

Article

Oral N-Acetylneuraminic Acid Promotes Spot Brightening and Enhances Hydration and Elasticity: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Background: N-Acetylneuraminic acid (Neu5Ac), the predominant form of sialic acid, exhibits potent antioxidant, anti-inflammatory, and anti-glycation properties in preclinical studies, suggesting potential dermatological benefits. However, robust clinical evidence supporting the efficacy of oral Neu5Ac supplementation on human skin conditions remains lacking. **Methods:** A randomized, double-blind, placebo-controlled trial was conducted with 55 Chinese women (40–65 years) who received 120 mg/day Neu5Ac ($n = 27$) or a matching placebo ($n = 28$) for 84 days. Skin pigmentation, hydration, biomechanical properties, and dermis echogenicity were evaluated at baseline, D28, D56, and D84 using standardized clinical and instrumental assessments. **Results:** Both clinical (Pantone color card) and instrumental (photographic) assessments showed that oral Neu5Ac supplementation significantly improved skin lightness on both pigmentary spots and surrounding normal skin compared with placebo at day 84. In addition, stratum corneum hydration and skin biologic extensibility were significantly increased in the Neu5Ac group. Dermal echogenicity showed numerical improvement but did not reach statistical significance. Self-assessment indicated that 100% of the Neu5Ac participants reported improvements in skin whiteness, radiance, elasticity, firmness, and hydration, with mean satisfaction scores of 9.1/10 versus 7.9/10 for placebo. **Conclusions:** Daily oral supplementation with 120 mg Neu5Ac for 84 days significantly promoted localized spot brightening, enhanced skin hydration, and improved skin elasticity, providing the first clinical evidence supporting Neu5Ac as a safe and effective oral cosmetic ingredient for skin anti-aging.

Keywords: N-acetylneuraminic acid; sialic acid; skin health; clinical trial; skin brightening; skin hydration; skin elasticity



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1. Introduction

Sialic acids are a structurally diverse family of nine-carbon α -keto acids comprising more than fifty naturally occurring derivatives, with N-acetylneuraminic acid (Neu5Ac) representing the most common and biologically significant member [1]. Neu5Ac generally occupies the terminal position of cell-surface glycans, oligosaccharides, and glycoconjugates, thereby participating in numerous physiological and pathological processes [2]. As a

functional food ingredient, Neu5Ac has demonstrated a variety of health benefits, including neuroprotection, anti-inflammatory activity, and immune modulation [3–6].

In recent years, the cosmetic and dermatological applications of Neu5Ac have attracted increasing attention. Preclinical studies have revealed that Neu5Ac possesses potent antioxidant activity and inhibits the expression of SOD1 and lipid peroxidation [7–10]. Given that oxidative stress is a primary driver of extrinsic skin aging, these antioxidant properties suggest a potential role for Neu5Ac in skin photoprotection and anti-aging [11]. Furthermore, Neu5Ac has been shown to inhibit advanced glycation end-product (AGE) formation, a process closely associated with dermal collagen cross-linking, loss of skin elasticity, and accelerated skin aging [12,13]. In vitro studies have demonstrated that Neu5Ac can suppress melanogenesis by inhibiting tyrosinase activity, the rate-limiting enzyme in melanin biosynthesis, indicating potential skin-lightening effects [10,14–16].

Despite these promising preclinical findings, clinical evidence supporting the dermatological efficacy of oral Neu5Ac supplementation in humans remains scarce. Most existing studies have focused on topical applications or in vitro models, and no well-designed randomized controlled trial has evaluated the effects of oral Neu5Ac intake on comprehensive skin parameters in human subjects.

To address this gap, we designed and conducted a randomized, double-blind, placebo-controlled clinical trial to evaluate the effects of 84-day oral Neu5Ac supplementation on multiple skin parameters, including skin radiance, pigmentation, dermis echogenicity, hydration, biomechanical properties, and wrinkle characteristics, in middle-aged Chinese women. This study provides the first clinical evidence for the efficacy of oral Neu5Ac as a cosmetic ingredient for skin health improvement.

2. Materials and Methods

2.1. Study Design

This study was a randomized, double-blind, placebo-controlled, parallel-group clinical trial conducted at COSderma China Laboratory (Wuhan, China; Study No. E23075/22065). The study was performed in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Shanghai Ethics Committee for Clinical Research (SECCR). All participants provided written informed consent prior to enrollment.

2.2. Participants

Chinese women were recruited and randomized into the Neu5Ac group and the placebo group. Inclusion criteria were as follows: (1) female sex; (2) age 40–65 years; (3) Asian ethnicity (Chinese); (4) dry, dry-combination, or normal skin type on the face; (5) presenting with a lack of elasticity to the face (inclusion grade ≤ 6 according to a 10-point scale); (6) presenting with a lack of density to the face (inclusion grade ≤ 6 according to a 10-point scale); (7) presenting with crow's feet wrinkles (grade ≥ 2 according to Skin Ageing Atlas Vol. 2, p. 41 [17]); (8) presenting with pigmentary spots with at least one spot of diameter ≥ 3 mm; (9) usual or occasional user of food supplements, with abstinence from all non-study supplements during the trial; (10) able to provide informed consent.

Subjects who meet any of the following exclusion criteria were excluded: (1) having a history of allergy or adverse reaction to cosmetic products; (2) undergoing hormone therapy or those with changes in hormone treatment within three months prior to the enrollment visit; (3) anticipated change in current hormone therapy during the study period; (4) treatment with anti-allergic, antibiotic, anti-inflammatory, dermatological medications, or corticosteroids within 2 weeks prior to the enrollment visit; (5) presence of chronic diseases; (6) participation in another clinical test during the study period or within the

washout period; (7) employees of Cosderma; (8) pregnant or lactating women; (9) women not using contraceptive measures.

A total of 58 subjects were enrolled and 55 participants completed the full 84-day study protocol for clinical scoring (placebo group, $n = 28$; Neu5Ac group, $n = 27$). Two participants were excluded due to erroneous inclusion at D0. One participant was excluded for personal reasons at D28 (Table S1). Randomization was performed using a minimization algorithm balancing baseline blood MDA levels, yielding comparable group means (placebo 2.346 vs. Neu5Ac 1.897 nmol/mL; $p > 0.05$). The mean age was 51.9 years (range: 40–62) in the placebo group and 53.6 years (range: 41–65) in the Neu5Ac group.

2.3. Investigational Products

The Neu5Ac group received oral pills containing N-acetylneuraminic acid (sialic acid) at a daily dose of 120 mg. The placebo group received matching pills identical in appearance, taste, and packaging but without the active ingredient. Both products were manufactured and provided by Wuhan CASOV Green Biotech Co., Ltd. (Wuhan, China). Participants were instructed to take the assigned pills once daily for 84 consecutive days. Daily facial cream (SOD-containing) and sunscreen (SPF 30+, PA+++), with placebo pills or Neu5Ac pills were distributed to all participants at D0. Other skincare products or cosmetics were not allowed to be used on the face during the study period.

2.4. Study Visits and Assessments

Assessments were performed at four time points: baseline (D0), D28, D56, and D84. The D0 inclusion visits were conducted between 25 and 28 September 2023; D28 visits between 23 and 27 October 2023; D56 visits between 20 and 24 November 2023; and D84 visits between 18 and 22 December 2023. All instrumental measurements were performed under controlled environmental conditions (temperature 20–23 °C; humidity 40–60%) after an appropriate acclimatization period. Before each post-baseline visit (D28, D56, D84), subjects were instructed to: (1) refrain from applying any skincare or cosmetic product on the face on the day of the visit; (2) wash the face only with water (no cleansers) on the morning of the visit; (3) apply the last topical product (if any) on the evening before the visit; and (4) take the last oral study product on the morning of the visit. These restrictions were consistently applied to all subjects to ensure reproducibility. Subjects were systematically asked about any health or skin reactions at each visit (by open-ended question and dermatologist inspection), and all reported events were recorded in the eCRF.

2.5. Outcome Measures

2.5.1. Clinical Scoring

Clinical evaluation was performed by a trained dermatologist using validated visual and tactile scoring scales [17]. The following parameters were assessed: (1) crow's feet visibility (0 = no wrinkles, 6 = very deep and visible wrinkles; referenced to Skin Ageing Atlas Vol. 2, p. 41); (2) ptosis of the lower part of the face (0 = clear lower facial contour, 6 = sagging skin with unclear contour; referenced to Skin Ageing Atlas Vol. 2, p. 67); (3) under-eye wrinkles (0 = no wrinkles, 9 = very deep and visible wrinkles; referenced to Skin Ageing Atlas Vol. 2, p. 47); (4) complexion radiance (0 = dull/dark complexion, 9 = very radiant; visual assessment); (5) skin moisturization (0 = very dry, 9 = very moisturized; visual assessment); (6) skin elasticity (0 = no elasticity/tight skin, 9 = very elastic; tactile assessment); (7) skin firmness (0 = very soft, 9 = very firm; tactile assessment).

2.5.2. Skin Color and Pigmentation—Pantone Color Card Scoring

Skin color was assessed using Pantone color card clinical scoring on both normal skin adjacent to pigmented spots and on the pigmented spots themselves. All Pantone clinical scorings were performed with subjects seated in front of an Evalux bench[®] (Orion concept) equipped with long-life calibrated LED lamps (color temperature 6500 K). The technician turned on three lamps—two longitudinal and one transverse—to ensure uniform, shadow-free illumination. The dermatologist performed the visual matching of the skin to the Pantone SkinTone[™] guide (Pantone LLC, Carlstadt, NJ, USA) under this standardized lighting. The L^* (lightness), a^* (redness), b^* (yellowness) values corresponding to each Pantone code were obtained from the manufacturer's reference database, and ITA° (individual typology angle) was subsequently calculated [18]. $ITA^\circ = [\arctan ((L^* - 50)/b^*)] \times 180/\pi$ [19]. a^* and b^* reflect the hue or undertone of the skin, and L^* and ITA° represent the tone or lightness and darkness of the skin. Higher L^* and ITA° values indicate lighter skin tone.

2.5.3. Skin Color and Pigmentation—Photographic Analysis

Standardized facial photographs were captured using the COSderma Photobench system (COSderma, Bordeaux, France) under cross-polarized light [20]. Quantitative image analysis was performed by Newton Technologies Company (Lyon, France, an independent image analysis company). Parameters assessed included: L^* (brightness), a^* (redness), b^* (yellowness), ITA° (skin pigmentation index) of both the spot and surrounding skin. $ITA^\circ = [\arctan ((L^* - 50)/b^*)] \times 180/\pi$.

2.5.4. Skin Hydration

Skin hydration was measured using a Corneometer CM825[®] (Courage+Khazaka, Cologne, Germany) operating at 0.9–1.2 MHz, which quantifies stratum corneum water content based on capacitance measurements. The Corneometer[®] probe has a contact area of 49 mm², and the measurement is performed on this area. The measurement was performed on the zygomatic (cheek) area opposite to the side of the selected pigmented spot, placed on the most prominent part of the zygomatic bone, approximately 1 cm below the lower eyelid. A transparent template was used to ensure the same position at all visits. Skin hydration was always measured first at each time point, before the instrumental test (e.g., ultrasound, skin elasticity), to prevent interference. For each site, three consecutive readings (M1, M2, M3) were taken, and the average $M = (M1 + M2 + M3)/3$ was used for analysis. Values are expressed in arbitrary units (a.u.) [21].

2.5.5. Biomechanical Properties

The biomechanical properties of the stratum corneum were assessed using a Dermal Torque Meter[®] (Dia-Stron Ltd., Andover, Hampshire, UK), which applies torsional stress to a circular skin area (20 mm diameter) between a movable central disk and a fixed concentric ring affixed to the skin surface with double-sided adhesive tape [22]. Upon activation of the motor coupler, the central disk rotates instantaneously, inducing immediate elastic deformation (U_e), followed by a gradual angular increase to produce viscoelastic deformation (U_v). Upon cessation of torsional coupling, skin recovery occurs in two phases: rapid elastic recoil to the initial state (U_r) followed by gradual recovery to baseline. The biologic extensibility (E) is calculated as the ratio of rapid recovery to immediate distension ($E = U_r/U_e$), while U_f represents the maximum distension achieved during the measurement cycle.

2.5.6. Dermis Echogenicity

Dermis echogenicity was assessed using a 25 MHz high-frequency ultrasound scanner named Dermcup[®] (Atys Medical, Soucieu-en-Jarrest, France). Data analysis was performed by an independent image analysis company, Orion Concept (Lyon, France). The ultrasound is transmitted in skin tissues and is reflected in different ways according to the acoustic properties of the crossed tissues. The reflected sound waves are collected by the probe, enabling visualization of the hyperechogenicity of the dermis' fibrous structure. The following parameters were quantified: sub-epidermal low-echogenicity band (SLEB) thickness, total thickness (full dermis thickness), skin damage depth, and the "age" of the dermis [23,24]. All measurements were performed at the temple area ipsilateral to the selected pigmentary spot. A transparent template was used to mark the exact location at baseline (D0) and to reposition the probe at D28, D56, and D84. Skin damage depth was calculated as SLEB thickness/total dermal thickness. Ultrasound-assessed "age" of the dermis is a composite parameter generated by Orion Concept based on a combination of SLEB thickness, total dermal thickness, and dermal echogenicity values, using a proprietary algorithm. A decrease in SLEB density and skin damage depth indicates improved dermal structure and reduced photoaging.

2.5.7. Self-Assessment Questionnaire

Participants completed a self-assessment questionnaire at D0, D28, D56, and D84, evaluating subjective perceptions of product efficacy across multiple dimensions including skin whitening, radiance, moisturization, elasticity, firmness, wrinkle improvement, and overall satisfaction. At D84, participants also filled a 4-point ordinal scale questionnaire (agree, partially agree, partially disagree, disagree) to evaluate if their skin felt better, became whiter, pigmentation faded or reduced, skin became radiant and more elastic, skin became firmer than before, wrinkles and fine lines improved, skin became hydrated, skin water–oil become balanced, and skin looked refreshed as if revitalized, sleep quality improved, and memory improved or not. A final overall product rating was provided on a 0–10 scale (0 = very poor, 10 = excellent).

2.6. Statistical Analysis

All data shown in figures are presented as mean $\pm$ Standard Error of the Mean (SEM). All data shown in the text are presented as mean $\pm$ Standard Deviation (SD). All statistical analyses were performed using R (version 4.5.3, R Foundation for Statistical Computing, Vienna, Austria). A two-sided p -value < 0.05 was considered statistically significant. Detailed statistical methods for each comparison are described in the respective figure legends.

3. Results

3.1. Participant Characteristics and Safety

A total of 55 participants completed the study for clinical scoring (Neu5Ac group, $n = 27$; placebo group, $n = 28$). The mean age was 53.6 years (range: 41–65) in the Neu5Ac group and 51.7 years (range: 40–62) in the placebo group. Skin types included normal (25.9%), dry (29.6%), and dry-combination (44.4%) in the Neu5Ac group, and normal (35.7%), dry (25.0%), and dry-combination (39.3%) in the placebo group. Sensitive skin was reported by 37.0% and 42.9% of participants in the Neu5Ac and placebo groups, respectively. At baseline (D0), no statistically significant differences in demographic characteristics or skin parameters were observed between the two groups (Table 1). No serious adverse events were reported during the study period.

Table 1. Subject basic information.

Index	Placebo Group	Neu5Ac Group
Number	28	27
Mean age (years)	51.7	53.6
Minimum age (years)	40	41
Maximum age (years)	62	65
Sensitive skin	12 (42.9%)	10 (37.0%)
Non-Sensitive skin	16 (57.1%)	17 (63.0%)
Normal skin	10 (35.7%)	7 (25.9%)
Dry skin	7 (25.0%)	8 (29.6%)
Dry combination skin	11 (39.3%)	12 (44.4%)

3.2. Clinical Scoring

The Neu5Ac group demonstrated statistically significant decreases in crow's feet wrinkles, ptosis of the lower part of the face, and under-eye wrinkles since D56 (Figure 1A–C). At D84, the crow's feet wrinkles, ptosis of the lower part of the face, and under-eye wrinkles decreased by 8.31%, 8.98%, and 8.52% from baseline (D0). The radiance of the complexion, moisturized aspect of the skin, and skin elasticity increased significantly since D28 in the Neu5Ac group (Figure 1D–F). At D84, the radiance of the complexion, moisturized aspect of the skin, and skin elasticity increased by 30.77%, 51.28%, and 33.33%, respectively. Inter-group comparisons revealed that D28 – D0 (placebo = 0 ± 0 ; Neu5Ac = -0.05 ± 0.13), D56 – D0 (placebo = -0.04 ± 0.13 ; Neu5Ac = -0.14 ± 0.21), and D84 – D0 (placebo = -0.07 ± 0.16 ; Neu5Ac = -0.23 ± 0.32) of the ptosis of the lower part of the face in the Neu5Ac group improved significantly compared with the placebo group (Figure 1H). In addition, the crow's feet wrinkles, ptosis of the lower part of the face, under-eye wrinkles, radiance of the complexion, moisturized aspect of the skin, and skin elasticity all improved at D56 – D0 and D84 – D0 compared with the placebo group, but had not yet reached statistical significance (Figure 1G,I–L).

3.3. Skin Color—Pantone Color Card Scoring

3.3.1. Normal Skin Adjacent to Pigmentary Spots

The L^* and ITA° parameters on normal skin adjacent to pigmentary spots increased significantly at D28, D56, and D84 compared with D0 in the Neu5Ac group (Figure 2A,D). At D84, the L^* and ITA° increased by 2.74% and 18.24% from baseline (D0). The b^* parameters decreased significantly at D28, D56 and D84 compared with D0 in the Neu5Ac group (Figure 2C). At D84, the b^* decreased by 7.92%. The parameter a^* also increased from D0 to D56 and D84 within the normal range, but the change was not significant (Figure 2B). Inter-group (Di – D0) comparison shows that the L^* (placebo = 1.69 ± 1.5 ; Neu5Ac = 2.46 ± 1.3) and ITA° (placebo = 6 ± 4.6 ; Neu5Ac = 7.6 ± 3.6) increased significantly in the Neu5Ac group at D84 – D0. The b^* showed a similar growth trend to that of the placebo group. Although the a^* tended to be higher in the placebo group compared with the Neu5Ac group, the difference was not statistically significant (Figure 2E–H).

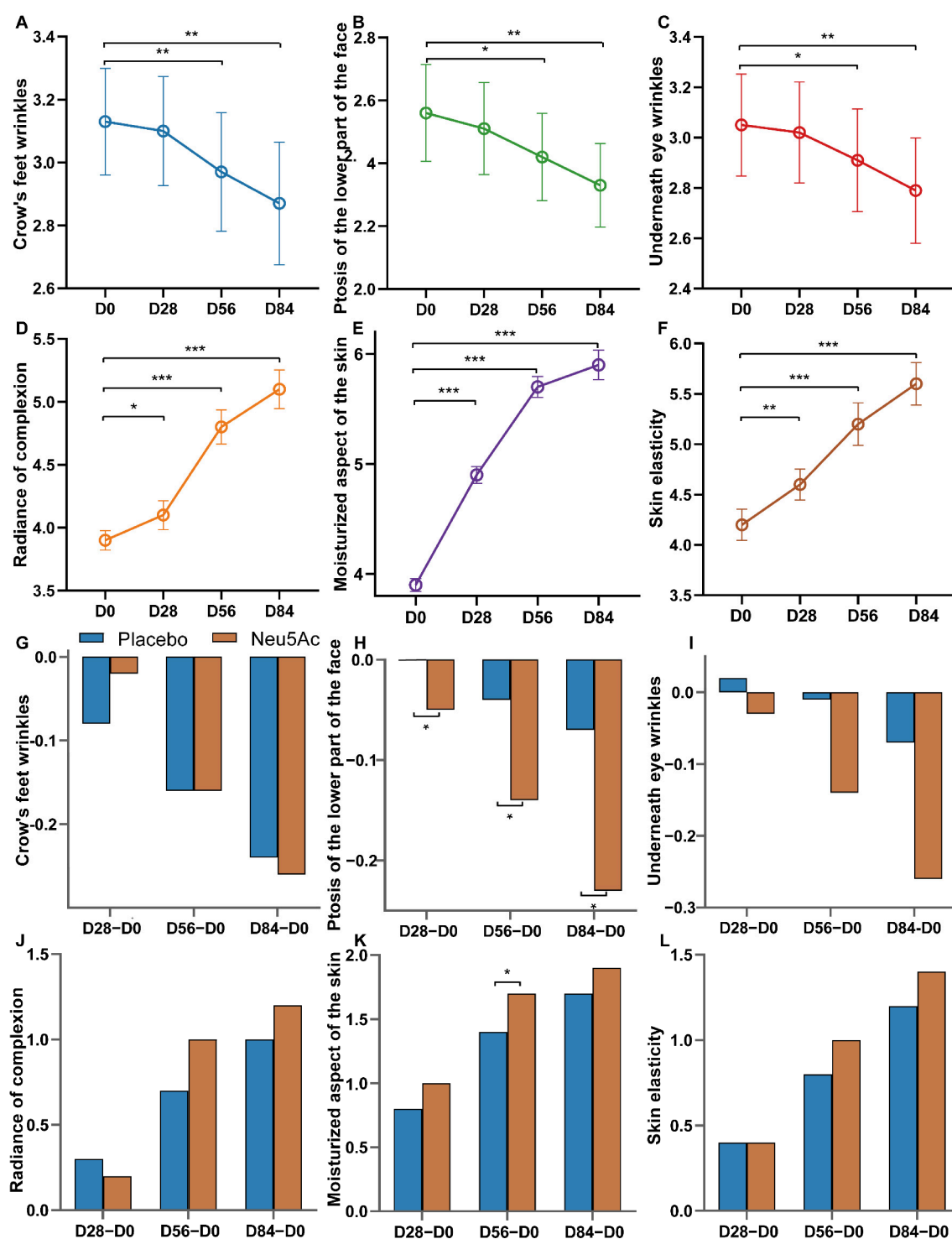


Figure 1. Clinical scoring results in skin parameters over the 84-day study period. (A–F). Participants' changes in crow's feet wrinkles, ptosis of the lower part of the face, and under-eye wrinkles, radiance of the complexion, moisturized aspect of the skin, and skin elasticity from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. The Friedman test was used as the omnibus test, followed by the Wilcoxon signed-rank test for pairwise comparisons against D0, with *p*-values adjusted using the Holm method (G–L). Average $D_i - D_0$ for the crow's feet wrinkles, ptosis of the lower part of the face, and under-eye wrinkles, radiance of the complexion, moisturized aspect of the skin, and skin elasticity in the placebo and Neu5Ac groups. The Mann–Whitney U test was applied for between-group comparisons. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

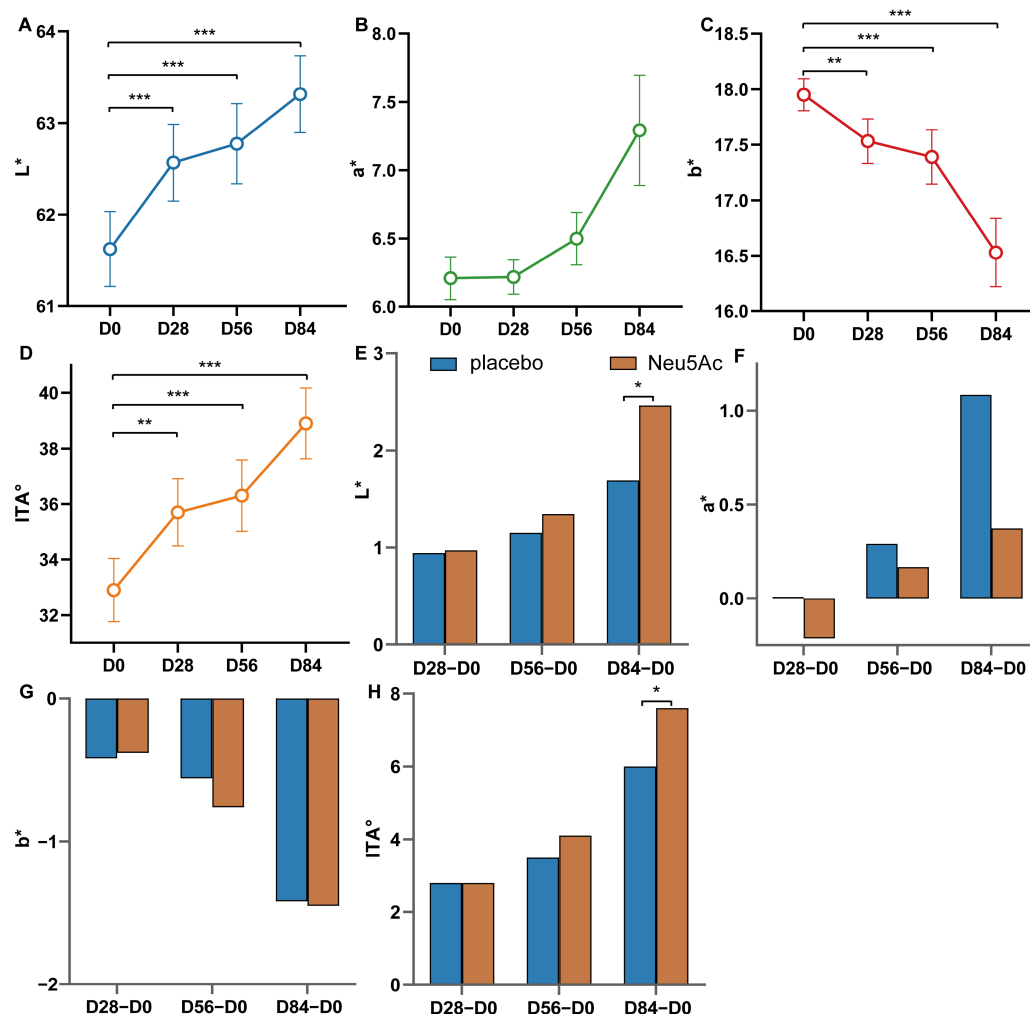


Figure 2. Skin color parameters on normal skin adjacent to pigmented spots. (A–D). Participant's changes in L*, a*, b*, and ITA° from D0 to D84 in the Neu5Ac group. Data shown as mean ± sem. The Friedman test was used as the omnibus test, followed by the Wilcoxon signed-rank test for pairwise comparisons against D0, with *p*-values adjusted using the Holm method. (E–H). Average Di – D0 for the L*, a*, b*, and ITA° in the placebo and Neu5Ac groups. The Mann–Whitney U test was applied for between-group comparisons. Statistical significance: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

3.3.2. Pigmentary Spots

From D28 onward, the Neu5Ac group showed significant increases in L* parameters on pigmentary spots (Figure 3A). From D56 onward, the Neu5Ac group showed significant increases in ITA° parameters on pigmentary spots (Figure 3D). At D84, the L* and ITA° increased by 3.89% and 1325%. The a* also increased significantly from D28 compared with D0 (Figure 3B). At D84, the a* increased by 12.46%. The b* first decreased and then increased from D0 to D84, but the changes did not reach statistical significance (Figure 3C). The increment of L* (placebo = 0.93 ± 1.84; Neu5Ac = 1.94 ± 2.02), a* (placebo = 0.33 ± 1.11; Neu5Ac = 0.94 ± 1.2), and ITA° (placebo = 2.7 ± 5.3; Neu5Ac = 5.3 ± 5.6) became statistically significant at D84 in inter-group comparison of changes from baseline (Figure 3E,F,H). The significant increases in L* and ITA° values indicated a brightening/lightening effect on the spot.

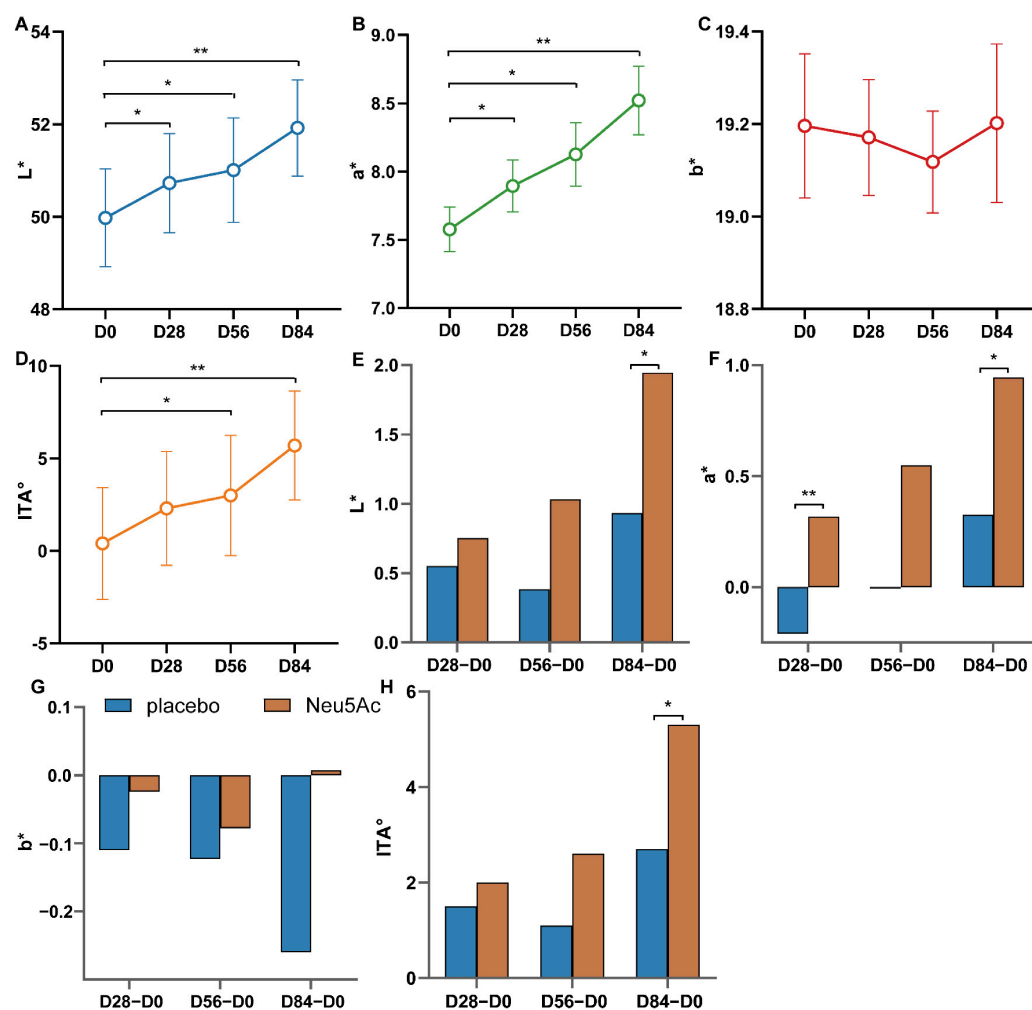


Figure 3. Skin color parameters on pigmented spots indicating skin-lightening effects. (A–D). Participant's changes in L*, a*, b*, and ITA° from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. The Friedman test was used as the omnibus test, followed by the Wilcoxon signed-rank test for pairwise comparisons against D0, with *p*-values adjusted using the Holm method (E–H). Average Di – D0 for the L*, a*, b*, and ITA° in the placebo and Neu5Ac groups. The Mann–Whitney U test was applied for between-group comparisons. Statistical significance: * *p* < 0.05, ** *p* < 0.01.

3.4. Skin Color—Photographic Analysis

3.4.1. Normal Skin Adjacent to Pigmentary Spots

The L* on normal skin adjacent to pigmentary spots increased significantly at D56, compared with D0 in the Neu5Ac group (Figure 4A). The b* parameters decreased significantly since D28 compared with D0 in the Neu5Ac group (Figure 4C). The parameter a* and ITA° increased from D0 to D84, but the changes did not reach statistical significance (Figure 4B,D). Inter-group (Di – D0) comparison shows that the L* (placebo = -0.96 ± 2.5 ; Neu5Ac = 0.73 ± 2.4) and ITA° (placebo = -2.35 ± 5.7 ; Neu5Ac = 1.39 ± 5.2) increased significantly in the Neu5Ac group at D84 – D0. The b* followed a similar trend to the placebo group. Meanwhile, the a* was higher in the placebo group than in the Neu5Ac group, but this difference was not statistically significant (Figure 4G). These results, together with Figure 2, demonstrated that the Neu5Ac had a superior skin lightening effect on normal skin adjacent to a pigmentary spot.

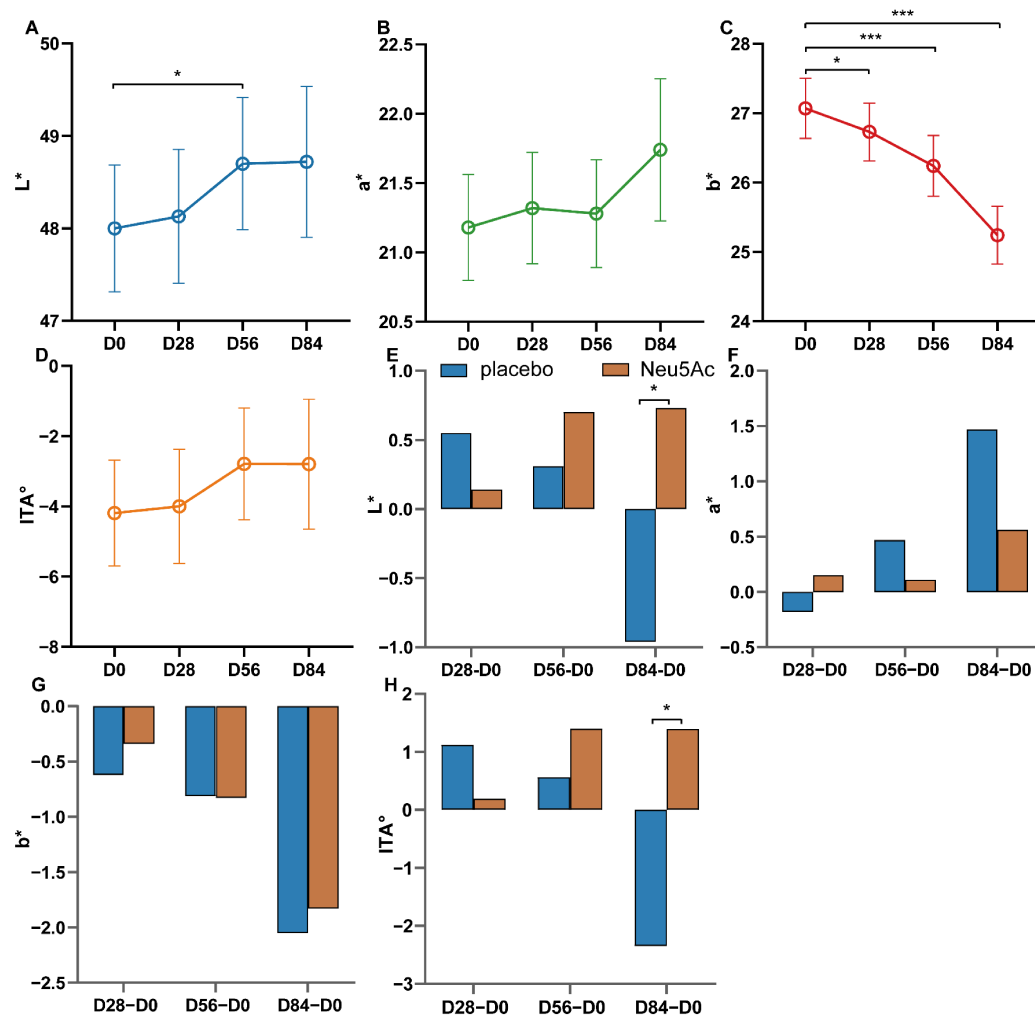


Figure 4. Photographic analysis results on normal skin adjacent to pigmented spots. (A–D). Participant's changes in L*, a*, b*, and ITA° from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. Repeated-measures analysis of variance (RM-ANOVA) was used as the omnibus test, followed by Dunnett's post hoc test for pairwise comparisons against D0. (E–H). Average Δ – D0 for the L*, a*, b*, and ITA° in the placebo and Neu5Ac groups. For between-group comparisons, normality of the Δ – D0 values was assessed using the Shapiro–Wilk test; if both groups passed normality ($p > 0.05$), Welch's *t*-test was applied, otherwise the Mann–Whitney U test was used. Statistical significance: * $p < 0.05$, *** $p < 0.001$.

3.4.2. Pigmentary Spots

L*, a*, and ITA° parameters tended to increase in the Neu5Ac group at pigmentary spots, but the changes were not statistically significant. (Figure 5A,B,D). The b* increased from D0 to D84 significantly (Figure 5C). The increments of L* (placebo = -0.69 ± 2.3 ; Neu5Ac = 0.95 ± 2.6), a* (placebo = 1.58 ± 1.5 ; Neu5Ac = 0.41 ± 1.5), and ITA° (placebo = -8.7 ± 4.2 ; Neu5Ac = 1.11 ± 4.7) reached statistical significance at D84 in the between-group comparison of changes from baseline. (Figure 5E,F,H). These results, together with Figure 3, demonstrated that the Neu5Ac had a superior brightening/lightening effect on the spot.

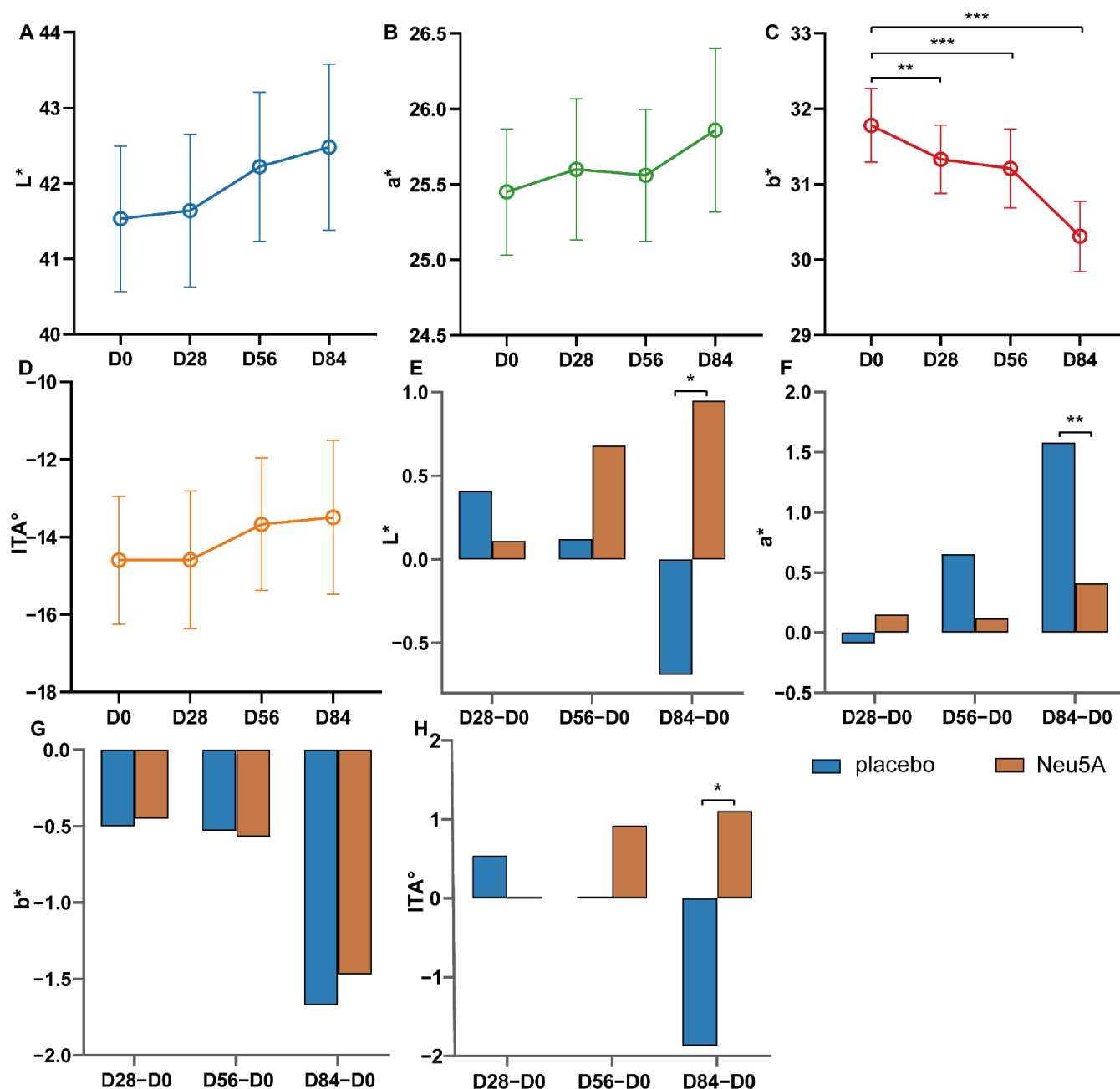


Figure 5. Skin color parameters on pigmented spots indicating skin-lightening effects in the photographic analysis. (A–D). Participant's changes in L^* , a^* , b^* , and ITA° from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. Repeated-measures analysis of variance (RM-ANOVA) was used as the omnibus test, followed by Dunnett's post hoc test for pairwise comparisons against D0. (E–H). Average $D_i - D_0$ for the L^* , a^* , b^* , and ITA° in the placebo and Neu5Ac groups. For between-group comparisons, normality of the $D_i - D_0$ values was assessed using the Shapiro–Wilk test; if both groups passed normality ($p > 0.05$), Welch's t -test was applied, otherwise the Mann–Whitney U test was used. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.5. Skin Hydration

Corneometer® measurements showed changes in both groups. The Neu5Ac group had a baseline corneometric stratum corneum hydration index (M) of 65.62 ± 10.95 a.u., which increased to 66.89 ± 8.91 a.u. at D84 (Figure 6A). Inter-group comparison of the ($D_i - D_0$) changes revealed that at D84, the M improvement increased significantly in the Neu5Ac group compared with the placebo (placebo = -3.57 ± 7.8 a.u.; Neu5Ac = 1.27 ± 6.88 a.u.).

It can also be observed that the average $M (D_i - D_0)$ of the placebo group decreased, likely due to the dry environment (Figure 6B).

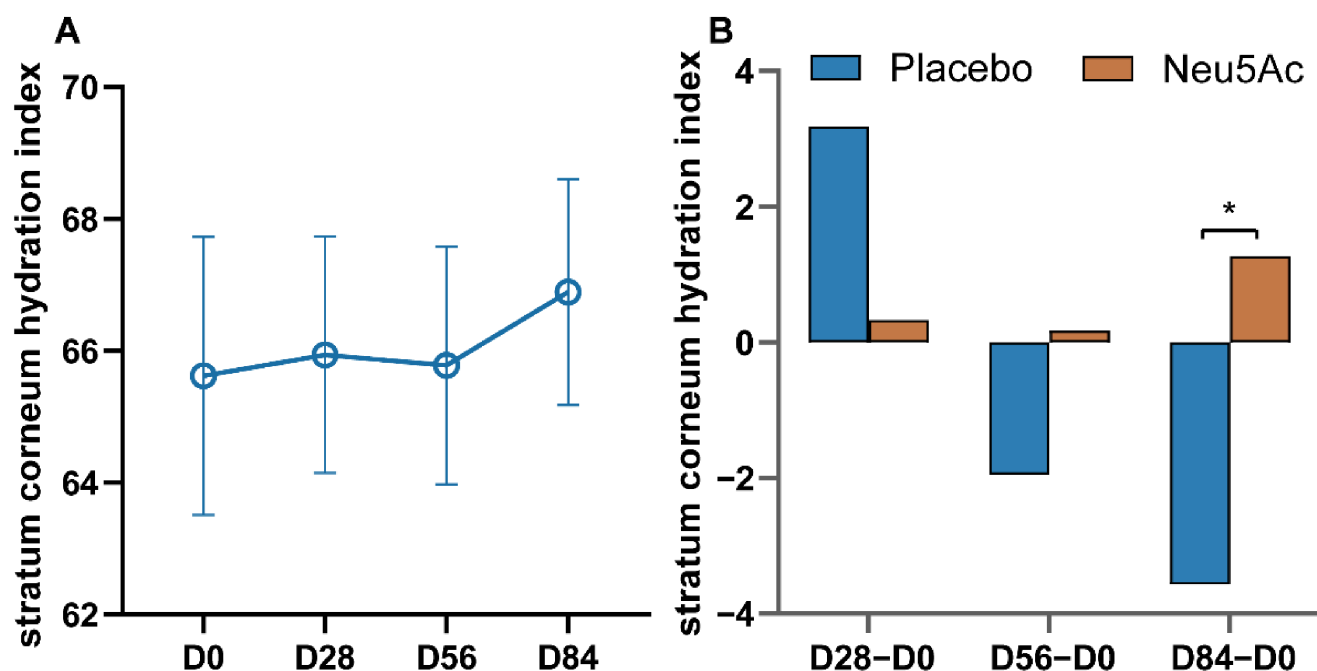


Figure 6. Skin hydration measurements over the study period. (A). Participant's changes in stratum corneum hydration index from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. Repeated-measures analysis of variance (RM-ANOVA) was used as the omnibus test, followed by Dunnett's post hoc test for pairwise comparisons against D0. (B). Average $D_i - D_0$ for the stratum corneum hydration index in the placebo and Neu5Ac groups. For between-group comparisons, normality of the $D_i - D_0$ values was assessed using the Shapiro–Wilk test; if both groups passed normality ($p > 0.05$), Welch's t -test was applied, otherwise the Mann–Whitney U test was used. Statistical significance: * $p < 0.05$.

3.6. Biomechanical Properties

Dermal Torque Meter[®] measurements assessed multiple biomechanical parameters. The Neu5Ac group showed improvements in skin elasticity parameters over the study period. The U_r was increased at D28, D56, and D84 significantly compared with D0 (Figure 7D). At D84, the U_r increased by 19.97%. Parameters U_v and U_f increased significantly at D84 compared with D0 (Figure 7B,C). At D84, the U_v and U_f increased by 10.12% and 8.06%. The ratio of U_r/U_e (E) was also increased significantly from D28 to D84 (Figure 7E). The U_e increased significantly at D28 and D84 (Figure 7A). At D84, the E and U_e increased by 12.56% and 6.39%, respectively. Inter-group comparison showed that the increment of E in the Neu5Ac group was significant during D56 – D0 (placebo = -0.01 ± 0.0 ; Neu5Ac = 0.02 ± 0.0), while $p = 0.05$ at D84 – D0 (placebo = 0.01 ± 0.0 ; Neu5Ac = 0.04 ± 0.1) compared with the placebo (Figure 7F). These results indicated statistically significant improvements in biomechanical parameters in the Neu5Ac group, with the overall conclusion supporting enhanced skin elasticity and firmness following Neu5Ac supplementation.

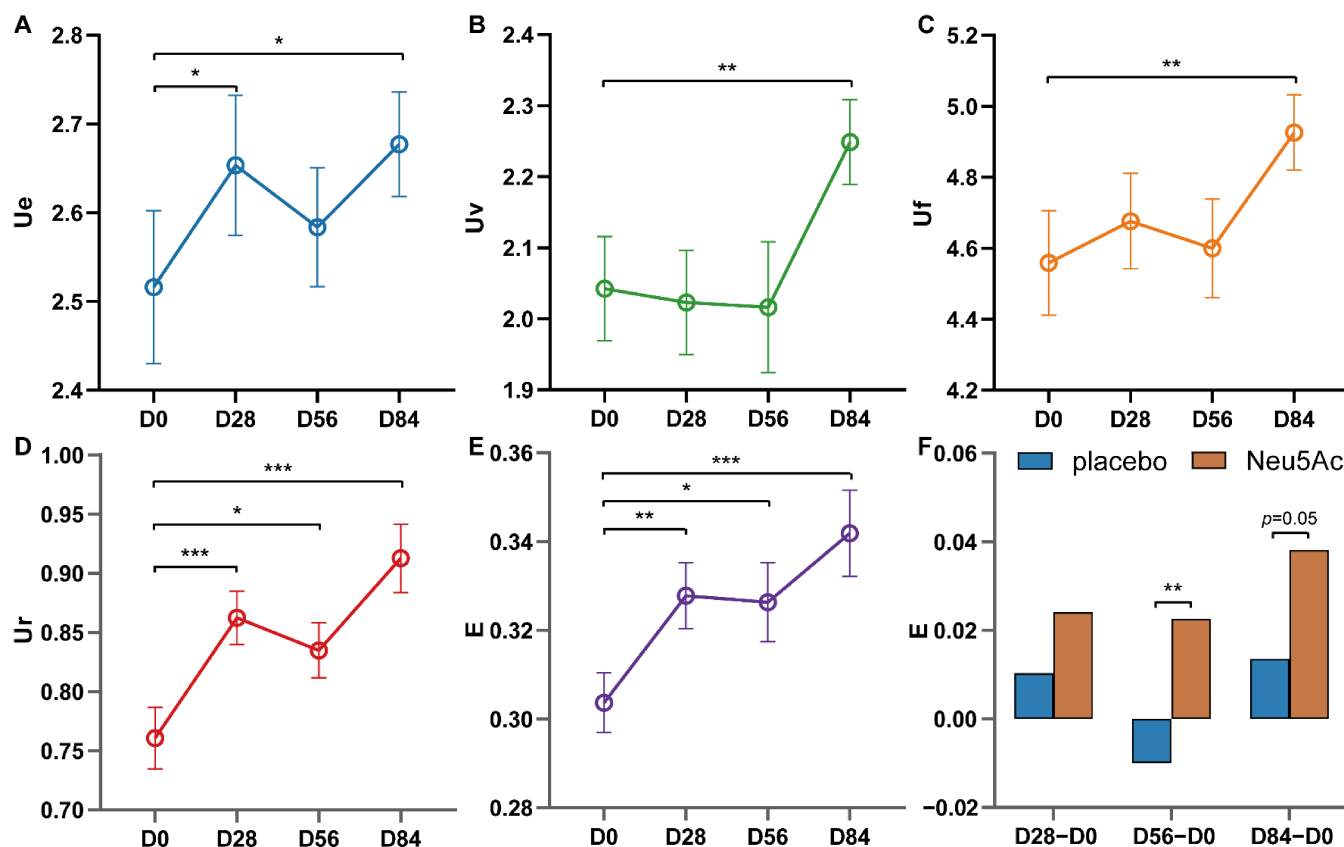


Figure 7. Biomechanical properties showing improvements in skin elasticity. (A–E). Participant's changes in Ue, Uv, Uf, Ur, and E from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. Repeated-measures analysis of variance (RM-ANOVA) was used as the omnibus test, followed by Dunnett's post hoc test for pairwise comparisons against D0. (F). Average Di – D0 for the E in the placebo and Neu5Ac groups. For between-group comparisons, normality of the Di – D0 values was assessed using the Shapiro–Wilk test; if both groups passed normality ($p > 0.05$), Welch's t -test was applied, otherwise the Mann–Whitney U test was used. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.7. Dermis Echogenicity

High-frequency ultrasound (25 MHz) analysis revealed changes in dermal structure over the study period. In the Neu5Ac group, the depth of skin damage and SLEB thickness decreased significantly from D0 to D84 (Figure 8A,B). At D84, the depth of skin damage and SLEB decreased by 26.83% and 23.35%. The “age” of the dermis in the Neu5Ac group decreased from D0 to D84, though the change was not statistically significant (Figure 8C). Inter-group comparison of the depth of skin damage, SLEB thickness, and “age” of the dermis changes (D84 – D0) showed that the improvement in dermis echogenicity was more favorable in the Neu5Ac group as time went by, but did not reach statistical significance (Figure 8E–H). While the data suggest a trend toward improved ultrasound-assessed dermal echogenicity in the Neu5Ac group compared with the placebo group, the evidence remains inconclusive and would benefit from extended study duration and/or alternative assessment modalities.

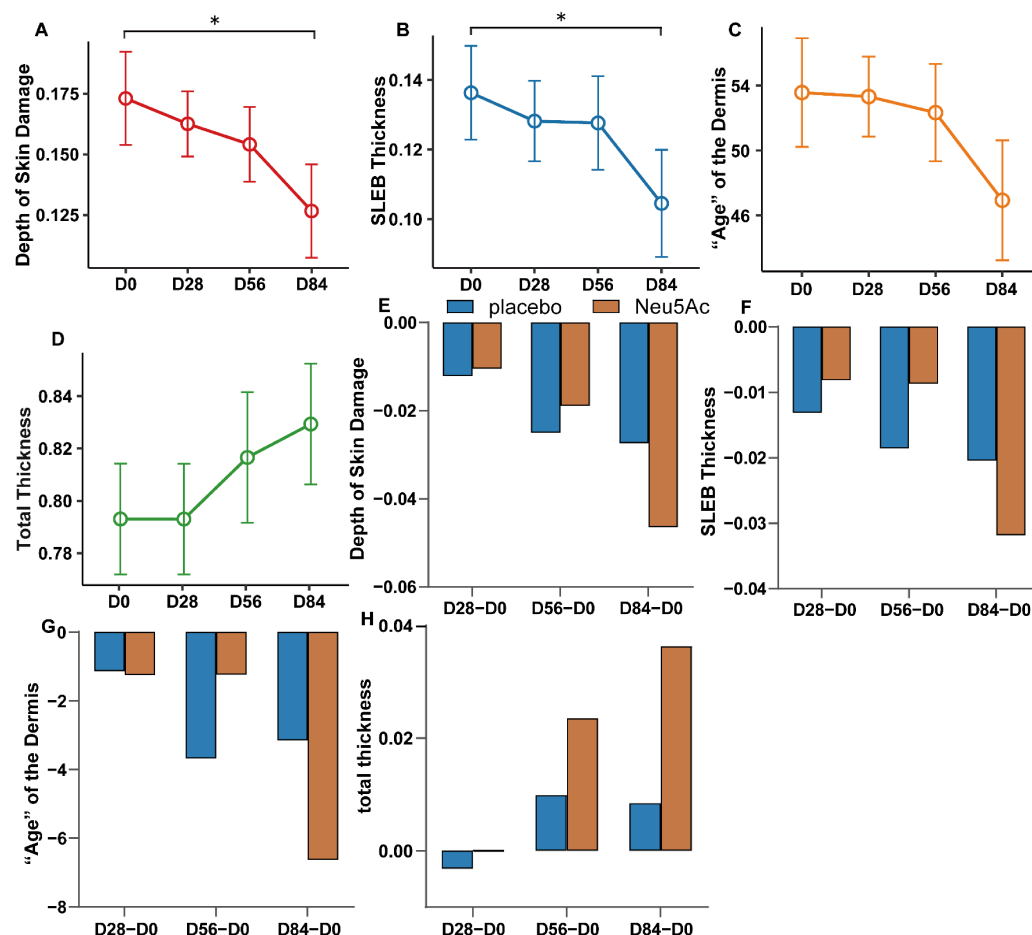


Figure 8. Changes in dermis echogenicity parameters over the 84-day treatment period. (A–D). The participant's depth of skin damage, SLEB thickness, "age" of the dermis, and total thickness from D0 to D84 in the Neu5Ac group. Data shown as mean $\pm$ sem. Repeated-measures analysis of variance (RM-ANOVA) was used as the omnibus test, followed by Dunnett's post hoc test for pairwise comparisons against D0. (E–H). Average Di – D0 for the depth of skin damage, SLEB thickness, "age" of the dermis, and total thickness in the placebo and Neu5Ac groups. For between-group comparisons, normality of the Di – D0 values was assessed using the Shapiro–Wilk test; if both groups passed normality ($p > 0.05$), Welch's t -test was applied, otherwise the Mann–Whitney U test was used. Statistical significance: * $p < 0.05$.

3.8. Self-Assessment

Participants' self-evaluation of skin beauty, complexion homogeneity, complexion radiance, and skin firmness all increased significantly from D28 onward (Figure 9A,C–E). At D84, the beauty of skin, complexion homogeneity, radiance of complexion, and firmness of the skin increased by 170.83%, 170.83%, 151.85%, and 123.33%. Participant's evaluation for the visibility of wrinkles decreased significantly since D28 (Figure 9B). At D84, the visibility of wrinkles decreased by 35%. Inter-group comparison indicated that the increment in complexion homogeneity (D56 – D0: placebo = 1.0 ± 0.9 ; Neu5Ac = 3.1 ± 1.9 ; D84 – D0: placebo = 2.4 ± 2.0 ; Neu5Ac = 4.1 ± 1.8), radiance of complexion (D56 – D0: placebo = 1.3 ± 1.4 ; Neu5Ac = 3.0 ± 1.8 ; D84 – D0: placebo = 2.9 ± 2.2 ; Neu5Ac = 4.1 ± 1.6), and firmness of the skin (D56 – D0: placebo = 1.1 ± 1.3 ; Neu5Ac = 3.1 ± 1.9 ; D84 – D0: placebo = 2.4 ± 2.0 ; Neu5Ac = 3.7 ± 1.6) in the Neu5Ac group was superior to placebo group significantly both D56 – D0 and D84 – D0. Beauty of skin (placebo = 1.5 ± 1.3 ; Neu5Ac = 3.0 ± 1.7), was improved significantly in the Neu5Ac group at D56 – D0. The visibility of wrinkles decreased at the same rate in both the placebo and Neu5Ac groups (Figure 9F–J).

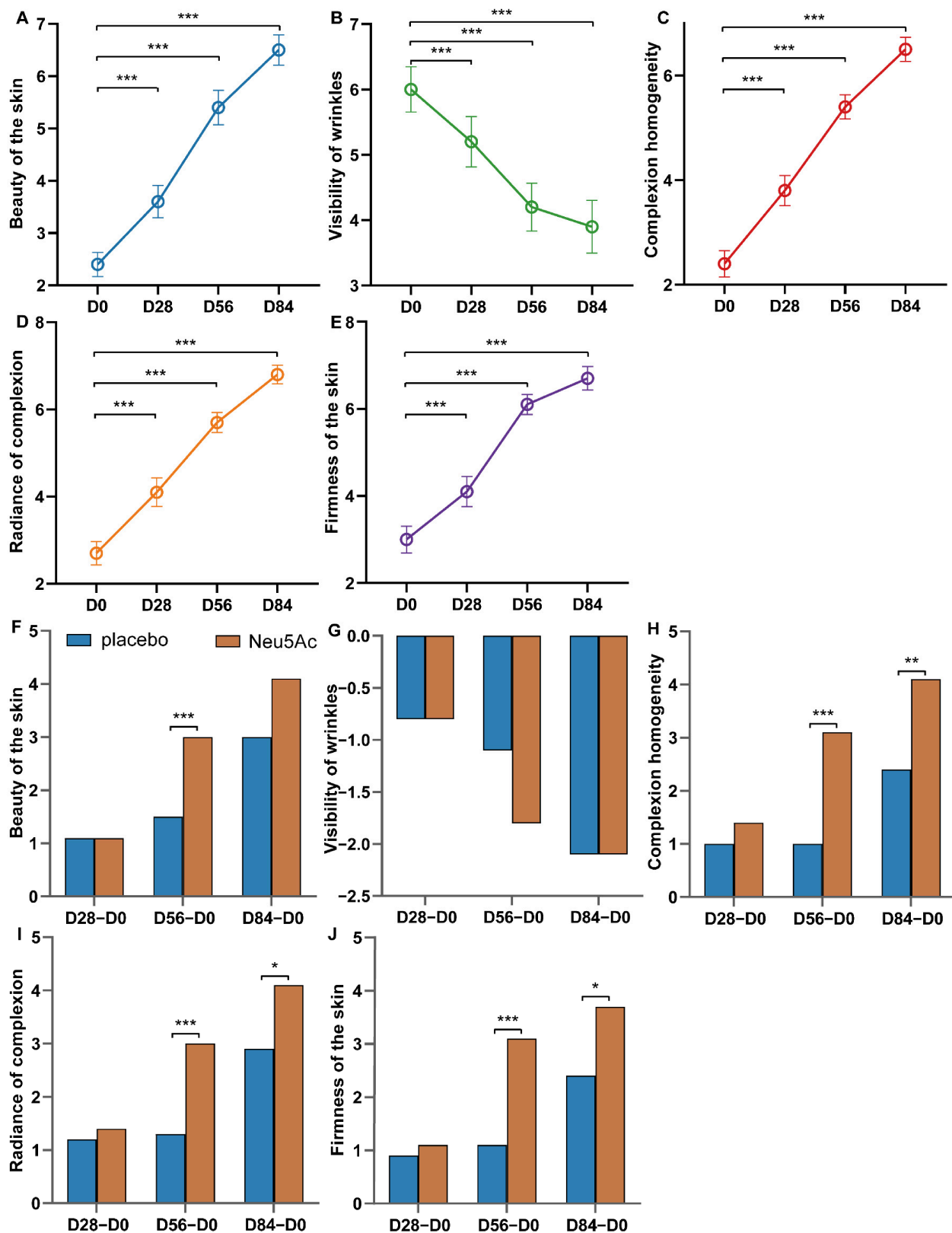


Figure 9. Self-assessment questionnaire results. (A–E). Participants’ self-grading in the Neu5Ac group from D0 to D84 in five different dimensions to evaluate their skin status. Data shown as mean $\pm$ sem. The Friedman test was used as the omnibus test, followed by the Wilcoxon signed-rank test for pairwise comparisons against D0, with p -values adjusted using the Holm method (F–J). Average self-grading of Di – D0 in the placebo and Neu5Ac groups. The Mann–Whitney U test was applied for between-group comparisons. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Self-assessment questionnaire revealed markedly superior subjective outcomes in the Neu5Ac group at D84. In the Neu5Ac group, 100% of the participants reported that their skin felt better, became whiter, pigmentation faded or reduced, skin became radiant and more elastic, skin became firmer than before, skin became hydrated, and skin looked refreshed as if revitalized. In the Neu5Ac group, 96% of the participants reported improved skin water–oil balance, and 93% reported improvements in wrinkles and fine lines. Furthermore, 89% of the participants reported improved sleep quality and memory and rated the Neu5Ac product higher than the placebo group (7.9 ± 1.46 for placebo and 9.1 ± 1.30 for Neu5Ac; Figure S1A,B).

4. Discussion

This randomized, double-blind, placebo-controlled clinical trial provides the first comprehensive clinical evidence that daily oral supplementation with 120 mg Neu5Ac for 84 days significantly improves multiple skin parameters in middle-aged Chinese women. The observed improvements encompassed complexion homogeneity, reduced pigmentation around pigmented spots and within the spots themselves, improved hydration trajectory, improved biomechanical properties, and superior subjective satisfaction.

The localized spot brightening effects observed in this study are particularly noteworthy. While all groups (including placebo) showed improvements in normal skin lightness parameters (L^* and ITA°), the Neu5Ac group demonstrated a significantly superior effect specifically on pigmented spots. At D84, the inter-group comparison of changes from baseline (D84 – D0) for both L^* and ITA° on pigmented spots and surrounding skin reached statistical significance in favor of the Neu5Ac group. This targeted skin-lightening effect on hyperpigmented lesions, rather than a generalized skin-lightening effect, is consistent with preclinical findings demonstrating the tyrosinase-inhibitory activity of Neu5Ac [10,14–16]. Tyrosinase catalyzes the rate-limiting step in melanin biosynthesis, and its inhibition represents a well-established mechanism for skin depigmentation [25]. The progressive nature of the effect, emerging from D56 and strengthening at D84, suggests a cumulative mechanism consistent with the gradual turnover of melanin-containing keratinocytes in the epidermis. The Newton photographic analysis also shows that L^* and ITA° increased significantly in both the pigment spot and the surrounding skin compared with the placebo at D84 – D0.

The improvement in dermis echogenicity, reflected by a decrease in skin damage depth and SLEB thickness on high-frequency ultrasound, is another important finding, suggesting that oral Neu5Ac supplementation may promote dermal matrix remodeling, potentially through its anti-glycation properties. Advanced glycation end-products (AGEs) are known to cross-link collagen fibers, leading to increased tissue stiffness and reduced echogenicity [12,13]. By inhibiting AGE formation, Neu5Ac may preserve the structural integrity of dermal collagen, thereby improving echogenicity. Notably, the magnitude of improvement in the Neu5Ac group appeared to increase from D56 – D0 to D84 – D0, suggesting a potential time-dependent effect. Longer treatment periods may yield more pronounced and statistically detectable changes. These findings, while preliminary, support the need for further studies with extended intervention durations.

The Corneometer® data revealed an interesting pattern: while the placebo group showed a decline in skin hydration over the study period (D84 – D0: -3.57 ± 7.81 a.u.), the Neu5Ac group maintained or improved hydration levels (D84 – D0: 1.27 ± 6.68 a.u.). This differential trajectory may be attributed to the glycosaminoglycan-related properties of Neu5Ac. As a terminal sugar on many glycoconjugates, Neu5Ac plays a critical role in maintaining the hydration capacity of the extracellular matrix [26,27]. The seasonal context of this study (September–December, transitioning from autumn to winter) likely

contributed to the hydration decline observed in the placebo group, making the relative preservation of hydration in the Neu5Ac group particularly meaningful.

The biomechanical improvements assessed by Dermal Torque Meter[®] indicated enhanced skin elasticity and firmness following Neu5Ac supplementation. These improvements are likely related to the combined effects of improved dermal collagen integrity (as evidenced by echogenicity data) and maintained hydration. The anti-glycation properties of Neu5Ac may contribute to preserving collagen fiber flexibility and reducing the stiffness associated with AGE-mediated cross-linking [12,13].

The self-assessment results were remarkably consistent with the instrumental findings. At D84, 100% of participants in the Neu5Ac group reported improvements across virtually all assessed dimensions, and the mean product satisfaction score ($9.1 \pm 1.30/10$) was substantially higher than that of the placebo group ($7.9 \pm 1.46/10$). This high level of subjective satisfaction, combined with the objective instrumental data, provides strong evidence for the real-world efficacy of oral Neu5Ac supplementation.

Several limitations of this study should be acknowledged. First, the sample size was relatively modest ($n = 55$ completers), and larger studies are warranted to confirm these findings. Second, the study population was limited to Chinese women aged 40–65 years, and the generalizability of these results to other ethnic groups and age ranges remains to be established. Third, the 84-day study duration, while sufficient to demonstrate significant effects, does not provide information on the long-term efficacy. Fourth, the molecular mechanisms underlying the observed clinical effects were not directly investigated in this study and warrant further exploration through mechanistic studies. Fifth, the seasonal transition during the study period (autumn to winter) may have influenced some skin parameters, particularly hydration, although this effect would be expected to affect both groups equally. Sixth, Menopausal status was not systematically recorded, which may have influenced skin parameter outcomes. Seventh, adherence to the standardized skincare regimen was not formally monitored beyond initial instructions, which may have introduced variability in skin outcomes.

5. Conclusions

This randomized, double-blind, placebo-controlled clinical trial demonstrates that daily oral supplementation with 120 mg N-acetylneuraminic acid (Neu5Ac) for 84 days in middle-aged Chinese women significantly reduces ptosis of the lower part of the face, promotes skin brightening on spots and surrounding normal skin, increases skin hydration, and improves skin elasticity. Dermal echogenicity also improved compared with placebo, although the change did not reach statistical significance. Furthermore, the Neu5Ac group achieved superior subjective satisfaction. These results provide the first robust clinical evidence supporting the efficacy of oral Neu5Ac as a novel cosmetic ingredient for skin anti-aging. Further studies with larger sample sizes, longer durations, and diverse populations are warranted to confirm and extend these findings.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/cosmetics13030152/s1>: Figure S1: Self-assessment questionnaire and product rating; Table S1: CONSORT flow diagram.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Shanghai Ethics Committee for Clinical Research (Approval Code: SECCR/2022-132-01, Approval Date: 22 October 2022) The study was conducted at COSderma China Laboratory (Wuhan, China), with the principal investigator being Ms. Yanqiu Wang (Dermatologist). The study was registered with ClinicalTrials.gov (Approval Code: NCT07640321, Approval Date: 9 June 2026).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

Neu5Ac	N-Acetylneuraminic acid
Ue	Immediate elastic deformation
Uv	Viscoelastic deformation
Ur	Rapid elastic recoil to the initial state
Uf	Total deformation
E	Ur/Ue
SLEB	Sub-epidermal low-echogenicity band

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