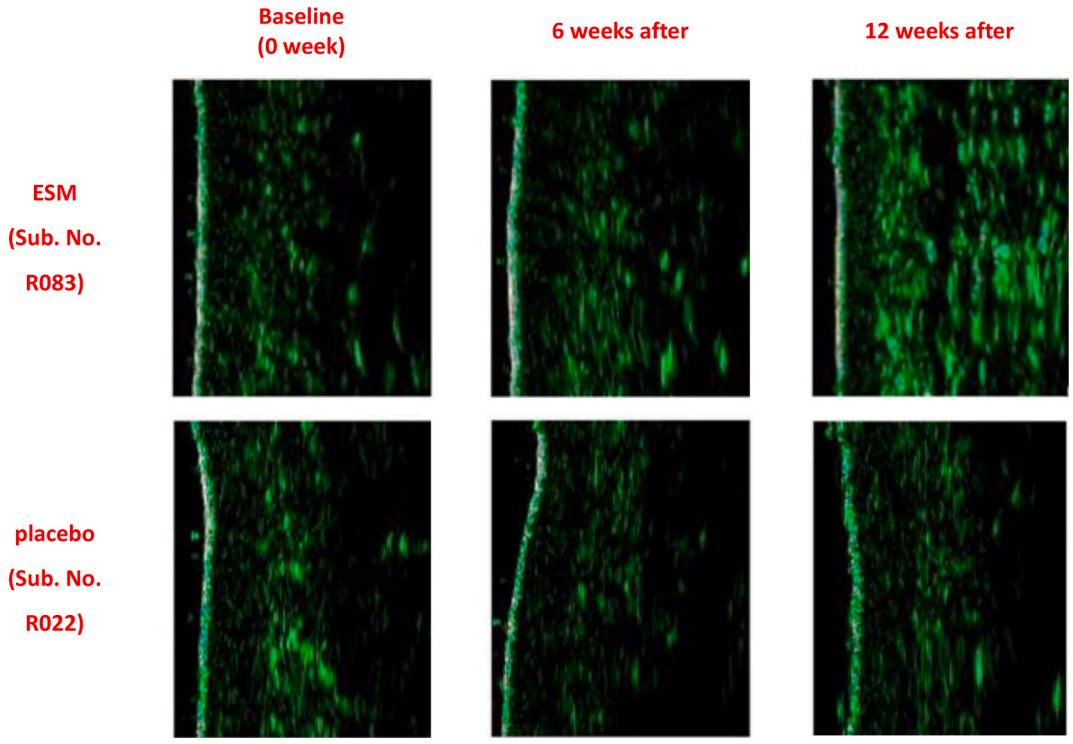

Fig. 6. Changes in Dermal density following product consumption.

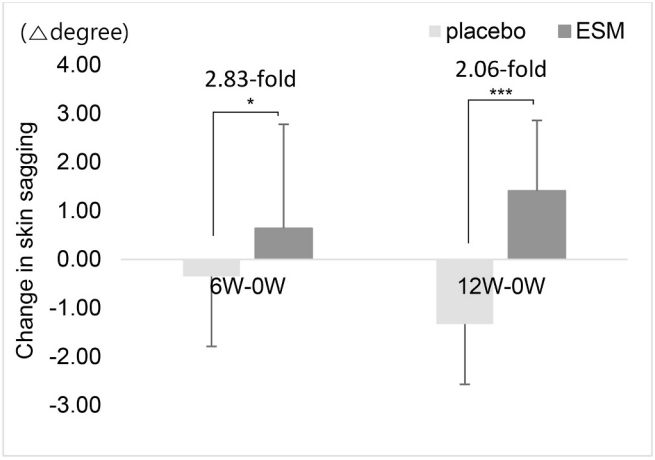

Fig. 7. Change in skin sagging after intake of ESM or placebo product. Skin sagging on cheek was measured with F-ray, and changes from baseline values are shown. Data are expressed as the mean ± S.D. * indicates significant differences between the ESM group and the placebo group (*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; RM-ANOVA (Group*Week)).

with no adverse effects reported.

4. Discussion

This study demonstrates the beneficial effects of oral supplementation with eggshell membrane (ESM, Ovoderm®-AS) on skin health, reinforcing the findings of previous research on ESM's bioactive properties. ESM contains essential components like collagen, hyaluronic acid, elastin, glucosamine, and chondroitin sulfate, which play critical roles in preserving skin moisture, elasticity, and structure (Baláž, 2014; Hincke et al., 2012; Tsai et al., 2006). The amino acid composition of ESM closely resembles that of collagen and elastin in the skin, further supporting its role in maintaining the extracellular matrix. Our findings align with previous research reporting ESM's efficacy in improving skin hydration, elasticity, and reducing wrinkles, positioning it as a promising ingredient in nutraceutical applications (Fladerer & Grollitsch, 2024; Solano, 2020).

ESM's potential in mitigating the effects of aging and UV-induced skin damage are well-supported by earlier studies (Candlish & Scougall, 1969). Its ability to inhibit collagenase, downregulate matrix metalloproteinases (MMPs), and restore hyaluronic acid synthesis contributes to wrinkle reduction and enhanced hydration (Karna et al., 2011). In our study, significant improvements in elasticity, dermal density, and sagging were observed as early as six weeks, suggesting that ESM offers a timely intervention for skin aging. However, we found that while serum levels of MMP-2 and MMP-9 tended to increase, these changes were not statistically significant, contrasting with previous animal studies that reported reduced MMP activity in localized skin

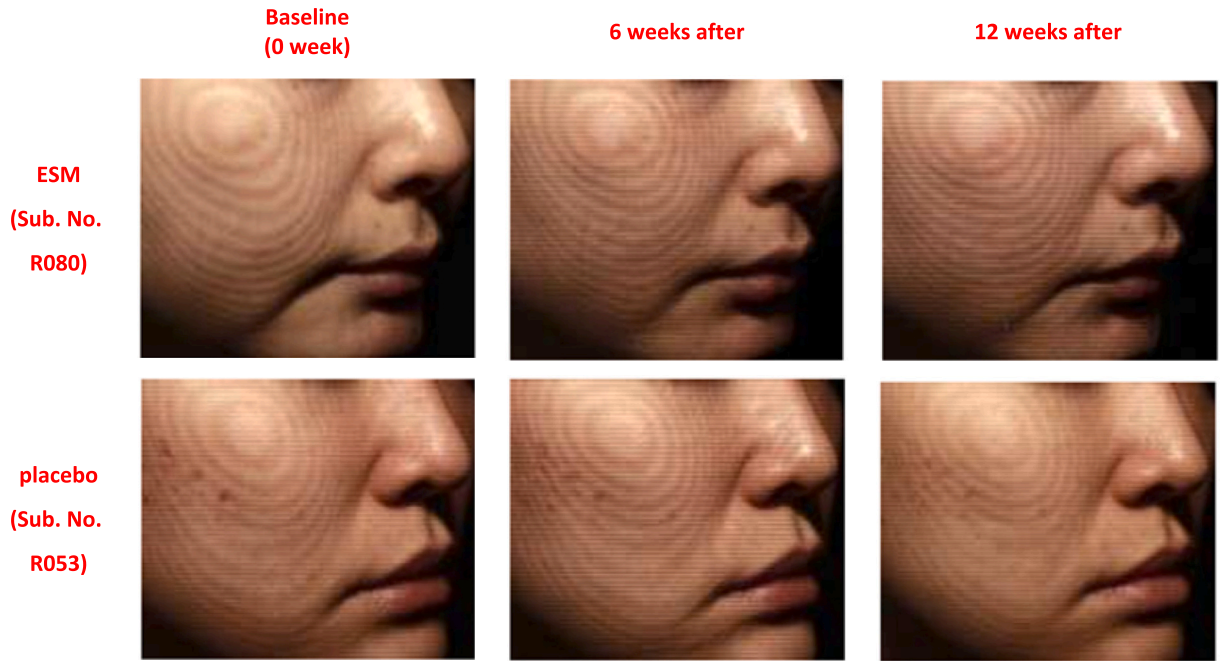

Fig. 8. Changes in cheek skin sagging following product consumption.

tissue (Sim et al., 2022). This discrepancy highlights potential differences in biological responses between humans and animal models, as well as the need to evaluate both tissue-specific and systemic markers in future research.

In animal studies, ESM has shown promising effects by reducing transepidermal water loss (TEWL) and reversing UV-induced declines in hyaluronic acid synthase mRNA expression, which promotes skin hydration and elasticity (Sim et al., 2022). These mechanisms are crucial for maintaining healthy skin and preventing the breakdown of structural components under oxidative stress. Given the variation between animal and human studies, future research should aim to harmonize methodologies by analyzing MMP expression in both skin biopsies and serum samples from human participants. Employing both ELISA and immunohistochemistry techniques would provide a comprehensive view of MMP regulation and collagen integrity across models, bridging the gap between preclinical and clinical findings.

The 12-week supplementation with ESM in this study produced significant improvements across multiple skin parameters, including reduced wrinkling and improved elasticity, hydration, and texture. These results not only confirm the safety and efficacy of ESM as a nutraceutical but also underscore its potential as a sustainable, bioactive ingredient for skincare formulations. The absence of adverse effects highlights ESM's suitability for long-term use, making it an attractive option for consumers seeking natural solutions to combat aging.

Future research should explore the synergistic effects of ESM with other skin-enhancing compounds, such as antioxidants and plant-based extracts, to optimize its application in both functional foods and cosmetic formulations. Additionally, further studies investigating molecular interactions between ESM components and skin cells will help elucidate the precise mechanisms involved, offering deeper insights for the development of targeted anti-aging strategies.

5. Conclusions

Our study shows that a 12-week oral intake of 600 mg/day of eggshell membrane (ESM, Ovoderm®-AS) significantly improves skin health in women aged 25–60. ESM improved the appearance of skin wrinkles, elasticity, dermal density, and sagging, as well as hydration, desquamation, and roughness, while reducing transepidermal water loss (TEWL), compared to the placebo. No adverse effects were reported during the study. These results highlight ESM's potential as a safe, sustainable nutraceutical for skin care. Future research should explore its underlying mechanisms and efficacy across diverse populations, contributing to innovations in functional foods and skincare applications.

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Ethical statement

All research procedures followed Good Clinical Practice (GCP) guidelines and the standard operating procedures of mariedm Co., Ltd.'s Skin Research Center in Seoul, Korea. The study protocol was approved by the mariedm Skin Research Center's Institutional Review Board (MDSRC-2200FR-1), with approval granted on February 10, 2022. The study was also registered in the Clinical Research Information Service (CRIS) under registration number KCT0009068.

Trial registration

KCT0009068; https://cris.nih.go.kr/cris/search/detailSearch.do?seq=26380&search_page=L

CRediT authorship contribution statement

Minhee Park: Writing – original draft, Investigation, Data curation. **Kyoungae Won:** Writing – original draft, Data curation. **Ji-Hoon Lee:** Writing – original draft, Investigation. **Yuri Park:** Software, Data curation. **Myeonghwan Oh:** Writing – review & editing. **Jun Chang:** Supervision. **Jeongrai Lee:** Supervision. **Miyeong Lee:** Writing – review & editing, Supervision.

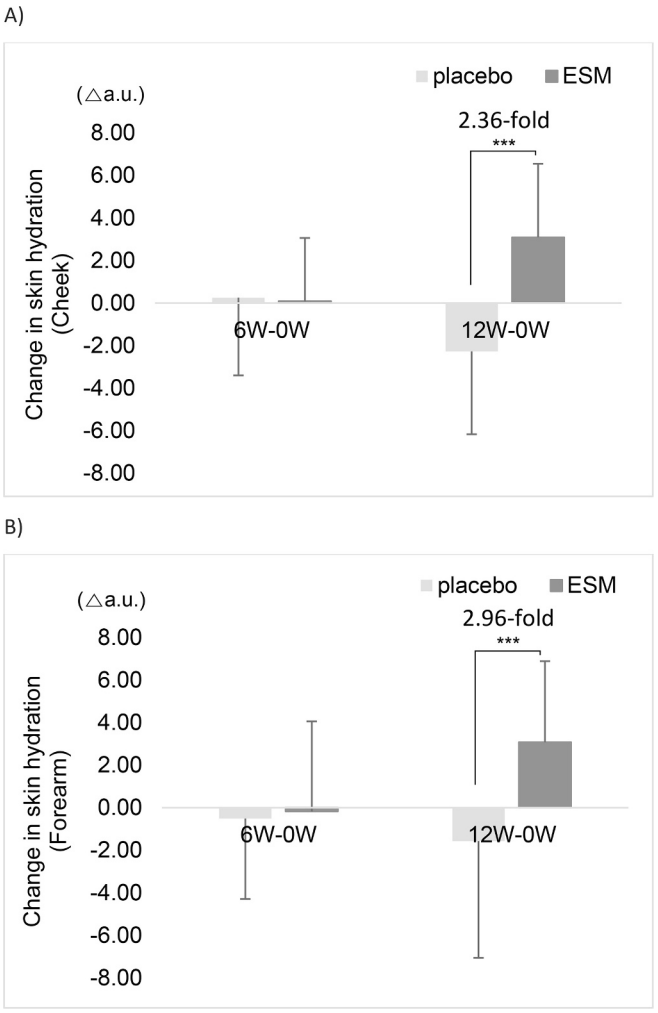

Fig. 9. Changes in skin hydration level after intake of ESM or placebo product. Skin hydration was measured with a Corneometer® CM 825, and changes from baseline values are shown, with arbitrary units (a.u.). (A) cheek area measurement, (B) Foream area measurement; Data are expressed as the mean ± S.D. * indicates significant differences between the ESM group and the placebo group (*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; RM-ANOVA (Group*Week)).

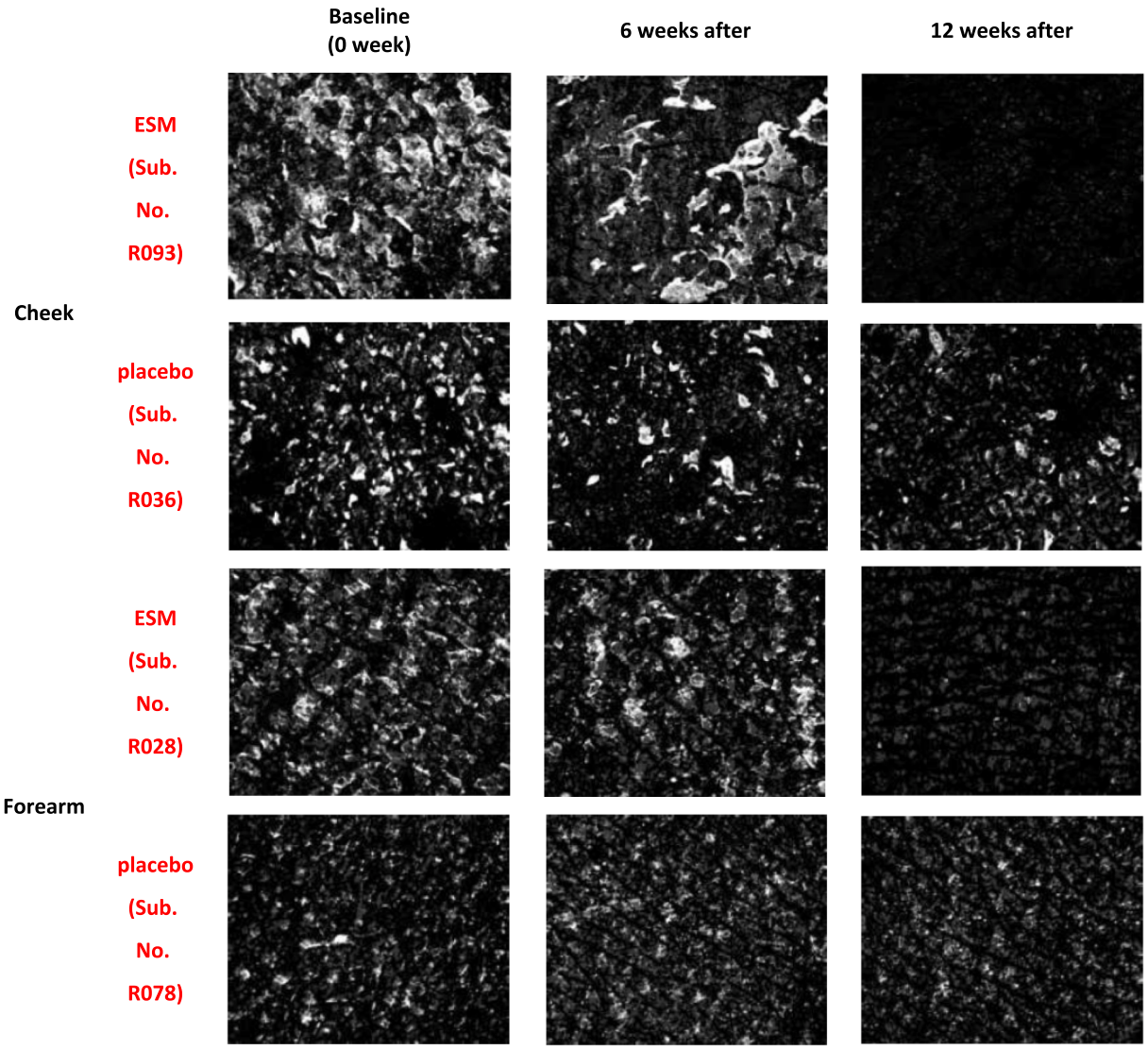

Fig. 10. Changes in skin desquamation on the cheek and forearm following product consumption.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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