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sensitive skin

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A fundamental investigation into aspects of the physiology and biochemistry of the stratum corneum in subjects with sensitive skin

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Abstract

BACKGROUND: Sensitive skin is a poorly understood skin condition. Defects in stratum corneum (SC) barrier function and/or extrasensory neuronal networks in the epidermis are believed to be involved in the problem.

OBJECTIVES: This study aimed to unravel the relationships between bleomycin hydrolase (BH) and calpain-1 (C-1), pyrrolidone carboxylic acid (PCA) levels, corneocyte maturation, transglutaminase (TG) and plasmin activities on the cheeks of subjects with sensitive skin.

METHODS: Forty-eight female Caucasian subjects, Fitzpatrick skin phototypes II–III, with self-perceived sensitive facial skin, were assessed and underwent a capsaicin reactivity test. Expert grading of skin condition was conducted as well as the measurement of transepidermal water loss (TEWL), skin capacitance, SC cohesion and SC integrity. BH, C-1 and plasmin activities were measured as well as PCA levels, plasmin and TG activity. Differential Nile red and involucrin immunostaining was performed to assess corneocyte maturation and size.

RESULTS: About 52% of the subjects reacted to capsaicin. There were no significant differences between the capsaicin-sensitive and non-capsaicin-sensitive subjects with reference to skin grading, TEWL, skin capacitance and SC cohesion. PCA levels and BH activity were lowest in the capsaicin-sensitive panel ($P < 0.05$) and were correlated in non-capsaicin-sensitive subjects ($r = 0.72$). The activity of TG was significantly lower (48%) in the capsaicin-sensitive subjects ($P < 0.001$) and their corneocytes were less mature and smaller ($P \leq 0.05$). SC was estimated to be thinner (6.87 ± 0.28 vs. $8.68 \pm 0.26 \mu\text{m}$; $P = 0.001$) in the capsaicin-sensitive subjects with a corresponding shorter SC path length ($83.2 \pm 4.4 \mu\text{m}$ and $113.1 \pm 4.5 \mu\text{m}$; $P = 0.001$).

CONCLUSIONS: Despite the physiological similarities between the two groups of sensitive skin subjects, differences in their biochemistry were clearly evident. Lower levels of PCA, BH and TG activities together with a greater number of smaller and immature corneocytes indicate inferior SC maturation in the capsaicin-sensitive subjects. The reduced maturation of corneocytes and thinner

SC likely contributes to a greater penetration of capsaicin and the associated increased skin sensitivity.

Résumé

CONTEXTE: La peau sensible est une condition de la peau mal comprise. Des défauts dans la fonction de barrière de la couche cornée (SC) et / ou dans les réseaux neuronaux extrasensoriels dans l'épiderme sont soupçonnés d'être impliqués dans le problème.

OBJECTIFS: Cette étude visait à démêler les relations entre bléomycine hydrolase (BH) et calpaïne-1 (C-1), du taux d'acide carboxylique (PCA) pyrrolidone, de la maturation des cornéocytes, de transglutaminase (TG) et des activités de la plasmin sur les joues de sujets avec peaux sensibles.

METHODES: Quarante-huit sujets caucasiens féminins, avec une peau de phototype Fitzpatrick II-III, avec autoperception d'une peau sensible au niveau du visage ont été évalués et ont subi un test de réactivité à la capsaïcine. Un classement Expert de l'état de la peau a été réalisé ainsi que la mesure de la perte d'eau transépidermique (TEWL/PIE), de la capacité de la peau, de la cohésion et de l'intégrité du SC. BH, C-1 et les activités de plasmin ont été mesurées, ainsi que les taux de l'APC, de plasmin et de l'activité TG. La coloration différentielle au Nil rouge et bar immunocoloration de l'involucrine a été réalisée pour évaluer la maturation et la taille descornéocytes.

RESULTATS: 52% des sujets ont réagi à la capsaïcine. Il n'y avait pas de différences significatives entre les sujets sensibles à la capsaïcine et non sensibles à la capsaïcine par rapport au classement de la peau, de la PIE, de la capacité de la peau et de la cohésion de SC. Les taux de l'APC et de l'activité BH étaient les plus bas dans le panel des personnes sensibles à la capsaïcine ($p < 0.05$) et ont été corrélés chez les sujets non-sensibles à la capsaïcine ($r = 0.72$). L'activité de TG était significativement plus faible (48%) chez les sujets sensibles à la capsaïcine ($p < 0.001$) et leurs cornéocytes étaient moins matures et plus petits ($p \leq 0,03$). Le SC a été à trouvé être plus mince ($6,87 \pm 0,28$ vs $8,68 \pm 0,26 \mu\text{m}$; $p = 0,001$) chez les sujets sensibles à la capsaïcine avec une longueur de trajet de SC plus courte correspondant ($83,2 \pm 4,4 \mu\text{m}$ et $113,1 \pm 4,5 \mu\text{m}$; $p = 0,001$).

CONCLUSION: Malgré les similitudes physiologiques entre les deux groupes de sujets à peaux sensibles, les différences dans leur biochimie étaient clairement évidentes. Les taux plus bas d'activités PCA, BH et TG ainsi qu'un plus grand nombre de cornéocytes petits et immatures indiquent une inférieure maturation du SC chez les

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sujets sensibles à la capsaïcine. La maturation réduite de cornéocytes et le SC plus mince contribuent probablement à une plus grande pénétration de la capsaïcine et à la sensibilité associée accrue de la peau.

Introduction

Subjects with sensitive skin experience a group of interrelated symptoms including hyper-reactive and irritable skin caused by physical, chemical, psychological or hormonal influences leading to symptoms of skin tightness, redness, burning or tingling sensations [1–3]. This rather ungrouped set of skin events, occurring in the absence of any clinical or histological evidence of skin lesions, are collectively described as 'sensitive skin syndrome' [4]. Studies conducted in recent years have shown that well over half of the population globally declare themselves to have sensitive skin [2, 5–13].

Various environmental factors such as UV radiation, extreme temperature, air pollution or climate have also been suggested to contribute towards the onset of sensitive skin [4, 7]. The low temperature and humidity in winter is also a predisposing factor compared with summer [14] although one study suggests that sensitive skin is more prevalent in summer [15]. Higher skin reactivity can be related to dermatological diseases such as eczema [16], and lower skin reactivity can be associated with subclinical sensitivity [17]. The diversity in methodological approaches employed in the various studies may have contributed to the lack of understanding of these conditions. There are many conflicting studies on sensitive skin with regard to gender differences. Some studies have shown that females are more reactive to skin sensitization than males [18], but other reports suggest no gender differences [19, 20]. However, changes in physiological responses during the menstrual cycle have been associated with variation in skin sensitivity [3, 21].

Ethnic variation in skin sensitivity is also reported [2, 5–13, 22–24] but this may be influenced by geographical location, sun exposure, the season of the year and cultural differences. However, despite similarities in perception of sensitive skin, Asians are reported to be more sensitive to stinging tests than Caucasians [25]. Lee *et al.* [26] also found regional and seasonal differences in the sensitivity of Asian subjects to 0.5% sodium lauryl sulphate (SLS), retinol, lactic and capsaicin. In addition, subjects living in China have been reported to have different skin reactivity depending upon their precise geographical location [27].

Sensitive skin symptoms are believed to be linked to hyperactive or dysfunctional sensory neurons, and as such, skin C-fibres possess sensory neuroreceptors such as the transient receptor potential (TRP) family of receptors that are thought to induce pain, burning and itching. In particular, the polymodal ion channel TRPV1 receptor (vanilloid receptor, VR1) is expressed on C-fibres and activated by low pH, noxious heat (>43°C), phorbol esters, anandamide, leukotriene B4 and capsaicin [28]. As a result, a topical capsaicin test can be used for the diagnosis of sensitive skin [reviewed in 13].

Although sensitive skin is linked with sensory perception through specific C-receptors in human skin, the evidence for increased epidermal nerve density in subjects with pruritus is limited [28]. Indeed, Buhe *et al.* [29] found the opposite demonstrating that the intra-epidermal c-fibre density is lower in subjects with sensitive skin. However, the skin biopsies were taken from the subjects' neck area. In contrast, Ehnis-Perez *et al.* [30] found an increased expression of the transient receptor potential vanilloid 1

from the nasolabial fold in subjects with sensitive skin. Increased prostaglandin E-2 levels have also been reported [31].

Several studies have also investigated some of the physiological, biochemical and cellular changes in sensitive skin [22]. Compromised barrier function is believed to be one of the factors that facilitates the onset of skin sensitivity [20, 32–37]. In this respect, some studies have suggested that a thinner SC with smaller corneocytes, [38–40] and reduced levels of SC ceramides [41] lead to impaired barrier function in sensitive skin. Most recently, differences in corneodesmosome functionality have been suggested [37], which may be related to the poor SC integrity known to occur in subjects with sensitive skin [3].

Increased SC protease activity can be associated with a thinner SC especially increased plasmin activities [42]. Increased activities of plasmin, as well as other serine proteases, have been shown to correlate with increasing transepidermal water loss (TEWL) values [43]. Nevertheless, the activities of plasmin in subjects with sensitive skin have not been examined.

Corneocyte envelope (CE) maturity bears some relationship with corneocyte size, but this parameter has not been examined in subjects with sensitive skin [44]. The induction of CE maturity by transglutaminases (TGase) is key for barrier function [45] and their phenotypes are assessed microscopically through Nile red lipid staining and involucrin antigen binding [44, 46, 47]. More rigid/polygonal-shaped, mature CEs are found in the superficial layers of SC, whereas, fragile/asymmetrical immature CEs are mainly found in the deeper layers of SC. Increased levels of immature CEs are known to occur in barrier-compromised skin conditions [46–48]. Thus, reduced TGase activity can lead to reduced maturation of CEs and this has especially been shown in dry skin conditions [48]. However, the activities of TGase have not been investigated in subjects with sensitive skin.

Hibino *et al.* [49] have reported on the reduced levels of the late stage filaggrin-processing enzyme bleomycin hydrolase (BH) and natural moisturizing factors (NMF) in subjects with self-perceived sensitive skin. Recently, we have reported methods to characterize the activities of BH, NMF, plasmin as well as an additional filaggrin-processing enzyme, calpain-1 in samples collected by tape stripping human subjects [50]. To our knowledge, there are no reports comparing SC NMF levels, activities of BH, calpain-1 (C-1), transglutaminase (TG) and plasmin activities together with corneocyte envelope (CE) maturation in subjects with capsaicin-sensitive

Table 1 Expert grading scale for facial skin dryness

Score	Judgement
1	No dryness, bright skin
2	No scaling squames, brightness loss
3	Slight dryness: visible relief lines, more or less presence of some scaling squames
4	Presence of more, larger scaling squames
5	Moderate dryness: presence of many scaling, more or less large squames, well visible relief lines
6	Appearance of some scales
7	Marked dryness: presence of many small scales, visible relief as grid pattern
8	Appearance of some large scales
9	Very marked dryness: presence of many large scales and/or irritation. Major disturbance of skin marking

Table II Expert grading scale of facial skin roughness, even numbers are used if needed

Score	Judgement
1	No roughness: perfectly smooth and soft surface
3	Slight roughness: slight unevenness and slight roughness when tangentially touching
5	Moderate roughness: clearly uneven and rough appearance and possible feeling of a slight skin roughness when vertically touching
7	Severe roughness: very marked roughness and unevenness feeling
9	Extreme roughness: very important unevenness, rough feeling

skin. Therefore, we set out to study these parameters in a group of subjects with self-perceived sensitive skin and subclassified them on the basis of a capsaicin stinging test.

Materials and methods

Materials

Sodium dodecyl sulphate (SDS) ACS reagent >99.0% (436143), ethylene diamine tetra-acetic acid, Triton X-100, acetic acid and Nile red were obtained from Sigma–Aldrich (Dorset, UK). High-performance liquid chromatography (HPLC) analytical grade water and acetonitrile were obtained from Fisher Scientific (Hertfordshire, UK). DL-dithiothreitol, trifluoroacetic acid and Tris-HCl (0.1 M) were purchased from Fluka (Buchs, Switzerland). Dimethylsulphoxide (DMSO) was obtained from VWR International Ltd (Bedford, UK), and phosphate-buffered saline (PBS) tablets were obtained from Oxoid (Hampshire, UK). The primary monoclonal antibody, anti-human involucrin (clone SY5), was purchased from Cambridge Scientific Products (Watertown, MA, USA). The rabbit anti-mouse IgG labelled with fluorescein isothiocyanate (FITC) was purchased from Abcam (Cambridge, UK). Aminomethyl coumarin (AMC) and protease substrates were donated by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland). All other materials were of reagent grade unless otherwise stated.

Study subjects

Caucasian subjects ($n = 48$; 39.9 ± 6.9 years old, Fitzpatrick skin phototypes II–III), with self-assessed sensitive facial skin participated in the observation study which took place in April, 2013 in Lyon, France. Subjects were recruited with a minimum score of 5 on a 9-point grading scale for dryness and roughness (Tables I and II) and with a TEWL $>15 \text{ g m}^{-2} \text{ h}$ on their cheeks. The study was approved by the Agence Régionale de Santé Rhône-Alpes (ARS) and was conducted in accordance with the Declaration of Helsinki Principles. Written informed consent was obtained from all participants before enrolment. For the 3-day skin conditioning phase prior to the data collection, the subjects were advised to refrain from any skin manipulations involving shaving, waxing or application of any cosmetics and drugs. Skin was carefully cleaned with a cotton pad soaked in distilled water 15 min before the bio-instrumental measurements and allowed to dry at room temperature. Subjects were acclimatized in a room under standard conditions ($21 \pm 1^\circ\text{C}$, $50 \pm 10\%$ relative humidity) prior to any measurements. The test site on the cheek (3 cm vertically beneath the outer edge of the eye) was delineated with a surgical marker to

ensure that the measurement probes, and the tapes were applied to the same area. All subjects were assessed with a cutaneous capsaicin reactivity test [51]. Briefly, nasolabial folds were cleansed using cotton pads impregnated with a 10% hydroalcoholic solution. Test subjects were excluded from the study if they reported any sensation of discomfort from this product. Diluted capsaicin solutions ($1.10 \times 10^{-4}\%$, $3.16 \times 10^{-4}\%$, $1.10 \times 10^{-3}\%$ and $3.16 \times 10^{-3}\%$ of capsaicin) were then applied over the nasolabial folds with cotton buds (10 times) sequentially until subjects reported any new sensation (stinging, burning, itching, other) and its intensity using the following scale: 0: no sensation, 1: doubtful, barely perceptible, 2: slightly perceptible, 3: moderately perceptible, 4: strongly perceptible, 5: painful. Twenty-five subjects (52%) showed a stinging sensation (stinger panel) and the other 23 subjects were not sensitive to capsaicin (non-stinger panel). All subjects participated in all stages of the study.

Sample collection and biophysical measurements

SC capacitance was measured on the cheek using a Corneometer CM825 (Courage & Khazaka, Electronic, Cologne, Germany), and basal TEWL was determined using an Aquaflux AF200 (Biox Systems, London, UK). All procedures were conducted following published guidelines [52–54]. Skin capacitance was expressed as the mean value of three recordings; TEWL was measured once.

Standard D-Squame[®] tapes, a D-Squame[®] pressure applicator and D-Squame[®] disc racks were obtained from Cuderm Corporation (Dallas, US). Six consecutive standard D-Squame[®] discs with a diameter of 2.2 cm and an area of 3.8 cm^2 were placed on the cheek at a pressure of 225 g cm^{-2} with a pressure device (CuDerm Corporation, Dallas, USA) for 5 s. To minimize variation, the procedure was conducted by the same technician for all volunteers, throughout the study. The interval between the tape strippings was $20 \pm 5 \text{ s}$ [55]. The SC protein content collected on tape strips was quantified by infrared absorption measurement at 850 nm using a SquameScan[™] 850A (Heiland electronic, Wetzlar, Germany) and the tapes were transferred to Eppendorf microtubes for storage (-20°C) until further analysis [56]. For SC protein quantification, the following equation was used [57].

$$\text{SC}_{\text{protein}} [\mu\text{g cm}^{-2}] = 1.366 \times \text{Absorption}[\%] - 1.557 \quad (1)$$

To determine the integrity of the SC, additional TEWL measurements were conducted after the 2nd, 4th and 6th tape strippings. Tapes 1, 2, 5 and 6 were used for biochemical and morphological evaluation.

PCA quantification

The second tape strip was used for PCA determination. Tapes were extracted at 1000 rpm in 750 μL , 0.1 mol L^{-1} KOH solution for

Table III Fluorogenic peptide substrates used to quantify BH, C-1 and plasmin

SC protease	Substrate	Source
BH-like activity	H-Cit-AMC	I-1170 (Bachem)
C-1-like activity	Suc-Leu-Leu-Val-Tyr-AMC	I-1395 (Bachem)
Plasmin-like activity	MeOSuc-Ala-Phe-Lys-AMC	I-1275 (Bachem)

2 h at 32°C with continuous shaking. The alkaline extracts were neutralized with 4.5 μL of perchloric acid (12 M), shaken again for 2 h and filtered through a 0.2- μm membrane. High-performance liquid chromatography (HPLC) at room temperature with UV detection at 210 nm was used to analyse the PCA content of the tape stripping extracts. A Synergi[®] 4 μm POLAR-RP-80A column, 4.6 \times 150 mm (Phenomenex, Macclesfield, UK) was used for all analyses. The volume of injection was 10 μL , and the flow rate was 0.4 mL min^{-1} . The mobile phase consisted of 20 mmol L^{-1} ammonium formate, 1.5 mmol L^{-1} tetrabutylammonium hydroxide and 3% acetonitrile at pH 7.3 [58]. The retention time was 7.5 min.

TG activity

TG activity of tape stripping extracts was measured using polystyrene microtitre plates (Thermo Fisher Scientific, Zug, Switzerland) coated with N,N'-dimethylcasein (VWR Chemicals, Dietikon, Switzerland). 5-(biotinamido)pentylamine (Thermo Fisher Scientific, Zug, Switzerland), which served as the substrate, was covalently incorporated into N,N'-dimethylcasein by TG in a calcium-dependent reaction. The biotinylated product was detected by streptavidin-alkaline phosphatase (GE Healthcare, Glattbrugg, Switzerland) and quantified by measuring the absorbance at 405 nm following the addition of p-nitrophenyl phosphate (VWR Chemicals, Dietikon, Switzerland) [59].

Protease activity

Protease activity was quantified using the methods previously described by Voegeli *et al.* [57]. Immediately after the SC protein measurement, each tape (tape 6) was transferred into a 1.5-mL Eppendorf tube and extracted for 25 min at 25°C and 1000 rpm in 750 μL of buffer composed of 0.1 M Tris-HCl and 0.5% Triton X-100 at pH 7.5. The extracts were treated with respective fluorogenic peptide substrates for BH, C-1 and plasmin, all from Bachem, Bubendorf, Switzerland (Table III). The mixtures were shaken and incubated at 37°C in an Orbital SI50 incubator (Stuart Scientific, Staffs, UK). The reaction was stopped after 2 h by adding 250 μL of acetic acid (1%). The released aminomethylcoumarin (AMC) was quantified by HPLC and a fluorescence detector. The wavelength for emission was 442 nm, and for excitation, it was 354 nm. The elution was conducted using reverse-phase HPLC with a mobile phase of 75% water, 25% acetonitrile, 0.01% trifluoroacetic acid (TFA). The flow rate was 1 mL/min , the injection volume was 20 μL , and the retention time of AMC was 6.5 min.

CE assay

Immature corneocytes were immunostained with anti-involucrin and mature corneocytes with Nile red using a modification of the method described in detail by Mohammed *et al.* [44]. The tapes were transferred to Eppendorf tubes and extracted using dissociation buffer consisting of SDS, DL-dithiothreitol, EDTA, Tris-HCl adjusted to pH 8. Using a dry water bath (Benchmark Scientific, NJ, USA), the tubes were heated at 75°C for 10 min, centrifuged and washed three times with dissociation buffer. The corneocytes were separated as pellets, which were then dissolved in dissociation buffer. The suspension was applied on a microscope slide and dried. Primary involucrin monoclonal antibody (anti-human involucrin, clone SY5) was added and incubated

overnight, and the next day, secondary antibody (FITC-labelled rabbit polyclonal to mouse antibody IgG (H&L)) was added and incubated for 1 h in a dark room. The slides were rinsed with PBS and incubated with Nile red for 10 min. The slides were subsequently rinsed with PBS and covered with a slide cover. Fluorescence was measured with a fluorescence microscope equipped with a Canon 70D 20.2 megapixel camera (Canon Inc, New York, USA). IMAGEJ[™] image analysis software (NIH, Bethesda, MD, USA) was used to analyse the red pixels obtained from the Nile red-stained cells and the green pixels from the immunostained cells. The ratio of red/green pixels corresponds to the corneocyte maturity. The Nile red-stained cell images were further processed using IMAGEJ[™] to analyse the cell surface area.

SC thickness and path length

The number of SC cell layers collected was estimated from the corneocyte surface area (A) using the relationship reported by Machado *et al.* [60]:

Table IV Facial dryness, roughness, capacitance, amounts of protein removed by tape stripping and basal TEWL values for capsaicin-sensitive and non-capsaicin-sensitive subjects. Mean \pm SEM

Evaluation	Non-capsaicin sensitive	Capsaicin sensitive
Number of subjects	23	25
Age of subjects [years]	40.3 \pm 1.5	39.7 \pm 1.5
Facial dryness, expert grading [scale 0–9]	4.6 \pm 0.4	4.9 \pm 0.4
Facial roughness, expert grading [scale 0–9]	4.0 \pm 0.3	4.2 \pm 0.3
SC Capacitance [AU]	47.0 \pm 2.6	47.2 \pm 2.3
Cumulative SC cohesion [$\mu\text{g SC protein cm}^{-2}$]	168.0 \pm 5.1	174.0 \pm 3.3
Basal TEWL	34.0 \pm 2.6	29.6 \pm 2.1

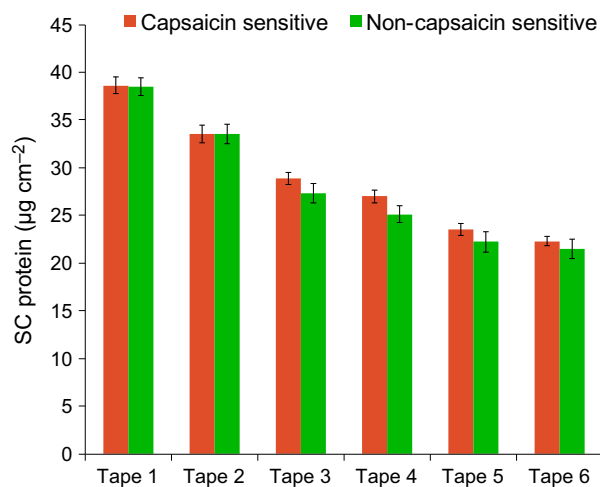


Figure 1 SC protein content in tape strips from cheek of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$). Data are mean \pm SEM.

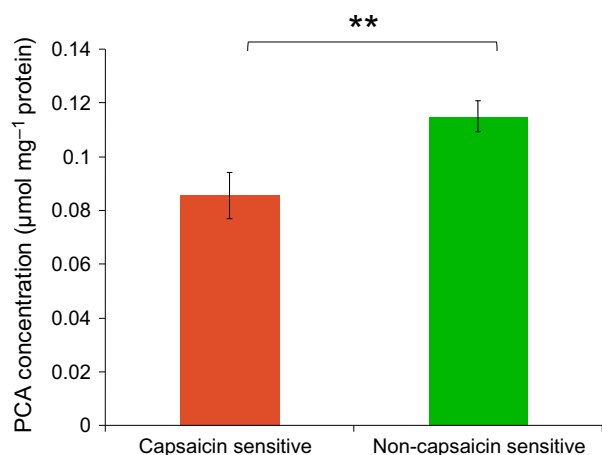


Figure 2 PCA concentration from cheek tape 3 of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$). Data are mean \pm SEM, Paired t -test, $**P < 0.001$.

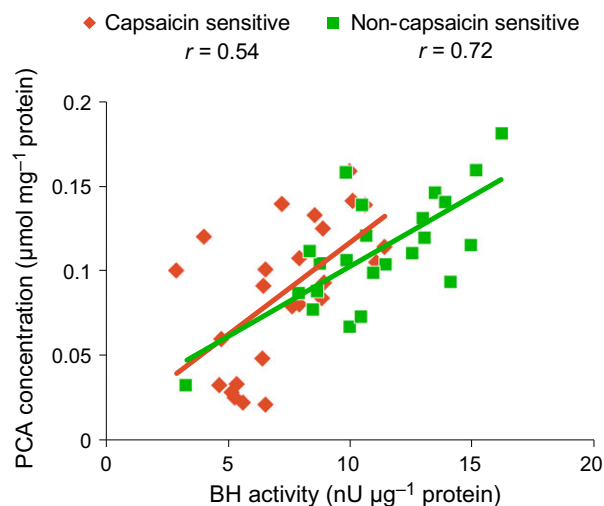


Figure 4 Pearson's coefficient correlation between BH activity and PCA concentration measured in cheek of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$).

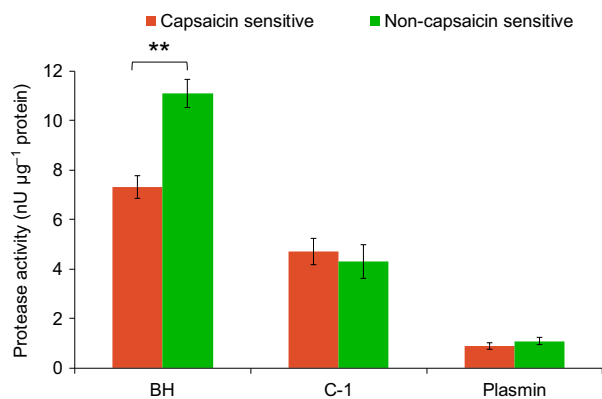


Figure 3 Proteolytic enzyme activities in cheek tape 6 of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$). Data are mean \pm SEM, $**P < 0.001$, one-way ANOVA.

$$\text{number of cell layers } (n) = \frac{\sqrt{A} - 21}{0.7} \quad (2)$$

where n is the number of cell layers of the SC and A is the area of the corneocyte.

The geometrical intercellular path length (μm) was calculated from equation 3:

$$\text{pathlength } (p) = n + \frac{\sqrt{A}(n-1)}{2} \quad (3)$$

where p is the calculated intercellular path length for intercellular diffusion in the SC.

Statistical analysis

All data are expressed as mean \pm SEM, and differences between means were analysed with SPSS software version 22 and Microsoft

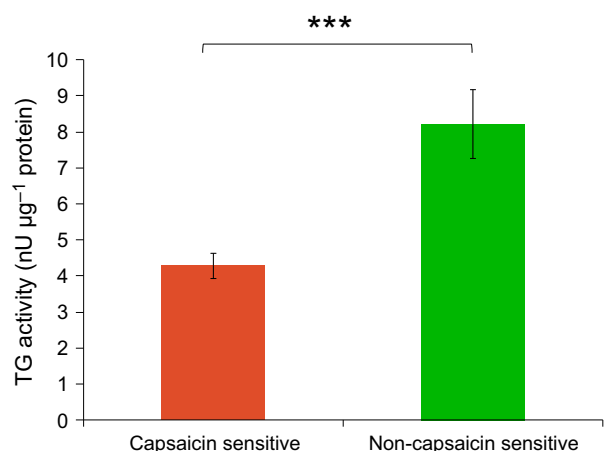


Figure 5 TG activity measured in cheek tape 5 of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$), mean \pm SEM, $***P < 0.0001$, Paired t -test.

Excel 2011. Normality was assessed using the Kolmogorov–Smirnov statistical test and the Shapiro–Wilk value. The following tests were applied: Student's paired t -test, analysis of variance (ANOVA) and nonparametric Mann–Whitney U -test with a two-tailed test of significance. Differences at $P < 0.05$ were considered to be statistically significant. The correlation coefficient (r) was calculated using Pearson's test.

Results

Skin grading, capacitance, TEWL and SC cohesion

Interestingly, there were no significant differences between the capsaicin-sensitive and non-capsaicin-sensitive subjects in expert grading, neither for facial roughness and dryness, nor for SC capacitance and TEWL (Table IV). The SC protein content was used

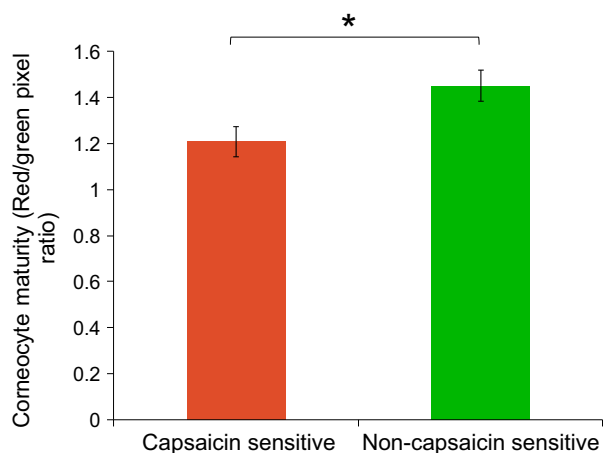


Figure 6 CE maturity in cheek tape 1 of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$). Data are mean \pm SEM, * $P < 0.05$, Paired t -test.

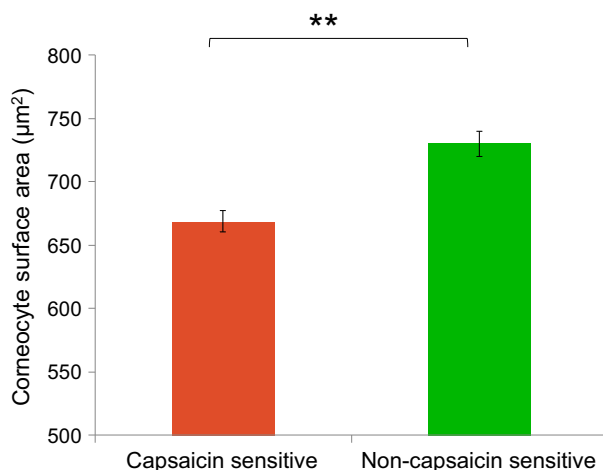


Figure 7 Corneocyte surface area from cheek tape 1 of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$). Data are mean \pm SEM, ** $P < 0.001$, Paired t -test.

to examine SC cohesion. However, there were no statistically significant differences between the two groups for cohesion of SC with sequential tape stripping (Fig. 1). With increasing number of tapes removed, the SC protein content collected in both groups decreased to the same extent, also indicating similar levels of protein density. There was also no difference in TEWL values between the two subject groups with increasing tape stripping (data not shown).

PCA quantification and protease measurements

Significantly lower levels of PCA were found for capsaicin-sensitive subjects compared with the non-capsaicin-sensitive subjects (Fig. 2). Significantly lower values of BH activity were found in capsaicin-sensitive subjects ($P < 0.001$) compared with non-capsaicin-sensitive subjects (-34%). However, there were no significant

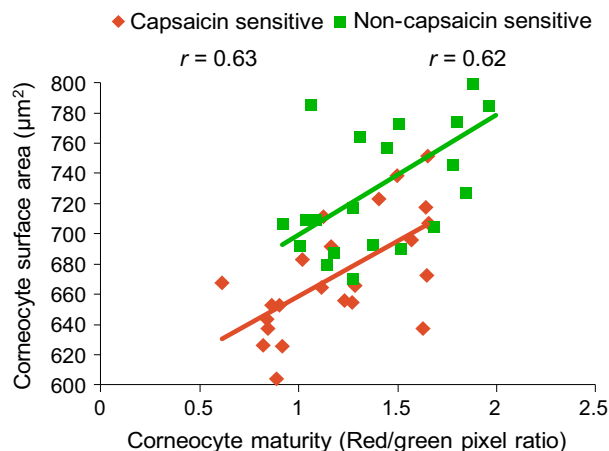


Figure 8 Pearson's coefficient correlation between corneocyte surface area and corneocyte maturity in cheek of capsaicin-sensitive ($n = 25$) and non-capsaicin-sensitive subjects ($n = 23$).

differences in levels of C-1 activity and plasmin activity between the two subject groups (Fig. 3). There was a higher correlation between PCA and BH activity in non-capsaicin-sensitive subjects compared with capsaicin-sensitive subjects (Fig. 4). There was no correlation between PCA levels and activity values for the other proteases (C-1 and plasmin). The values of TG activity were significantly decreased ($P < 0.001$) in the capsaicin-sensitive subjects (-48%) compared with non-capsaicin-sensitive subjects (Fig. 5).

CE assay and determination of SC path length and thickness

The maturity of corneocytes differed significantly between the two panels. The red/green (Nile red/involucrin) ratio was significantly lower ($P < 0.05$) in the capsaicin-sensitive panel (-20%) compared with the non-capsaicin-sensitive panel (Fig. 6). Similarly, the corneocyte surface area was also significantly smaller (-9%) as shown in Fig. 7. There was a positive correlation between corneocyte maturity and surface area in both groups ($r > 0.62$); that is, the greater the maturity, the larger the corneocyte surface area (Fig. 8). The SC path length and the number of SC cell layers were estimated to be significantly lower in capsaicin-sensitive subjects compared with the non-capsaicin-sensitive subjects ($P = 0.001$). The respective values for path length and number of cell layers for the capsaicin-sensitive subjects were 83.2 ± 4.4 and 6.87 ± 0.28 , and the corresponding values for the non-capsaicin-sensitive subjects were 113.1 ± 4.5 and 8.68 ± 0.26 .

Discussion

Our study aimed to explore the biochemical and morphological characteristics of the SC of capsaicin-sensitive subjects within a group of self-perceived sensitive skin subjects. We quantified BH, C-1, plasmin and TG activities, corneocyte maturity and the surface area of the corneocytes. In addition, the facial skin of the subjects was graded for dryness and roughness and basal TEWL, SC integrity, SC cohesion and skin capacitance were measured.

The two groups of subjects had comparable levels of skin dryness, roughness, basal TEWL, SC integrity, skin capacitance and SC cohesion. Basal TEWL values were similar to those obtained by

Yokota *et al.* [61]. We observed that the capsaicin-sensitive panel had lower levels of BH activity but not C-1 activity which was consistent with the lower NMF values in the former panel. BH is the terminal enzyme in the filaggrin degradation pathway, which leads to formation of NMF [49]. Hence, in capsaicin-sensitive subjects, the lower NMF formed may be due to the lower activity of BH. This is also supported by our finding of a positive correlation between higher BH activity and higher NMF in the non-capsaicin-sensitive panel ($r = 0.72$). Reductions in BH activity in self-perceived sensitive skin have been reported [49], but we show it is further decreased in subjects who also have a chemosensory skin problem. Reduced NMF levels and BH activity in the SC are normally associated with dry skin [49, 62, 63]. Nevertheless, the lowered PCA level in the capsaicin-sensitive subjects was not associated with further increased skin dryness or roughness and skin capacitance.

The accelerated proliferation of keratinocytes and abnormal corneocyte morphology is characteristic of inflammatory diseases of skin such as psoriasis vulgaris and eczema [46, 64–66]. Similarly, it has been found that dry skin has excessive amounts of immature corneocytes [48]. All these conditions are characterized by impaired SC maturation. Thus, fragile corneocytes with nuclei can be used as hallmarks of abnormal SC maturation [47]. The higher red/green ratio obtained in the capsaicin-sensitive subjects suggests impaired SC maturation is occurring in these subjects. We did not, however, observe any parakeratosis.

One group of enzymes involved in the corneocyte maturation process is catalysed by TG [45]. These enzymes rigidify the corneocyte envelope and improve its hydrophobicity by esterifying lipids to its surface proteins. A lower level of TG is observed in dry skin and is associated with increased numbers of immature corneocytes [48]. A similar finding was observed in our study. The capsaicin-sensitive panel had a significantly lower activity of TG compared with the non-capsaicin-sensitive panel. However, there was no further induction of dry skin. It is possible that the lower levels of PCA are a contributing factor as it is reported that there is an inverse relationship between filaggrinolysis and corneocyte

maturation [67]. The relationship between CE maturation and skin barrier function provides insight into the pathophysiology of sensitive skin

Smaller corneocytes as observed in the capsaicin-sensitive subjects are associated with impaired barrier function. This may not be detected by TEWL measurements, but a reduced penetration pathway for capsaicin may be contributing to the increased sensitivity. Using the equation of Machado *et al.*, we estimated the SC to be thinner which will also contribute to this effect [60].

Elevated plasmin activities and mass levels are usually associated with barrier-compromised skin conditions [42,43,68]. However, we did not observe any differences in plasmin activity between the two groups of subjects probably because their TEWL values were not different. Plasmin activities should be further investigated in other sensitive skin subjects where basal TEWL indicates a barrier problem.

Neurosensory disturbances are recognized in subjects with sensitive skin especially for the TRPV1 receptor [30]. From our results, we believe that the reduced chemosensory threshold in the capsaicin-sensitive subjects is related to an inferior barrier function driven by poor SC maturation events and possibly premature desquamation. The resulting reduced barrier function then exposed cutaneous TRPV1 receptors to the exogenously applied capsaicin.

To conclude, a greater penetration of capsaicin through the SC may be associated with the increased sensitivity observed in the capsaicin-sensitive subjects. The reduced penetration pathway may be due to the presence of smaller immature corneocytes and thinner SC. The biological events involved in the cornification process are not complete in these subjects as a result of lower TG activity which may be related to the lower PCA levels and BH activity.

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