International Journal of Cosmetic Science, 2004, **26**, 231–238

Topical niacinamide reduces yellowing, wrinkling, red blotchiness, and hyperpigmented spots in aging facial skin[1]

D. L. Bissett, K. Miyamoto, P. Sun, J. Li and C. A. Berge
The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH, U.S.A.

Received 6 May 2004, Accepted 9 June 2004

Keywords: aging, niacinamide, sallowness, wrinkling, yellowing

Synopsis

Previous clinical testing of topical niacinamide (vitamin B3) has revealed a broad array of improvements in the appearance of aging facial skin. The study reported here was done to confirm some of those previous observations and to evaluate additional end points such as skin anti-yellowing. Caucasian female subjects ($n = 50$, aged 40–60 years) participated in a 12-week, double-blind, placebo-controlled, split-face, left–right randomized clinical study assessing two topical products: moisturizer control product versus the same moisturizer product containing 5% niacinamide. Niacinamide was well tolerated by the skin and provided significant improvements versus control in end points evaluated previously: fine lines/wrinkles, hyperpigmentation spots, texture, and red blotchiness. In addition, skin yellowing (sallowness) versus control was significantly improved. The mechanism by which this array of benefits is achieved with niacinamide is discussed.

Correspondence: Donald L. Bissett, The Procter & Gamble Company, Miami Valley Laboratories, 11810 East Miami River Road, Cincinnati, OH 45252, U.S.A. Tel.: +1 513 627 2471; fax: +1 513 627 0139; e-mail: bissett.dl@pg.com

[1]This work was presented in part at the International Academy of Cosmetic Dermatology (IACD) meeting in Beijing, China (December 2003).

Résumé

Un précédent test clinique portant sur l'application topique de Niacinamide (Vitamine B3) a révélé le large potentiel de cette matière première pour améliorer l'aspect du visage. La présente étude a pour but de confirmer quelques-unes des observations déjà réalisées ainsi que d'évaluer d'autres propriétés comme l'anti-jaunissement de la peau. Des femmes de type caucasien ($n = 50$, d'âge compris entre 40 et 60 ans) ont participé pendant 12 semaines à une étude clinique contrôlée, portant sur l'application de deux produits en double aveugle, par demi-visage, aléatoirement répartis à droite ou à gauche. Le premier produit, ou témoin, était un soin hydratant, le second était le même produit, contenant 5% de Niacinamide. Le niacinamide n'a provoqué aucune intolérance cutanée et des améliorations significatives par rapport au témoin ont été observées sur les paramètres évalués: rides, ridules, tâches pigmentaires, texture de la peau, rougeur cutanée. De plus, le jaunissement de la peau par rapport au témoin a été significativement amélioré. Le mécanisme par lequel la Niacinamide agit sur ces différents paramètres est discuté.

Introduction

The nutritional value of niacinamide (vitamin B3) is well recognized, and its utility as a topical agent to provide skin-care benefits is being elucidated based on recently published studies. There are reports of topical niacinamide providing beneficial

effects in prevention of photoimmunosuppression and photocarcinogenesis [1], prevention of the loss of dermal collagen that accompanies photoaging [2], reduction in acne severity [3, 4], and improvement in bullous pemphigoid [5]. More recent studies [6–8] have noted additional beneficial effects of topical niacinamide in aging skin, such as improved barrier function, decreased appearance of signs of facial photoaging (e.g. texture, hyperpigmented spots, red blotchiness), and reduced sebum production. Some mechanistic effects were also noted in those studies to suggest how niacinamide provides this array of observed skin benefits.

The physiologic role of niacinamide is as a precursor to important co-factors: nicotinamide adenine dinucleotide (NAD) and its phosphate derivative (NADP). These cofactors and their reduced forms (NADH and NADPH) serve as redox co-enzymes in many enzymatic reactions [9], and the reduced forms are anti-oxidants [10, 11] and have other signaling properties [12]. Thus, it is possible that niacinamide has these multiple effects on skin indirectly as a result of its role as a co-enzyme precursor, although defining specifically how these dinucleotides fit mechanistically into all the observed skin effects is not clearly defined.

Since NADH and NADPH are anti-oxidants and their levels can be increased with niacinamide [9], a possible effect of topical niacinamide is inhibition of oxidative processes such as protein oxidation (glycation). Glycation (Maillard reaction) is a spontaneous oxidative reaction between protein and sugar [13–15], resulting in cross-linked proteins (Amedori products) that are yellowish-brown in color and are fluorescent. These products can accumulate in matrix components such as collagen that have long biological half-lives. For example, published data indicate a fivefold increase in collagen oxidation products in human skin from age 20 to 80 [16] and in glycation products and accompanying fluorescence in aging rat skin collagen [17]. An 'experiment of nature' that illustrates the impact of glycation on the appearance of skin is diabetes, where sugar levels are elevated. This leads to increased glycation and visibly more yellow appearance described as 'yellow skin syndrome' and 'yellow nail syndrome' [18–22]. There is thus potential for glycation to have a significant role in the normal aging-induced changes in skin appearance (e.g. yellowing of skin, also more technically described as skin sallowness) and for niacinamide to inhibit the appearance change.

This report presents data on clinical effects (e.g. skin anti-yellowing) not previously reported for topical niacinamide and also presents data confirming previous benefit observations of this topical vitamin. Mechanistic information relevant to these effects is also presented.

Materials and methods

Clinical testing

Before participating in the clinical study, each subject signed a written informed consent that contained all the basic elements outlined in 21 Code of Federal Regulations (CFR) 50.25. It explained the type of study, the procedures to be followed, the general nature of the materials being tested, and any known or anticipated adverse reactions that might result from participation. Due to the cosmetic nature of this study, a formal external IRB or ethics committee review was not done. However, the protocol was reviewed and approved by qualified Procter & Gamble clinical, toxicology, and regulatory personnel and by corresponding personnel at the clinical site. The study was monitored for compliance with the protocol.

Facial aging study

Healthy Caucasian female subjects (age: 40–60 years; $n = 50$) were enrolled in a double-blind, placebo-controlled, split-face study with left–right randomization. Forty-nine of the enrolled subjects completed the study, with the one drop being for personal reasons (unrelated to the study treatments). All subjects were graded at baseline (0–5 grading scales: 0 being normal skin), and were eligible for study participation with grades of 2.0 or greater in both facial fine lines/wrinkles (primarily in the eye or 'crow's feet' area), texture (in the cheek area), and facial hyperpigmented spots. Our definition of poor texture encompasses two factors: enlarged pore size and 'pebbly, rippled' appearance of skin in the cheek area.

Prior to study start, there was a 2-week washout period in which subjects were instructed to discontinue use of their normal facial cleanser and moisturizer products for the duration of the study, and all were instructed to use the same supplied facial cleanser commercial product and the same

facial oil-in-water moisturizer commercial product twice daily. After the 2-week wash-out period, this cleanser product was also used throughout the subsequent 12-week study period, but the moisturizer product was replaced with two oil-in-water moisturizer test formulations (placebo control and the same formulation containing 5% niacinamide) which were packaged in blind-coded 30-g opaque tubes, labeled 'left' or 'right'. To each side of the face was applied a pea-sized amount (approximately 0.4 g) of each assigned test formulation, twice daily for 12 weeks, with the evening application occurring at least 1 h before bedtime. Subjects were supplied with new containers of test formulations at baseline and at weeks 4 and 8 during the 12-week study. Subject compliance with instructions was performed by having subjects complete a daily product use diary, in a return visit to the study site after 1 week of test product usage to review the diary and their product use habits, and by weighing the returned product containers (at weeks 4, 8, and 12). These compliance checks indicated subjects were following product use instructions. This study was conducted from February to May in Cincinnati.

All skin measurements were done on untreated skin (skin was not treated with test products that day) at least 30 min after washing with the assigned facial cleanser commercial product. Subjects acclimated their skin in a controlled temperature (21 ± 2°C) and relative humidity (30–50%) room for 30 min prior to measurements (taken under the same temperature and humidity conditions). All measurements were done in the morning, with each subject having an assigned time of day to return to the clinical site for all visits. They had their hair and clothing covered with black drapes. The images were taken using the same imaging equipment under the same conditions (lighting, distance, head position, etc.) at all time points.

Digital images of each side of the face of all subjects were captured at baseline and at weeks 4, 8, and 12. The facial images were recorded using a Wrinkle Imaging System (WIS) and Facial Color Imaging System (FaCIS). WIS (Fig. 1) captures subject facial images with a high-resolution video camera (Sony DXH-537H 3 CCD video camera equipped with a Canon J15 × 9.5 BKRS lens) under highly controlled reproducible lighting (quartz tungsten halogen light source located above the head) and facial positioning conditions. It involves reflected illumination using reflection boards to create shadows to enhance topographical features (fine lines and wrinkles). Calibration was performed at the start of each day of imaging in a study. The subject's head was positioned using a chin rest and bite stick, and re-positioning was done by displaying the baseline image on a monitor while simultaneously superimposing the subject's live image on the monitor to adjust the subject's head position to exactly match the base-

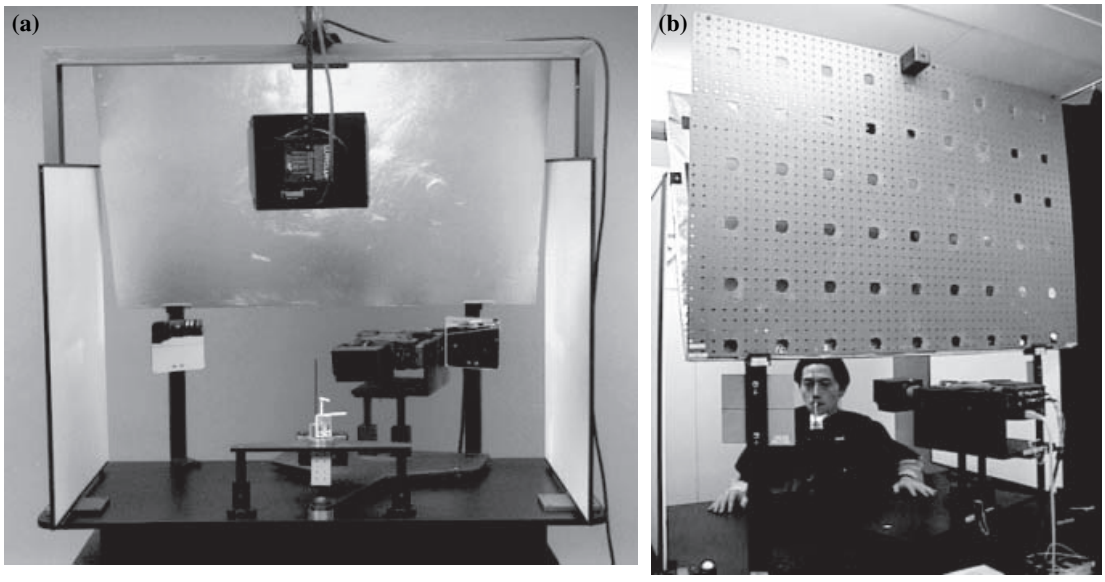

Figure 1 Views of Wrinkle Imaging System. (A) front view and (B) back view.

line positioning. Then the image for that subsequent time point is captured. The mounted camera and illumination can be rotated 45° left or right of the subject's head to image capture the right or left side, respectively, of the face. The FaCIS for capturing color images has been described elsewhere [23, 24]. After each imaging session, subjects resumed test product application.

In the captured images, the facial region of interest for analysis is defined and analyzed to quantify the parameter of interest. Using non-commercial algorithms (developed within Procter & Gamble) for the image analysis, total linear (fine line/wrinkle) depression area in mm^2 around the eye (crow's feet area), total hyperpigmented spot area in mm^2, and skin yellowness (b values, excluding the area of hyperpigmented spots) are determined. The wrinkle image analysis process is overviewed in Fig. 2. As noted above, the color image analysis process has been described elsewhere [23, 24].

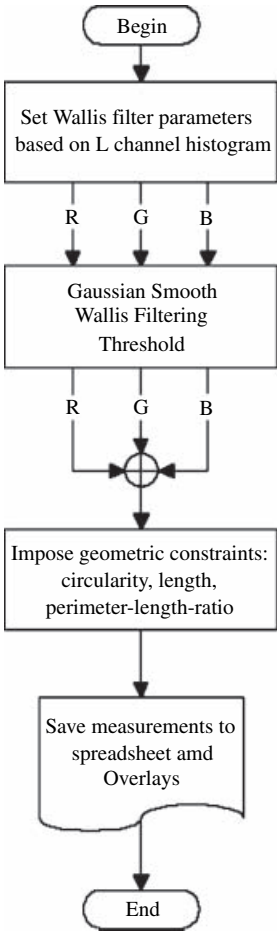

Figure 2 Overview of wrinkle image analysis algorithm.

For red blotchiness, test product effects were measured by a Visual Perception Study (VPS), wherein expert graders assessed the FaCIS facial images photos for several aging skin attributes. Trained and qualified graders graded the CIS images. Blind-coded baseline and either 4, 8 or 12 week color images were viewed simultaneously on color-calibrated Barco monitors, randomized as to treatment and side of screen. Graders determined which side looked better for a specific skin attribute and how much better (0–100 scale). Graders had the option of selecting the left-hand image, the right-hand image, or no difference. Three graders independently judged the images. The three grades for each image pair were averaged.

Statistical analysis

Change from baseline values for fine lines/wrinkles, hyperpigmented spots, and texture were analyzed using a mixed model (SAS Proc Mixed) with subject (random), treatment, side (left and right), and baseline as covariate for each post-treatment time point (weeks 4, 8, and 12). Red blotchiness was analyzed the same way except that baseline was not included in the model due to no baseline values (Visual Perception Systems comparisons versus baseline). Differences between adjusted treatment means were considered significant if the P-value was less than or equal to 0.05. Sample sizes of up to 50 were historically used for this type of facial benefits studies with sufficient power. Error bars presented in the figures represent one standard error above or below the treatment means.

Results

Skin improvement effects confirming previous observations

As in previous facial testing [7], in the present study topical 5% niacinamide was found to be extremely well tolerated by test subjects, i.e. it does not induce skin irritation responses (no redness, dryness, burn, sting, or itch responses). In the present study, there was an increase in red blotchiness (Fig. 3) and in hyperpigmented spots (Fig. 4) in both the control and niacinamide treated skin.

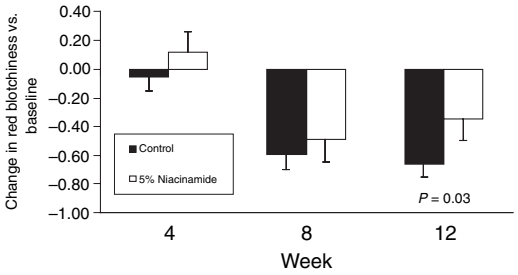

Figure 3 Topical 5% niacinamide prevented an increase in facial skin red blotchiness vs. the placebo control. The data (mean ± SE) are presented as VPS (Visual Perception System) scores, with higher values indicating less red blotchiness. VPS data were obtained by treatment-blinded expert grader assessment of facial images (image at each time point versus the baseline image).

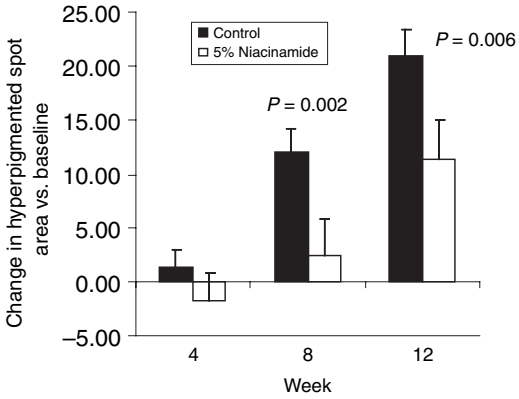

Figure 4 Topical 5% niacinamide prevented an increase in hyperpigmented spot area (mm^2) in facial skin versus the placebo control. The data (mean ± SE) are presented as area change from baseline (the average baseline spot area for the study was 183 mm^2), with lower values indicating less total spot area. Spot area data were obtained from quantitative computer image analysis.

However, niacinamide significantly prevented the increase versus the control. Such effects on these two parameters are consistent with previous observations [6–9].

Additional skin improvement effects in the present study

In addition to the aging skin benefits discussed above, in the present study there were improvements in other aging skin end points. After 8 weeks of treatment, there was a small but significant reduction in fine lines/wrinkles (Fig. 5). By 12 weeks of treatment, the reduction versus control was approximately 5.5%.

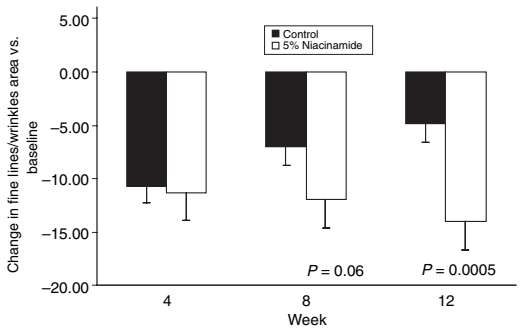

Figure 5 Topical 5% niacinamide reduced facial skin fine lines/wrinkles (measured as linear depression area in mm^2) versus the placebo control. The data (mean ± SE) are presented as change from baseline (the average baseline area for the study was 181 mm^2), with lower values indicating less fine lines/wrinkles. The area data were obtained from quantitative computer image analysis.

An additional assessment from clinical testing of topical niacinamide was determination of the effect on skin yellowing (sallowness). Yellow color analysis ('*b*' value from image analysis) of the clinical facial images revealed that niacinamide was significantly effective in preventing an increase in skin yellow color at the 8 and 12-week time points (Fig. 6).

Discussion

As overviewed above, topical niacinamide (vitamin B3) provides a variety of beneficial effects to skin,

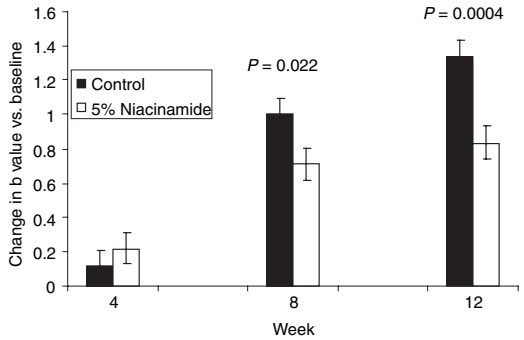

Figure 6 Topical 5% niacinamide prevents skin yellowing (quantitative '*b*' value determination from images) versus placebo control. The data (mean ± SE) are presented as change from baseline, with lower values indicating less yellow color.

such as improvement in the appearance of facial skin texture, fine lines/wrinkles, hyperpigmentation, red blotchiness, and yellowing (sallowness). In addition, the treatment is extremely well tolerated by the skin (no irritation, redness, burn, sting, itch issues). This is in contrast to other topical technology such as *trans*-retinoic acid (tretinoin) which provides appearance improvements (particularly wrinkles and hyperpigmentation) but at the expense of barrier, leading to skin sensitivity and redness [25]. Since niacinamide is non-irritating to facial skin, easily formulated, chemically stable, and compatible with other formulation components, it is an ideal agent for use in cosmetic products. And in particular, since it improves the appearance of both hyperpigmentation and yellowing without inducing irritation, it is especially useful for providing overall skin tone improvement.

In this study, some parameters (e.g. hyperpigmented spots and red blotchiness) increased from baseline in both treatment groups during the course of the study. This study was conducted between February and May, a time period in which there is increasing sun exposure potential. Since these parameters are increased by sun exposure, it is anticipated that the measured values would increase. Topical 5% niacinamide was effective in preventing the seasonal-induced increase in these parameters. From previous testing in fall-winter [6–9] where there is no increase from baseline in these parameters in the control group, we have observed that topical niacinamide will also reduce existing skin redness and spots.

The mechanisms by which niacinamide provides this array of skin benefits are not completely defined, but in general may be via niacinamide's role as a precursor to the NAD(P) family of coenzymes. These coenzymes are key to many metabolic enzyme reactions in the skin [9], and the reduced forms [NAD(P)H] also have anti-oxidant properties [10, 11]. Niacinamide as a precursor has been shown to increase NADPH levels in aged skin cells [7]. There is thus potential to impact many processes in the skin. While this coenzyme precursor role for niacinamide may explain in general how it can have multiple effects on clinical appearance and function of skin, the precise mechanism by which NAD(P)H is involved in pathways relevant to these clinical end points has not been definitively defined. However, there is some mechanistic information relevant to several skin effects.

For skin yellowing which is at least in part because of the oxidative protein glycation process (discussed in the Introduction section), a niacinamide-induced elevation of the endogenous antioxidant NAD(P)H level would be expected to modulate the yellowing phenomenon. An anti-glycation effect for niacinamide has been reported [26, 27].

The mechanism by which red blotchiness is improved may be related to barrier function. Niacinamide increases both the lipid and protein stratum corneum components of the skin's barrier and enhances the skin's barrier properties [7, 8, 28]. This enhancement is observed by both reduction in *trans*-epidermal water loss (TEWL) and increased resistance of the skin to damage from barrier destructive agents such as surfactant and solvent [7]. This should translate to less irritation and redness when the skin encounters environmental insults such as detergents and soaps.

For fine lines/wrinkles, a couple of mechanisms may be involved. One is increased dermal matrix collagen production. The literature discusses this mechanism in regard to topical retinoic acid [29], which is well recognized as providing skin wrinkle improvement [30]. Some previous work [9] has indicated that niacinamide does increase collagen production.

Another mechanism relevant to wrinkle reduction is reduction in the excess dermal glycosaminoglycans (GAG's). Elevation of dermal GAG's is a characteristic of photodamaged or wrinkled skin [31]. While a low level of GAG is required for normal structure and function of the dermal matrix, excess levels are associated with poor visible appearance of skin, e.g. the wrinkled skin of Shar Pei dogs is the result of excess dermal GAG [32]. In clinical testing with chemical peel [33] and in mice treated topically with *trans*-retinoic acid [34], reduction in wrinkles is also associated with reduction in excess dermal GAG content. Other testing [35] has indicated that niacinamide reduces excess GAG's production by old human dermal fibroblasts, thus supporting the potential involvement of this mechanism in fine line and wrinkles appearance effects.

For pigment spot reduction, niacinamide has been observed to inhibit the process of melanosome transfer from melanocytes to keratinocytes [6]. This process involves a number of steps [36], and the specific site of action of niacinamide has not yet been elucidated.

References

1. Gensler, H.L. Prevention of photoimmunesuppression and photocarcinogenesis by topical niacinamide. *Nutr. Cancer* **29**, 157–162 (1997).
2. Yu, J.-M., Liu, Y., Xie, N. *et al.* Effects of nicotinamide on levels of dermis hydroxyproline in photoaging skin. *Huanjing Yu Jiankang Zazhi* **19**, 102–104 (2002).
3. Shalita, A.R., Smith, J.G., Parish, L.C., Sofman, M.S. and Chalker, D.K. Topical nicotinamide compared with clindamycin gel in the treatment of inflammatory acne vulgaris. *Int. J. Dermatol.* **34**, 434–437 (1995).
4. Griffiths, C.E.M. Nicotinamide 4% gel for the treatment of inflammatory acne vulgaris. *J. Dermatol. Treat.* **6S**, 8–10 (1995).
5. Berk, M.A. and Lorincz, A.L. The treatment of bullous pemphigoid with tetracycline and niacinamide: a preliminary report. *Arch. Dermatol.* **12**, 670–674 (1986).
6. Hakozaki, T., Minwalla, L., Zhuang, J. *et al.* The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer. *Br. J. Dermatol.* **147**, 22–33 (2002).
7. Bissett, D.L., Oblong, J.E., Saud, A. *et al.* Topical niacinamide provides skin aging appearance benefits while enhancing barrier function. *J. Clin. Dermatol.* **32S**, 9–18 (2003).
8. Bissett, D.L. Topical niacinamide and barrier enhancement. *Cutis* **70S**, 8–12 (2002).
9. Matts, P.J., Oblong, J.E. and Bissett, D.L. A review of the range of effects of niacinamide in human skin. *Intl. Fed. Soc. Cosmet. Chem. Magazine* **5**, 285–289 (2002).
10. Wozniacka, A., Sysa-Jedrzejowska, A., Adamus, J. and Gebicki, J. Topical application of NADH for the treatment of rosacea and contact dermatitis. *Clin. Exp. Dermatol.* **28**, 61–63 (2003).
11. Shindo, Y., Witt, E., Han, D., Epstein, W. and Packer, L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J. Invest. Dermatol.* **102**, 122–124 (1994).
12. Berger, F., Ramirez-Hernandez, M.H., and Ziegler, M. The new life of a centenarian: signaling functions of NAD(P). *Trends Biochem. Sci.* **29**, 111–118 (2004).
13. Vlassara, H. Recent progress on the biologic and clinical significance of advanced glycosylation end products. *J. Lab. Clin. Med.* **124**, 19–30 (1994).
14. Wolff, S.P., Jiang, Z.Y. and Hunt, J.V. Protein glycation and oxidative stress in diabetes mellitus and aging. *Free Rad. Biol. Med.* **10**, 339–352 (1991).
15. Wu, J.T. Advanced glycosylation end products: a new disease marker for diabetes and aging. *J. Clin. Lab. Anal.* **7**, 252–255 (1993).
16. Dyer, D.G., Dunn, J.A., Thorpe, S.R. *et al.* Accumulation of maillard reaction products in skin collagen in diabetes and aging. *J. Clin. Invest.* **91**, 2463–2469 (1993).
17. Odetti, P., Pronzato, M.A., Noberasco, G. *et al.* Relationships between glycation and oxidation related fluorescence in rat collagen during aging. *Lab. Invest.* **70**, 61–67 (1994).
18. Sueki, H., Nozaki, S., Numazawa, S. *et al.* Effect of non-enzymatic glycosylation and heating on browning of human stratum corneum and nail. *Dermatologica* **183**, 197–202 (1991).
19. Prasad, T.R. Yellow nail syndrome. *Chest* **77**, 580 (1980).
20. Huntley, A.C. Cutaneous manifestations of diabetes mellitus. *Diabetes Metab. Rev.* **9**, 161–176 (1993).
21. Nozaki, S., Sueki, H., Fujisawa, R., Aoki, K. and Kuroiwa, Y. Glycosylated proteins of stratum corneum, nail, and hair in diabetes mellitus: correlation with cutaneous manifestations. *J. Dermatol.* **15**, 320–324 (1988).
22. Nelson, L.M. Yellow nail syndrome. *Arch. Dermatol.* **10**, 499–500 (1969).
23. Miyamoto, K., Takiwaki, H., Hillebrand, G.G. and Arase, S. Development of a digital imaging system for objective measurement of hyperpigmented spots on the face. *Skin Res. Technol.* **8**, 227–235 (2002).
24. Miyamoto, K., Takiwaki, H., Hillebrand, G.G. and Arase, S. Utilization of a high-resolution digital imaging system for the objective and quantitative assessment of hyperpigmented spots on the face. *Skin Res. Technol.* **8**, 73–77 (2002).
25. Tanno, O., Ota, Y., Kitamura, N., Katsube, T. and Inoue, S. Nicotinamide increases biosynthesis of ceramides as well as other stratum corneum lipids to improve the epidermal permeability barrier. *Br. J. Dermatol.* **143**, 525–531 (2000).
26. Griffiths, C.E. and Voorhees, J.J.T. Topical retinoic acid for photoaging: clinical response and underlying mechanisms. *Skin. Pharmacol.* **6S**, 70–77 (1993).
27. Weiss, J.S., Ellis, C.N., Headington, J.T. and Voorhees, J.J. Topical tretinoin in the treatment of aging skin. *J. Am. Acad. Dermatol.* **19**, 169–175 (1988).
28. Gonzalez, S., Moran, M. and Kochevar, I.E. Chronic photodamage in skin of mast cell-deficient mice. *Photochem. Photobiol.* **70**, 248–253 (1999).
29. Dunstan, R.W. and Kennis, R.A. Selected heritable diseases of domesticated animals. In: *Handbook of Mouse Mutations with Skin and Hair Abnormalities* (Sundberg, J.P., ed.), pp. 509–532. CRC Press, Boca Raton (1994).
30. Kligman, A.M., Baker, T.J. and Gordan, H.L. Long-term histologic follow-up of phenol face peels. *Plast. Recon. Surg.* **75**, 652–659 (1975).
31. Schwartz, E. and Kligman, L.H. Topical tretinoin increases the tropoelastin and fibronectin content of

photoaged hairless mouse skin. *J. Invest. Dermatol.* **104**, 518–522 (1995).
32. Bissett, D.L., Oblong, J.E. and Berge, C.A. Niacinamide: a B vitamin that improves aging facial skin appearance. *J. Dermatol. Surg.* in press (2004).
33. Reber, F., Geffarth, R., Kasper, M. *et al.* Graded sensitiveness of the various retinal neuron populations on the glyoxal-mediated formation of advanced glycation end products and ways of protection. *Graefes Arch. Clin. Exp. Ophthalmol.* **241**, 213–225 (2003).
34. Thornalley, P.J. Glycation in diabetic neuropathy: characteristics, consequences, causes, and therapeutic options. *Int. Rev. Neurobiol.* **50**, 37–57 (2002).
35. Kang, S., Duell, E.A., Fisher, G.J. *et al.* Application of retinol to human skin *in vivo* induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels of irritation. *J. Invest. Dermatol.* **105**, 549–556 (1995).
36. Boissy, R.E. Melanosome transfer to and translocation in the keratinocyte. *Exp. Dermatol.* **12**(S2), 5–12 (2003).