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Reduction in the appearance of facial hyperpigmentation after use of moisturizers with a combination of topical niacinamide and *N*-acetyl glucosamine: results of a randomized, double-blind, vehicle-controlled trial

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Conflicts of interest
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Summary

Background Topical niacinamide and N-acetyl glucosamine (NAG) each individually inhibit epidermal pigmentation in cell culture. In small clinical studies, niacinamide-containing and NAG-containing formulations reduced the appearance of hyperpigmentation.

Objectives To assess the effect of a combination of niacinamide and NAG in a topical moisturizing formulation on irregular facial pigmentation, including specific detection of changes in colour features associated with melanin.

Methods This was a 10-week, double-blind, vehicle-controlled, full-face, parallel-group clinical study conducted in women aged 40–60 years. After a 2-week washout period, subjects used a daily regimen of either a morning sun protection factor (SPF) 15 sunscreen moisturizing lotion and evening moisturizing cream each containing 4% niacinamide + 2% NAG (test formulation; $n = 101$) or the SPF 15 lotion and cream vehicles (vehicle control; $n = 101$). Product-induced changes in apparent pigmentation were assessed by capturing digital photographic images of the women after 0, 4, 6 and 8 weeks of product use and evaluating the images by algorithm-based computer image analysis for coloured spot area fraction, by expert visual grading, and by chromophore-specific image analysis based on noncontact SIAscopy™ for melanin spot area fraction and melanin chromophore evenness.

Results By all four measures, the niacinamide + NAG formulation regimen was significantly ($P < 0{\cdot}05$) more effective than the vehicle control formulation regimen in reducing the detectable area of facial spots and the appearance of pigmentation.

Conclusions A formulation containing the combination of niacinamide + NAG reduced the appearance of irregular pigmentation including hypermelaninization, providing an effect beyond that achieved with SPF 15 sunscreen.

Irregular facial hyperpigmentation is a substantial contributor to the aged appearance of skin.[1] As a result, technology that can effectively address pigment appearance is of interest, especially given proposed regulatory changes limiting the use of hydroquinone.[2] Niacinamide is a member of the vitamin B3 family and can be used as an ingredient in cosmetic moisturizers. It is a precursor to a group of enzyme cofactors: nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), and their reduced forms (NADH and NADPH). These cofactors are involved in many reactions in the body, including several in the skin which appear to contribute to a reduction in the appearance of hyperpigmentation.[3–5] The results of *in vitro* cell coculture testing suggest that the inhibition of melanosome transfer from melanocytes to keratinocytes by niacinamide is a relevant mechanism in this effect.[6,7]

N-acetyl glucosamine (NAG) is an amino hexose that occurs throughout nature and in all human tissues. It is recognized primarily for its function as a precursor to hyaluronic acid, a polymer composed of alternating NAG–glucuronic acid disaccharide units. This polymer plays a significant role in the structure and hydration of the extracellular matrix in joints and skin,[8–10] in both the epidermis and the dermis. Studies have shown that glucosamine[11–13] and NAG[14] effectively reduce the production of melanin in human cell culture systems. These agents inhibit the glycosylation of tyrosinase, a required step in the activation of the human enzyme, which is central to formation of melanin. Initial clinical tests of moisturizer formulations containing NAG have shown that this ingredient can reduce the appearance of hyperpigmentation.[15,16]

Because niacinamide and NAG have different mechanisms of action, it was proposed that their combination could be more effective than the individual materials alone in reducing hyperpigmentation. Indeed, in small clinical studies, the combination was more effective than the individual constituents.[17] We conducted this large clinical trial to evaluate and compare the effect of a topical facial regimen containing niacinamide in combination with NAG with that of a vehicle control regimen on the appearance of facial hyperpigmentation and uneven skin tone in female subjects.

Materials and methods

Study design

This was a 10-week, prospective, randomized, double-blind, vehicle-controlled clinical study consisting of a 2-week preconditioning period followed by an 8-week test product use period. Because of the cosmetic nature of this study, conducted in the U.S.A., regulations did not require institutional review board review. The conduct of the study was consistent with Good Clinical Practices standards. Each subject gave written informed consent to participate in the study. The study was conducted from March to May 2005 in Cincinnati, OH.

Subjects

Subjects eligible to participate in the trial were white-skinned women aged 40–60 years with moderate to moderately severe irregular hyperpigmentation due primarily to solar lentigines. Subjects were randomly assigned to receive the niacinamide plus NAG ($n = 101$) or vehicle control ($n = 101$) regimen; study groups were balanced with respect to hyperpigmentation and age (40–50 and 51–60 years) at baseline.

Test regimens

During the 2-week preconditioning period, all subjects used the same commercial facial cleanser, topical night-time moisturizing cream, and topical daytime moisturizing lotion. Subjects were permitted to apply their usual brand of foundation, blushers, and eye and lip makeup provided these products did not contain any ingredients known to affect skin pigmentation.

After 2 weeks, the night-time cream and daytime lotion used during the preconditioning period were replaced with blind-coded test formulations, which were used for the duration of the product use period. Subjects in the niacinamide plus NAG group received a topical night-time cream formulation containing 4% niacinamide plus 2% NAG and a topical daytime lotion formulation which also contained 4% niacinamide plus 2% NAG, plus sufficient sunscreen agents [1% Parsol HS (phenylbenzimidazole sulphonic acid) and 2% Parsol 1789 (avobenzone)] to achieve sun protection factor (SPF) 15, broad-spectrum protection. Subjects in the vehicle control group received the vehicles for the night-time cream and daytime lotion (including sunscreen) formulations. The vehicle control and niacinamide plus NAG formulations were packaged in identical 50-g blind-coded pump jars labelled with subject number and the time of day at which they should be applied ('morning' or 'night-time').

In the morning and evening, each subject applied approximately 1·0 g of her assigned test formulation to her entire face (total daily product usage ~2 g). Subjects applied the daytime lotion in the morning and the night-time cream in the evening at least 1 h before bedtime for 8 weeks. Subjects received new containers of test formulation at baseline and at week 4 and returned used containers at weeks 4 and 8. Subjects recorded each application of the test formulation in a diary.

Compliance with the product use regimen was assessed on the basis of diary records and the weight of returned containers.

Clinical measurements

Subjects underwent evaluation at screening and baseline and again at weeks 4, 6 and 8 during the test product use period. Evaluation included facial colour imaging and analyses and noncontact SIAscopy™ (Astron Clinica Ltd, Cambridge, U.K.).

Facial colour imaging and analysis

Standardized digital photographic images were taken of untreated skin (skin not treated with any products on that day) on each side of the face of all subjects. Before imaging, subjects washed their face with the assigned facial cleanser and underwent equilibration for 30 min in a room with controlled temperature (21 ± 2 °C) and relative humidity (30–50%). Subjects' hair and clothing were covered with black drapes. Images were taken with the same imaging equipment in a reproducible manner (lighting conditions, distance, head position etc.) at all time points.

Images were taken with the REAL (Rapid Evaluation of Anti-aging Leads) 3.0 digital imaging system with a cross-polarized lighting system as generally described elsewhere.[18] Briefly, the REAL 3.0 imaging system is equipped with a combination of two cameras to capture both study images and repositioning images. Study facial images were captured with a 6.2-megapixel Fuji S2 Pro digital SLR camera with a 60-mm Nikor lens equipped with a polarizing filter. Repositioning images were captured with a SuperCircuits PC-33C CCD video camera with a Computar 10–25 mm f1·4 CS-mount lens. Facial illumination was provided by a JTL Versalight D 1000 flash unit with a colour temperature of approximately 5600 K. The flash unit also provided lighting for the repositioning images via a built-in 250 W diffused halogen quartz modelling light. The cameras and flash units were mounted on to the imaging head of the REAL 3.0 system. The subject's head position was fixed via a bite stick mounted on a positioning arm. Colour calibration was performed by placing a selected Gretag Macbeth colour chart on the positioning arm prior to each image collection. During image capture, the baseline repositioning image was displayed on a monitor simultaneously with the subject's live image so that her head position could be adjusted to match the baseline image. The imaging head was adjusted on the system table to an angle of approximately 40° either left or right to capture the left or right side facial images.

On each image, the region of interest on the cheek from the jaw line to the periorbital area was defined, and proprietary algorithms were used to determine the total area of hyperpigmented spots in the selected region. The percentage spot area fraction was then calculated for each image as the ratio of total hyperpigmented area to total skin measurement area, multiplied by 100. Subjects resumed application of their study formulation after each imaging session.

Visual perception study

The effects of the test formulations were also assessed in a visual perception study (VPS) wherein two expert graders blinded to test product identities assessed the REAL facial images for changes in hyperpigmented spots and evenness of skin colour. The graders viewed and compared pairs of blind-coded images taken at baseline (identified as 'before') and at 4, 6 or 8 weeks (identified as 'after'). Paired images were displayed on a calibrated colour monitor. Each pair of images was graded according to the magnitude of the colour attribute change (−8 to +8 scale). Negative grades indicated worsening, positive grades indicated improvement, and zero (0) indicated no difference between the two images. Final grades were determined by consensus of two graders.

Melanin-specific imaging and analysis

Noncontact SIAscopy™, described elsewhere,[19,20] was performed to determine skin melanin content and distribution. This method employs a digital camera and cross-polarized lighting system to acquire large-field eumelanin chromophore maps. For every pixel of the original raw image, noncontact SIAscopy™ calculations are performed automatically to yield exclusive concentrations of eumelanin. When these concentrations are recombined as an array, a parametric grey scale concentration map is produced. These maps are then analysed by custom algorithms. The melanin-specific images are used to calculate percentage melanin spot area fraction as described previously for calculation of percentage spot area fraction. The melanin content of each image element (pixel) is represented by a grey scale value from 0 to 255. Melanin evenness is calculated as the SD of the mean individual pixel grey scale values over the total skin measurement area. Hence, a smaller SD is indicative of more even pigmentation across the image. The thresholds used to define hyperpigmented spots in both the colour image analysis and the melanin-specific image analysis were identical.

Statistical analysis

The primary outcome measures were the mean changes from baseline in percentage spot area fraction based on analysis of REAL facial images, the VPS grades assigned to REAL images by expert graders, and the percentage melanin spot area fractions and melanin evenness as determined by melanin-specific image analysis. For each of these clinical efficacy variables, the values for the two sides of the face were averaged, and the changes from baseline were analysed with a mixed model (SAS 8.2 Proc Mixed; SAS Institute, Cary, NC, U.S.A.) for repeated measures with the subject effect fitted as random, and the other effects [treatment, side (left vs. right), time (week 4 vs. week 8), treatment-by-time interaction, age, and baseline] fitted as fixed. Baseline was not included in the model for VPS as there were no baseline measures. For spot area fraction analysis, square root transformation was used to satisfy the normality assumption of the analysis model. Pairwise differences between adjusted means were considered significant if $P \leq 0{\cdot}05$ (two-sided). The analyses were performed on all subjects on an intention-to-treat basis.

The sample size of 100 subjects per regimen was based on the assumption of a 0·05 (two-sided) level of significance, an effect size of 0·40% on image analysis of percentage spot area fraction, and > 90% power.

Results

Subject disposition and compliance

In total, 202 subjects were enrolled; 188 completed the study and 14 discontinued early (Fig. 1). On average, the subjects consumed 90–108% of the amount of test formulation expected (Fig. 2). The amount of daytime lotion used was significantly lower in the niacinamide plus NAG group than in the vehicle control group during the period from week 4 to week 8.

Effect on hyperpigmentation

Computer algorithm-based analysis of the REAL facial images revealed that the mean percentage spot area fraction increased from baseline during the study in both groups. However, the increases were smaller in the niacinamide plus NAG group than in the vehicle group at each time point, and these differences were significant at 6 and 8 weeks (Fig. 3). When the values for all time points were incorporated in a repeated measures statistical analysis, the overall change from baseline in mean percentage spot area fraction was significantly lower in the niacinamide plus NAG group (P = 0·031).

Expert VPS grading also indicated that hyperpigmentation worsened from baseline at each time point in both study groups. However, repeated measures analysis demonstrated that the overall mean change from baseline in the expert VPS grade was significantly less in the niacinamide plus NAG group (−0·87) than in the vehicle control group (−1·12). This finding reflected a smaller increase in hyperpigmentation in the niacinamide plus NAG group than that in the vehicle control group (P = 0·041). Example images of subjects showing improvement in the appearance of hyperpigmented spots are displayed in Figure 4.

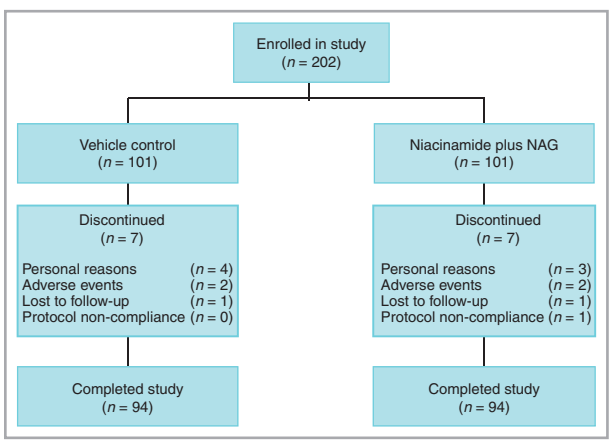

Fig 1. Flow diagram of study subjects. NAG, N-acetyl glucosamine.

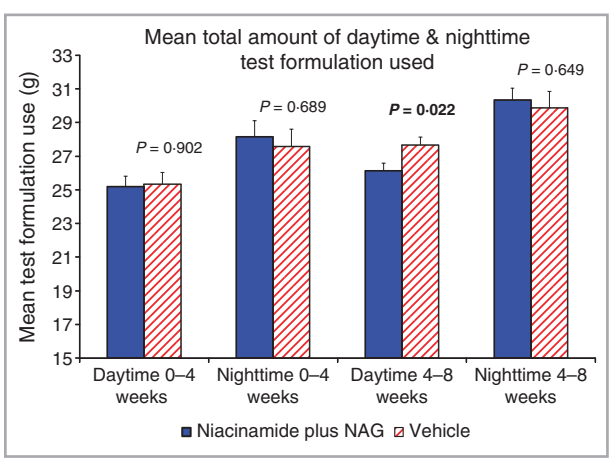

Fig 2. Mean ± SEM total amount of daytime and night-time test formulation used by study subjects from week 0 to week 4 and from week 4 to week 8. NAG, N-acetyl glucosamine.

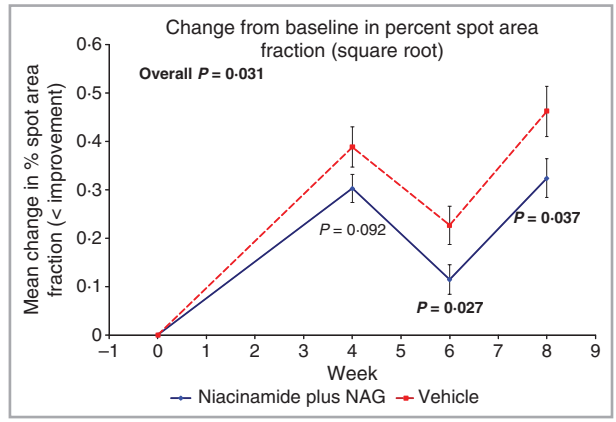

Fig 3. Mean ± SEM changes from baseline in percentage spot area fraction as determined by computer algorithm-based analysis of REAL images for the formulation with niacinamide plus N-acetyl glucosamine (NAG) and the vehicle control. Smaller numbers indicate a smaller increase in the percentage of hyperpigmented spot area. As noted in the text, overall, subjects appeared more hyperpigmented, probably because of the seasonal increase in sun exposure. Use of the combination product attenuated that hyperpigmentation increase.

Effect on melaninization

The results of the melanin-specific image analysis showed that the combination of niacinamide plus NAG also reduced the percentage melanin spot area fraction and increased the evenness of melanin distribution in the skin (Figs 5 and 6). In this analysis, these measures improved with use of the niacinamide plus NAG formulation but worsened with use of the vehicle alone.

Regimen tolerability

Both product regimens were well tolerated by study subjects. Only seven (3·5%) of the 202 subjects reported product-related adverse events. Two subjects in each study group experienced moderate skin irritation reactions that led to with-

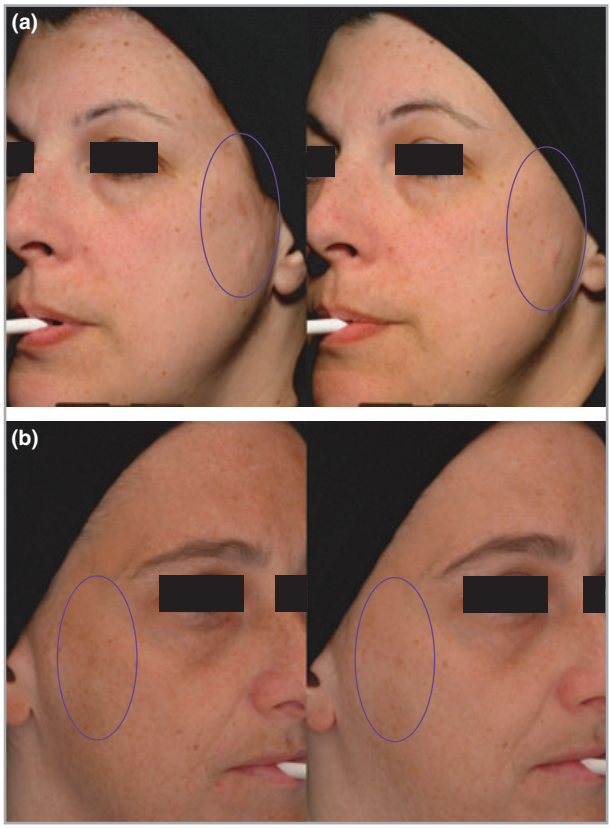

Fig 4. Two sets of images (a, b) illustrating the effect of the formulation with niacinamide plus N-acetyl glucosamine (NAG) regimen on the appearance of hyperpigmented spots. The left-hand images were captured at baseline, and the right-hand images were captured after 8 weeks of the niacinamide plus NAG regimen. (a) Note reduced appearance of hyperpigmented spots in the encircled area in the right-hand after-treatment image. (b) Note reduced appearance of hyperpigmented spots and overall pigmentation in the encircled area in the right-hand after-treatment image.

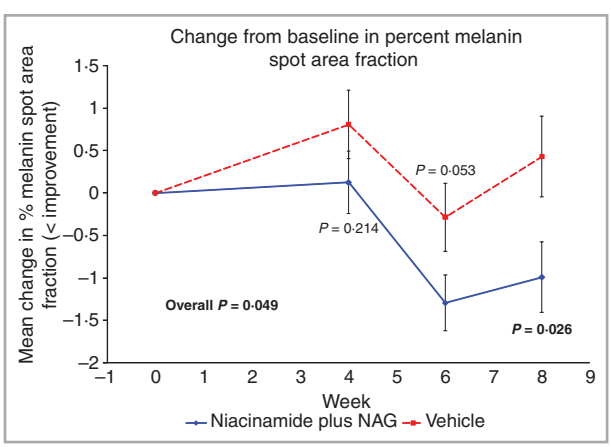

Fig 5. Mean ± SEM change from baseline in percentage melanin spot area fraction determined on the basis of full-face noncontact SIAscopy. Percentage melanin spot area fraction decreased after use of the formulation with niacinamide plus N-acetyl glucosamine (NAG) and increased after use of the vehicle.

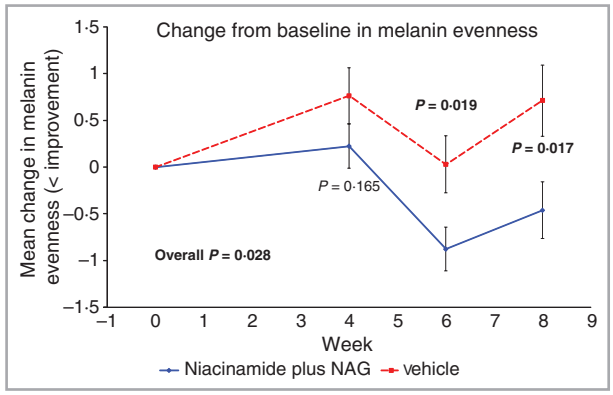

Fig 6. Mean ± SEM change from baseline in melanin evenness determined via SIAscopy. Appearance of melanin evenness increased after use of niacinamide plus N-acetyl glucosamine (NAG) and decreased after use of the vehicle. Lower numbers indicate more evenness of melanin distribution.

drawal from the study. Two subjects in the niacinamide plus NAG group and one in the vehicle control group experienced mild irritation reactions, but these three subjects remained in the study.

Discussion

In this large trial, which used several different imaging tools to measure pigmentation, a formulation combining 4% niacinamide with 2% NAG was consistently more effective than the vehicle in reducing the appearance of hyperpigmentation and was well tolerated. Further, this combination formulation provided an ameliorative effect beyond that achieved with SPF 15 sunscreen. These findings are consistent with those from an earlier 10-week clinical study in 35 white-skinned women treated with 4% niacinamide plus 2% NAG.[17] In both studies, the results of computer analysis of REAL images for hyperpigmented spot area and blinded VPS assessment of the images by expert graders showed that the niacinamide plus NAG formulation was more effective than the vehicle control in reducing hyperpigmentation.

During the course of this study, the percentage spot area fraction increased relative to baseline in both study groups, and expert VPS grading also indicated that hyperpigmentation appearance worsened in general. These results are likely to be due to increased incidental environmental ultraviolet (UV) exposure during the March–May study period, leading to increased pigmentation despite good compliance with daily SPF 15 sunscreen. Data from the U.S. Weather Service collected approximately 100 miles west of the study site confirm that UV radiation, and therefore potential exposure, increased during the study period (Fig. 7a).[21] The diminutions in the changes from baseline in mean percentage spot area fraction, percentage melanin spot area fraction and melanin evenness that occurred at week 6 of the study are likely to be due to a dip in UV radiation during late April and early May, as shown in a more detailed graph of the UV radiation data from the U.S. Weather

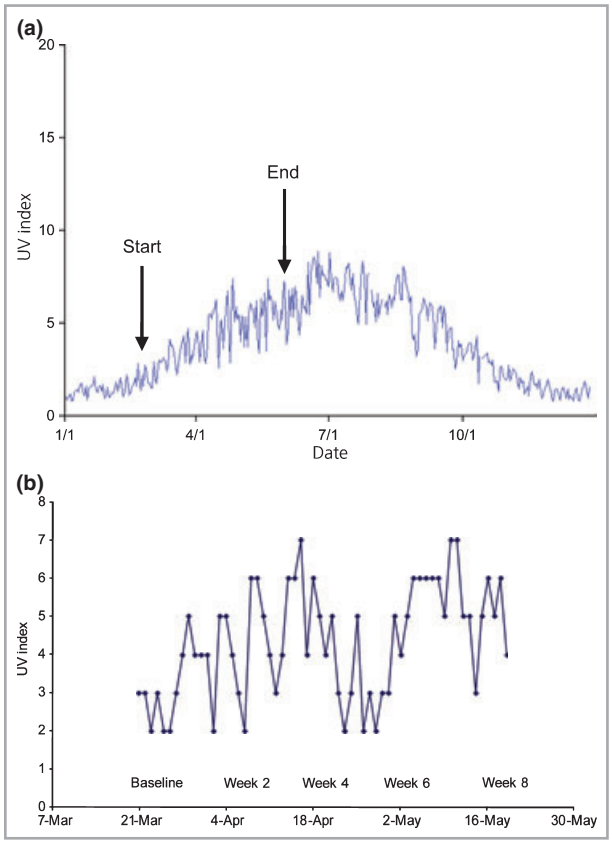

Fig 7. (a) Ultraviolet (UV) index in Indianapolis, IN in 2005. UV radiation increased during the course of the clinical study.[21] Dates are given in the format month/day. (b) UV index in Indianapolis, IN in 2005. UV radiation dipped somewhat in late April and early May (week 4 to week 6 of the study).[22]

Service during this period (Fig. 7b).[22] The effect of waning environmental UV exposure was seen in the earlier small study by Bissett *et al.*, which was conducted in Cincinnati, OH from October to December.[17] In that study, pigmentation was substantially reduced from baseline in both the niacinamide plus NAG and the vehicle control groups. The authors attributed this reduction to seasonal fading of hyperpigmentation during the autumn and winter months. Nevertheless, the formulation containing 4% niacinamide in combination with 2% NAG was significantly more effective than the vehicle in reducing hyperpigmentation appearance in that study as well. Thus, under conditions of both increasing and decreasing background UV intensity, the formulation containing niacinamide plus NAG was significantly more effective than the vehicle in reducing the apparent area of hyperpigmented spots. In addition, the results of the present study show that the NAG plus niacinamide combination formulation augments the effect of sunscreen, enhancing the value of sunscreen alone.

Some of the measurements used here were based on the principles of noncontact SIAscopy™, a recently described method to measure skin melanin content and distribution.[19,20] As noncontact SIAscopy™ is a tool which involves rapid capture of facial chromophore maps, it has potential to be used widely in clinical evaluation. The additional capability of the methodology to differentiate melanin from other skin chromophores (i.e. haemoglobin) provides an even greater potential utility to analyse skin condition and the effects of products on skin. This approach can be particularly important in the evaluation of product-induced appearance changes related to melanin features because it may give different results than traditional colour image analysis, which incorporates all forms of colour including erythema. Increased vascular content associated with hyperpigmentation has been described for melasma,[23] and the same may be the case for hyperpigmented spots, resulting in increased redness in colour images.

In this study, the formulation containing the combination of niacinamide and NAG performed better than the vehicle formulation by all measures, although in the colour image analyses both spot area fraction and skin appearance in both groups worsened from baseline. In contrast, SIAscopy™ showed that both melanin spot area fraction and evenness in the niacinamide plus NAG group improved compared with baseline. This finding supports that colours other than brown, such as red, were affecting the objective and subjective analyses of the colour images and may therefore also be important to address clinically when designing cosmetic products.

In this study, the amount of the daytime lotion applied was greater in the vehicle control group than in the niacinamide plus NAG group between weeks 4 and 8. Because product use was lower in the combination product group, this difference would not be expected to exaggerate the study findings. As this study tested a product with the combination of niacinamide plus NAG, and not the individual components, the effects observed can be attributed only to the combination formulation. The seasonally related changes in hyperpigmentation observed in this study and in the earlier study by Bissett *et al.*[17] underscore the importance of comparisons against a vehicle control, as well as a baseline measurement, in studies assessing the effect of skincare products. Inclusion of dates and location in reports of these studies is also important as seasonal changes appear to influence the effect of treatment.

In summary, a formulation containing the combination of 4% niacinamide plus 2% NAG reduced the appearance of irregular pigmentation including hypermelaninization, providing an effect beyond that achieved with SPF 15 sunscreen. This study demonstrates the importance of careful selection of ingredients when formulating cosmetic products to improve the appearance of facial pigmentation. As new regulations affect the current armamentarium available to approach this problem (e.g. proposed change in over-the-counter status of hydroquinone), this well-tolerated combination of niacinamide plus NAG, which also provides some hydration and wrinkle appearance improvement effects,[24,25] has broad potential applicability.

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