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The impact of different hair-removal behaviours on the biophysical and biochemical characteristics of female axillary skin

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Abstract

OBJECTIVE: The impact of hair removal on the biophysical and biochemical characteristics of human axillary skin is not fully understood. This study investigated the effect of different hair-removal techniques on biophysical parameters and the concentrations of key inflammatory biomarkers in the axillae of female Thai subjects. Axillary hair was removed by shaving, plucking or waxing.

METHODS: Following a 2-week washout phase without hair removal, subjects underwent visual assessment for erythema and skin dryness in one (randomized) axilla, then hair was removed from the axilla by shaving, plucking or waxing according to each subject's established habit. Erythema and dryness were assessed again 30 min after hair removal, and buffer scrubs collected from depilated and non-depilated axillae and analysed for inflammatory cytokines; after a further 48 h, erythema, dryness and post-inflammatory hyperpigmentation (PIHP) were assessed in the depilated axilla. Biophysical assessments (skin hydration, barrier integrity, elasticity and roughness) were made in depilated and non-depilated axillae.

RESULTS: All three hair-removal techniques induced an increase in axillary erythema and skin dryness. Shaving was associated with significantly less erythema ($P < 0.01$), but significantly greater skin dryness ($P < 0.05$) versus the other techniques 30 min after hair removal. There were no between-technique differences in PIHP or biophysical parameters. Interleukins IL-1α and IL-1RA concentrations increased, and IL-8 concentration decreased following hair removal by each technique.

CONCLUSION: This is the first study to identify the principal cytokines associated with the inflammatory process triggered by axillary hair removal. A single hair-removal treatment did not appear to induce PIHP or further biophysical changes to the skin.

Résumé

OBJECTIF: L'impact de l'épilation sur les caractéristiques biophysiques et biochimiques de la peau axillaire humaine n'est pas entièrement compris. Cette étude a examiné l'effet de différentes techniques d'épilation sur les paramètres biophysiques et les concentrations de biomarqueurs inflammatoires clés dans les aisselles de sujets thaïlandais de sexe féminin. Les aisselles ont été épilées par rasage, à la pince ou à la cire.

MÉTHODES: Après une phase de sevrage de 2 semaines sans épilation, les sujets ont subi une évaluation visuelle de l'érythème et de la sécheresse cutanée dans une aisselle (randomisé), puis l'aisselle a été épilée par rasage, à la pince ou à la cire selon l'habitude établie de chaque sujet. L'érythème et la sécheresse ont été évalués à nouveau 30 minutes après l'épilation, et des frottis tampons ont été prélevés dans les aisselles épilées et non épilées et analysés pour détecter les cytokines inflammatoires; puis, après 48 heures, l'érythème, la sécheresse et l'hyperpigmentation post-inflammatoire (PIHP) ont été évalués dans les aisselles épilées. Des évaluations biophysiques (hydratation cutanée, intégrité de la barrière cutanée, élasticité et rugosité de la peau) ont été réalisées sur les aisselles épilées et non épilées.

RÉSULTATS: Les trois techniques d'épilation ont entraîné une accentuation de l'érythème axillaire et de la sécheresse de la peau. Le rasage a été associé à un érythème nettement moins important ($P < 0,01$), mais à une sécheresse cutanée nettement plus importante ($P < 0,05$) par rapport aux autres techniques 30 min après l'épilation. Aucune différence entre les techniques n'a été observée en ce qui concerne le PIHP ou les paramètres biophysiques. Les concentrations en interleukines IL-1α IL-1RA ont augmenté, et la concentration en IL-8 a diminué après l'épilation par chaque technique.

CONCLUSION: Cette étude est la première à identifier les cytokines principales associées au processus inflammatoire déclenché par l'épilation des aisselles. Une seule épilation n'a pas semblé entraîner de PIHP ou d'autres modifications biophysiques de la peau.

Introduction

Axillary skin has distinct properties compared with the skin at other body sites. Evaluation of trans-epidermal water loss (TEWL) and corneosurfametry has demonstrated reduced skin barrier integrity in the axilla compared with other body sites [1, 2]. Axillary skin is also characterized by elevated levels of cholesterol, ceramide 3 and lactic acid, and a lower concentration of natural moisturizing factor, when compared with the forearm [3]. The axillary skin is thus likely to be more susceptible to damage. In many cultures, women prefer to remove underarm hair. The axillary skin may therefore be subjected to repeated shaving, plucking or waxing to remove hair, which may induce irritation and further compromise skin barrier function.

Shaving is a frequently used axillary hair-removal technique. This causes visible irritation in the vault and fossa areas [4] and

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removes the uppermost layers of the skin (i.e. the top layers of the stratum corneum), which can lead to reduced hydration and increased skin dryness [5]. Cuts caused by shaving may also contribute to the irritation and further weaken the natural barrier [4, 5]. The effects of plucking and waxing on axillary skin have been less well studied. Little is known about the inflammatory process underlying the visible irritation and weakened skin barrier associated with axillary hair removal. Damage inflicted to the axillary skin barrier, such as removal of corneocytes by shaving, is likely to cause the release of pro-inflammatory mediators from epidermal keratinocytes and mast cells, which in turn will lead to skin irritation and erythema [6]. In contrast, hair plucking and waxing may trigger a deeper inflammatory response at the level of the hair follicle.

Another concern associated with hair removal in some consumers, particularly those with Fitzpatrick skin types III and above, is post-inflammatory hyperpigmentation (PIHP), that is skin darkening caused by epidermal melanin production in skin irritated by hair removal [7–9]. Histological evaluation of axillary skin in Filipino women demonstrated that hair plucking is associated with melanosome leakage into the dermis, leading to increased pigmentation [9]. This effect was accompanied by mononuclear cell and macrophage infiltration, which is usually associated with the production of inflammatory cytokines, which in turn stimulate melanocytes to produce more pigment-containing melanosomes [6, 9].

The current study was conducted to investigate potential differences in the response of the axillary skin barrier to different hair-removal techniques. The study objectives were to investigate the effect of different hair-removal techniques (shaving, plucking and waxing) on a variety of biophysical parameters and the concentrations of key inflammatory biomarkers in the axillae of female Thai subjects. Selected endpoint measurements included the level of visible irritation (erythema) and PIHP of the axilla immediately (30 min) and 48 h after hair removal, biophysical properties (i.e. hydration, barrier function, elasticity and roughness), and concentrations of inflammatory biomarkers in the axillary skin before and immediately after hair removal.

Methods

Impact of different hair removal techniques on the biophysical and biochemical properties of axillary skin

This was a non-interventional single-centre exploratory study that comprised a screening visit, a 2-week washout period and two subsequent test visits, 48 h apart (see Fig. 1). The study was conducted by Intertek CRS at Spincontrol Asia Co., Ltd, Huay Kwang, Bangkok, Thailand and approved by the Unilever R&D Institutional Review Board standard ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of Unilever and the local institutional research board and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

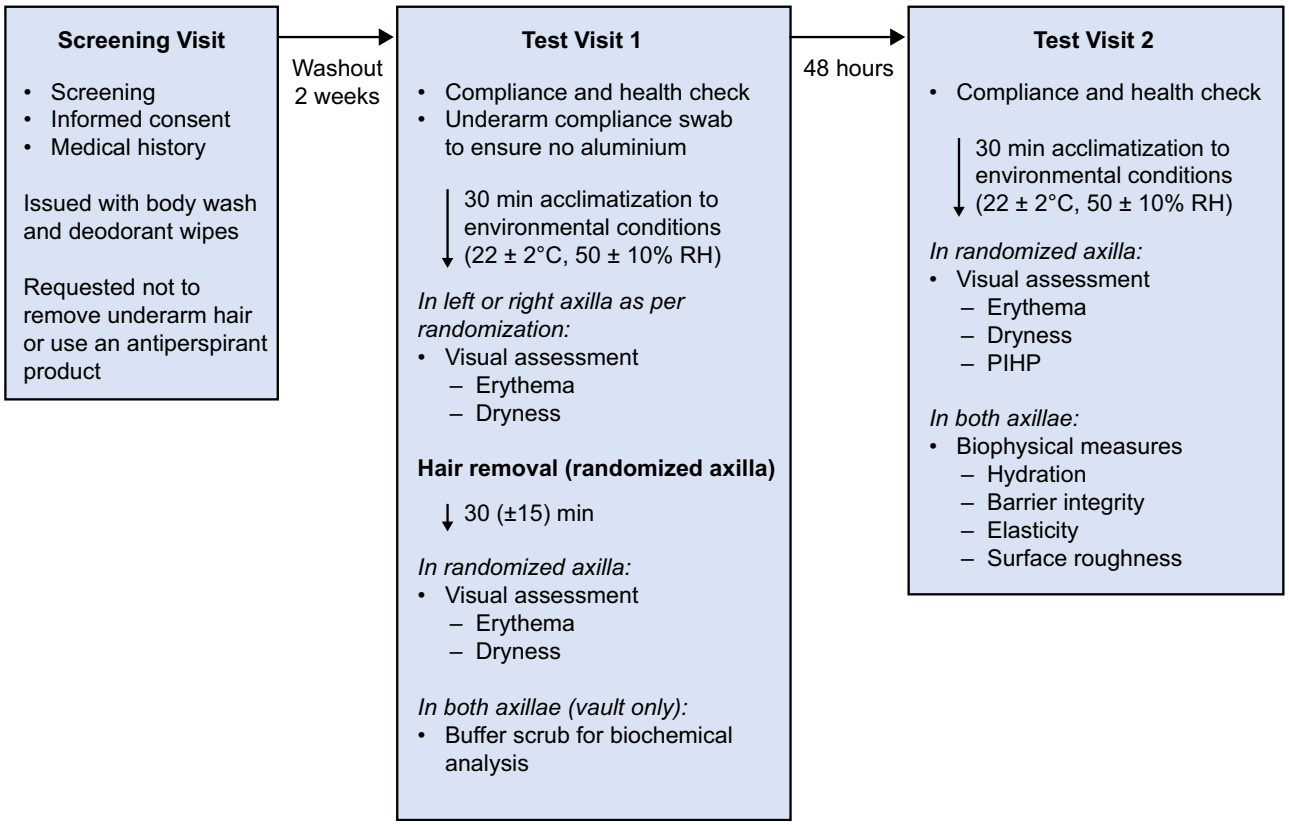

Figure 1 Study visits and procedures. PIHP, post-inflammatory hyperpigmentation; RH, relative humidity.

Subjects

The study recruited healthy women of Thai origin, aged 30–40 years, who had continuously used one of three methods of hair removal (i.e. shaving, plucking or waxing) for the past 2 years. Eligible subjects were non-smokers and had axillary skin free from cuts and abrasions. Exclusion criteria included pregnancy or breastfeeding; allergies or sensitivities to study product components, soaps, fragrances, deodorants or any other topically applied products; latex allergy; any skin conditions or factors that might affect the response of the skin or interpretation of the test results, including any active generalized skin diseases; current or previous medication that might influence the response of the skin or interpretation of the data, such as topical and systemic corticosteroids, regular use of antihistamine or anti-inflammatory medication; participation in another study involving the specific skin site as the target area within 3 weeks of the start of the current study; diabetes mellitus; any medical problems or active skin diseases affecting the axillae; use of self-prescribed or prescribed topical drugs in the axillae; or underarm shape or unsuitable underarms (e.g. excessive hair) that would render technical assessment difficult. Employees of Unilever, Intertek CRS and Spincontrol Asia Co., Ltd were also excluded. The safety population comprised all patients who enrolled in the study and completed the first test visit. Adverse events were monitored throughout the study. Some level of irritation (dryness and erythema) was expected owing to the nature of the study. Subjects were closely monitored at regular intervals to ensure that irritation levels remained within the acceptable limits for an axillary clinical study.

A total of 66 subjects were to be recruited for this study with the aim of 60 completing both test visits (20 subjects per hair-removal method). A sample size of 20 subjects provides a total of 40 test sites (two per subject) at each timepoint for buffer scrubs. Statistical power analysis of the biochemical sampling techniques has shown that this number of subjects is optimal for reducing noise in the analysed data. A sample size of 20 subjects also provided a total of 80 test sites for biophysical measures, which is sufficient data to identify any statistically significant differences between study groups.

All of the 66 subjects completed the washout phase and 64 completed the test visits (21 shavers, 22 pluckers and 21 waxers). Two subjects were withdrawn due to an excessive degree of irritation resulting from the waxing procedure; no other safety issues were reported. The mean age for the study population was 35.8 years (range 30.0–40.0 years) and was similar across the study groups: shavers 36.7 years (range 31.0–40.0 years), waxers 35.3 years (range 30.0–40.0 years) and pluckers 35.3 years (range 31.0–40.0 years).

Study design

At the screening visit, each eligible subject provided written informed consent to participate in the study before their demographic characteristics and medical history were recorded and their underarms were assessed to confirm suitability for the study. Eligible subjects were issued with deodorant (Boots Deo wipes; The Boots Company PLC, Nottingham, UK) and shower gel (Johnson's® Baby Bath; Johnson & Johnson Ltd, New Brunswick, NJ, USA) to use at home instead of their regular antiperspirant products for at least 14 days as a washout before Test Visit 1. This was to eliminate any traces of antiperspirant-derived aluminium from the skin, which may adversely affect biochemical analyses. The washout products were also to be used at home between Test Visits 1 and 2.

For the duration of the study, subjects were requested to refrain from swimming, using sunbeds, and from tanning the underarm area, and to not remove hair from the armpit area except when requested to do so at the test centre. Subjects were also required not to apply any product to their underarms or consume any alcohol for at least 12 h before test visits; not to consume green tea, cranberry juice, caffeine-containing food and drinks, and any spicy foods for at least 1 h before arrival at the study site; and to wash their underarms with water 2 h before the visit. At both test visits, subjects initially underwent a check for compliance with study requirements and for any changes in medical history. Subjects then acclimatized with their underarms exposed for a minimum of 30 min at 22 ± 2°C, 50 ± 10% relative humidity. All measurements and assessments were made with the subject lying supine on an examination bed with the axillae exposed such that their elbow was in line with their ear and the index finger in contact with the spine at the base of the skull. This ensured that the underarm was in a consistently open position.

At Test Visit 1, an assay was conducted to confirm that no aluminium was present in the underarm. Hair removal was randomized to the left or right axilla in each subject according to the randomization schedule supplied by the study statistician. Hair removal in the randomized axilla was performed according to the method regularly used by the subject. Shaving group subjects ('shavers') were provided with a BIC® Twin Lady razor (Société BIC S.A., Paris, France) and requested to shave the underarm using water only according to their usual procedure. In the plucking group ('pluckers'), subjects were requested to bring their own tweezers to the study site to remove the underarm hair. For subjects in the waxing group ('waxers'), a professional beautician removed hair using a hot waxing procedure with locally marketed wax. Visual assessment was completed in the randomized axilla of each subject both before and 30 min (±15 min) after hair removal. Biochemical sampling procedures were conducted in the axillae of each subject. Buffer scrubs were collected from the vaults of the randomized axilla (left or right) and contralateral axilla 30 min (±15 min) after hair removal from the randomized axilla.

Test Visit 2 was completed 48 h after Test Visit 1. Following acclimatization, visual assessment and assessment of hyperpigmentation of the randomized axilla were performed, and biophysical characteristics (skin hydration, skin barrier integrity and elasticity) were assessed in both axillae. Silicone-elastomer replicas were taken to assess skin roughness in both axillae.

Assessments

Underarm compliance swab

A cotton bud was used to wipe the subject's left axilla using five strokes; this was repeated using the opposite end of the bud for the right axilla. The cotton bud was stored in a plastic bag until tested. To test for the presence of aluminium, three or four drops of test reagent [containing 0.00625% (w/v) Eriochrome® cyanine RC detector dye; Merck Chemicals Ltd, Gillingham, UK] were placed onto the ends of the cotton bud. The swabs were left for several hours to develop. The appearance of a pinkish-purple colour, however slight, indicated the presence of antiperspirant-derived aluminium and hence non-compliance with study instructions.

Visual assessments

Erythema and dryness were assessed in the vault and fossa of the randomized axilla under shadow-free illumination by a trained examiner at Test Visits 1 and 2. A Luxo Circus magnifier lamp (22W, circular T5, shadow-free cool white, fluorescent light with a 3.5-dioptre lens; Glamox Ltd, Borehamwood, Hertfordshire, UK) was used for dryness assessments. Erythema and dryness were each graded according to the Axillary Irritation Grading Scale [10]: an 8-point scale ranging from 0.0 (no apparent cutaneous involvement) to 3.5 (for erythema: moderate to severe erythema with moderate oedema, or moderate to severe erythema with isolated eschar formations or vesicles; for dryness: severe scaling/uplifting flakes and/or moderate fissuring).

Post-inflammatory hyperpigmentation in the randomized axilla was assessed at Test Visit 2 by comparison with a colour ruler. The assessor determined whether any hyperpigmentation was uniform across the axilla. If uniform, the assessor placed the ruler alongside the coloured skin and allocated a score from the ruler. If hyperpigmentation was non-uniform, the assessor scored the single most predominant colour.

Biophysical assessments

Hydration was measured using a corneometer skin hygrometer [11] in six regions for both the vault and fossa of both axillae in each subject (Fig. 2). TEWL was measured using an open chamber evaporimeter (DermaLab, Cortex Technology, Hadsund, Denmark) [12] to assess skin barrier integrity. Two measurements were taken in each axilla: one in the centre of the vault and one in the lower centre of the fossa. Skin elasticity was measured using a Cutometer® MPA 580 (Courage + Khazaka Electronic GmbH, Cologne, Germany) with a standard 2 mm diameter aperture and using the following settings: time–strain mode (Modus 1); pre-time 0 s; on and off time 2 s each; pressure 450 mbar. Two measurements were made in each axilla: one in the centre of the vault and one in the lower centre of the fossa. Two parameters were assessed: 'biological elasticity' (R2) and 'viscoelasticity' (R6). R2 is the ability of the skin to return its original form and dimensions when the deforming forces are removed. As the skin's elastic recovery decreases, R2 decreases (perfect recovery R2 = 1). R6 is a measure of how the skin responds over time when deformed with force; R6 values are low (≈0) in highly elastic material.

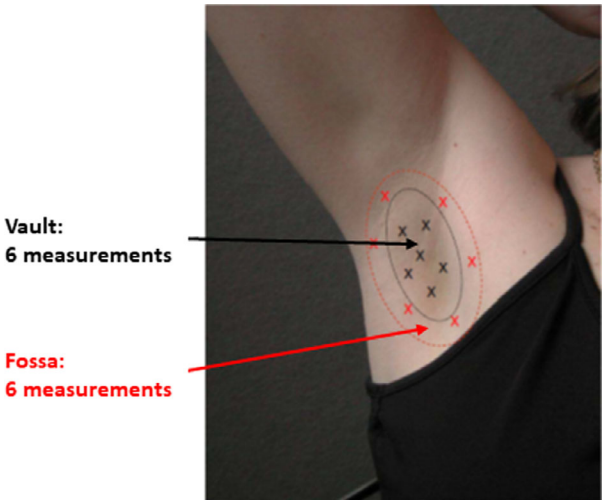

Figure 2 Sites of corneometer measurements in vault and fossa.

Surface roughness was evaluated by taking replicas of the axillary skin surface in the vault and fossa in both axillae using Silflo® (Flexico Developments Ltd, Stevenage, Hertfordshire, UK). A 20-mm circular adhesive ring was placed on the skin and the Silflo mixture smeared uniformly over the inner circle of the adhesive ring and left to cure before being removed. The replicas were analysed using bespoke PRIMOS software (GF Messtechnik, Teltow, Germany) from which a range of topographical parameters were extracted including overall roughness, average peak to valley distance and density of furrows.

Biochemical assessments

At Test Visit 1, buffer scrubs were collected from the axillae vaults, with the after hair-removal sample taken from the randomized axilla and the before hair-removal sample taken from the contralateral axilla (with hair still present) 30 min ± 15 min after hair removal from the randomized axilla. A sterile Teflon ring (2 cm internal diameter) was held firmly against the selected sampling site, 0.75 mL of phosphate-buffered saline containing 0.2% TWEEN® 20 was added and the skin was gently massaged with a sterile Teflon rod for 1 min. The suspension was recovered for analysis using a sterile plastic pipette. The process was immediately repeated at the neighbouring site within the same vault. Samples were held on dry ice and shipped to the analysis contractor (Quotient Bioanalytical Sciences Ltd, Fordham, Cambridgeshire, UK) on dry ice. All sample handling and shipping were completed in accordance with the UK Human Tissue Act. The samples were analysed for cytokines (interleukin [IL]-1α, IL-1 receptor antagonist [RA], IL-1β, IL-4, IL-6 and IL-8) using the Luminex® Multiplex® human cytokine/chemokine panel (Merck KGaA, Darmstadt, Germany) and for histamine via enzyme-linked immunosorbent assay (Aviva Systems Biology, San Diego, CA, USA) according to the manufacturers' instructions. All signals were normalized via protein concentration quantification using a Pierce™ BCA assay (Thermo Fisher Scientific, Waltham, MA, USA).

Data analysis

Assessment data were analysed for the intent-to-treat population (i.e. all patients who completed the study) by an Intertek CRS statistician using SAS® statistical software (version 9.2; SAS Institute Inc., Cary, NC, USA). Basic summary statistics (number, mean, standard deviation, median, minimum, maximum) were presented, according to hair-removal method, for the vault, fossa and whole axilla.

Visual irritation was calculated as the maximum score (visual erythema or visual dryness) in each axilla. Visual assessment data were analysed using analysis of covariance, where the test site level was 2 (vault or fossa region) or 1 (whole axilla; comprising combined vault and fossa data) and the covariate was pre-hair-removal score. Model factors were pre-hair-removal score (covariate), hair-removal method, site and interaction of hair-removal method by site.

Trans-epidermal water loss, hydration, elasticity and roughness data were analysed using two-way analysis of variance (ANOVA), where the test site level was 2 (vault and fossa) and the model factors were hair-removal method, site and interaction of hair-removal method by site. A one-way ANOVA was used to analyse the data for the whole axilla (test site level was 1) for each parameter. PIHP data were analysed using one-way ANOVA, where the test site level was 1 (whole axilla) and the model factor was hair-removal method.

Duncan's multiple range test was used as the multiple comparison procedure for comparing each pair of group means; a significant *F*-test indicated that at least one pair of means differed.

As all buffer scrub data sets were skewed, analysis was by determination of the median. For inflammatory biomarkers from buffer scrub samples, IL-1α, IL-1RA and IL-8 had a small number of values below the limit of quantification. These were treated as both missing and censored observations. Comparing concentrations between hair-removal groups by both approaches produced the same conclusions. A linear mixed model was employed, which modelled the log normalized values. The data for histamine included a high proportion of values either above or below the limit of quantification. To mitigate for this, a non-parametric analysis was adopted (Kruskal–Wallis).

Results

Visual and biophysical assessments

Erythema

Some degree of erythema was noted in all study groups before hair removal (Fig. 3). Significantly higher levels were observed in the vault compared with the fossa ($P < 0.02$). Both plucking and waxing were associated with a statistically significant ($P < 0.005$) rise in erythema in both the vault and fossa 30 min after hair removal, with levels returning to baseline after 48 h (Fig. 3). With regard to shaving, although erythema increased after the procedure, there was no significant difference in the values for either the vault or fossa across the three timepoints. Shaving was, however, associated with less erythema than waxing in the vault ($P < 0.06$) and significantly less erythema than plucking in the vault ($P < 0.01$) and waxing in the whole axilla ($P < 0.01$), 30 min after hair removal.

Skin dryness and PIHP

Skin dryness was noted before hair removal in all study groups (Fig. 4). In shavers, a significant ($P < 0.0001$) increase from baseline in vault-skin dryness was observed 30 min after hair removal, with levels returning to baseline by 48 h after shaving (Fig. 4). No significant change in dryness was seen following plucking or waxing. At 30 min, shaving resulted in significantly higher levels of dryness compared with plucking in the vault ($P < 0.05$), and with plucking ($P < 0.01$) and waxing ($P < 0.05$) in the whole axilla.

No statistically significant differences in PIHP scores were observed between the groups using different hair-removal techniques 48 h after hair removal.

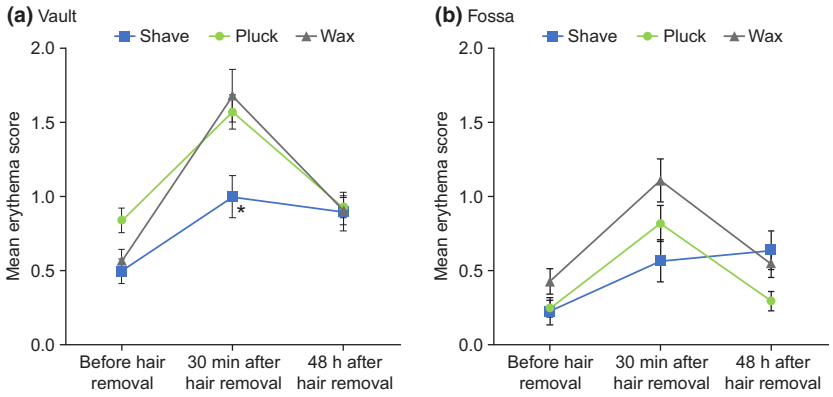

Figure 3 Erythema (mean score ± standard error) in (a) the vault and (b) the fossa before hair removal and at 30 min and 48 h after hair removal. *$P < 0.01$.

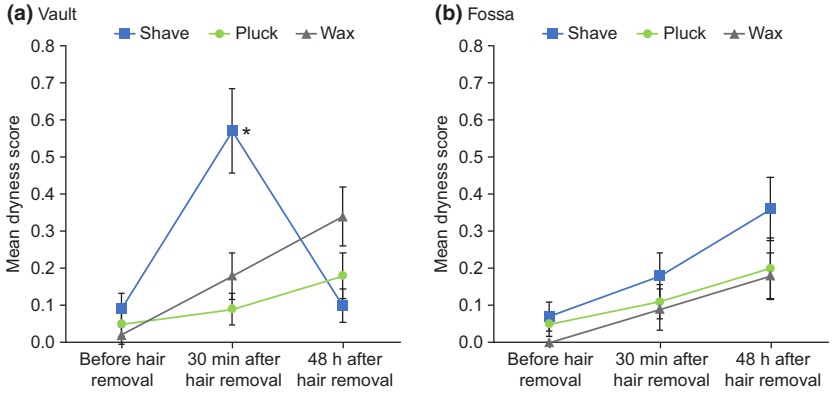

Figure 4 Visible dryness (mean score ± standard error) in (a) the vault and (b) fossa before hair removal and at 30 min and 48 h after hair removal. *$P < 0.05$.

Hydration and TEWL

No significant differences in mean hydration (Fig. 5) or TEWL (Fig. 6) levels were observed between hair-removal techniques or axilla regions at 48 h after hair removal. For all hair-removal techniques combined, TEWL at 48 h was higher in the vault than in the fossa ($P < 0.05$). There were no statistically significant within-technique differences in TEWL for the vault compared with the fossa.

No significant between-technique differences were observed in mean R2 or R6 elasticity values either in the vault or the fossa at 48 h after hair removal. For all hair-removal techniques, R2 (biological elasticity) was significantly higher in the fossa than in the vault ($P < 0.0001$). R6 (viscoelasticity) was significantly higher in the vault than in the fossa in shaved axillae and plucked axillae (both $P < 0.02$).

No significant differences in skin surface roughness were observed between hair-removal techniques. Higher roughness values were recorded in the vault than the fossa for all hair-removal techniques (significant for peak to valley distance only [$P < 0.0001$]).

Biochemical assessments

Of the six inflammatory cytokines measured, only three were detected in buffer scrub samples with enough regularity both before and after hair removal to enable further analysis. IL-1α, IL-1RA and IL-8 were frequently detected both before and after hair removal in buffer scrub samples from subjects in all three groups. In contrast, although IL-1β, IL-4 and IL-6 were detected occasionally, in the majority of samples their concentrations were below the level of detection. Histamine was not detected or only detected at very low concentrations in a small number of samples.

IL-1α concentrations increased following hair removal by each technique; the largest rise compared with pre-hair removal was observed in shavers (2.1-fold median rise vs. 1.4-fold for pluckers and 1.6-fold for waxers; Fig. 7a). IL-1RA concentrations also increased following hair removal by each technique. The largest increase from pre-hair removal concentrations was observed in waxers (3.3-fold median rise) and shavers (2.8-fold), followed by pluckers (1.3-fold; Fig. 7b). IL-8 concentrations decreased following

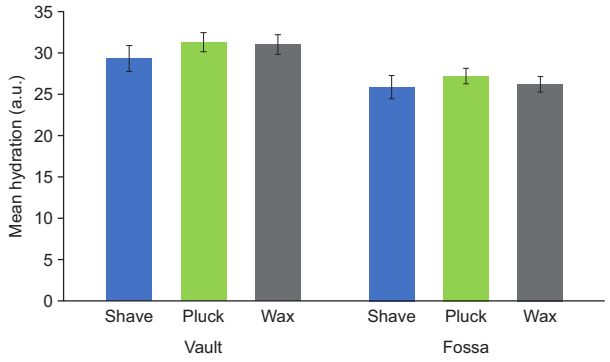

Figure 5 Hydration levels (mean score ± standard error) in the vault and fossa 48 h after hair removal. a.u., arbitrary units.

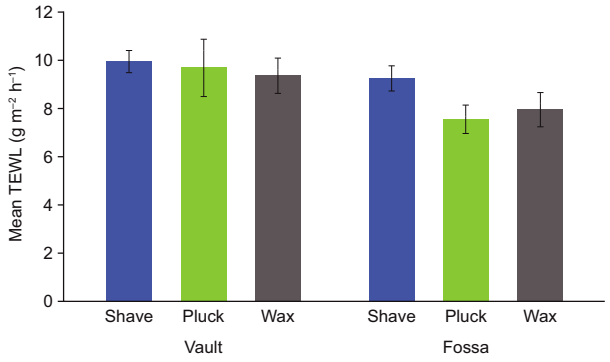

Figure 6 TEWL (mean score ± standard error) in the vault and fossa 48 h after hair removal. TEWL, trans-epidermal water loss.

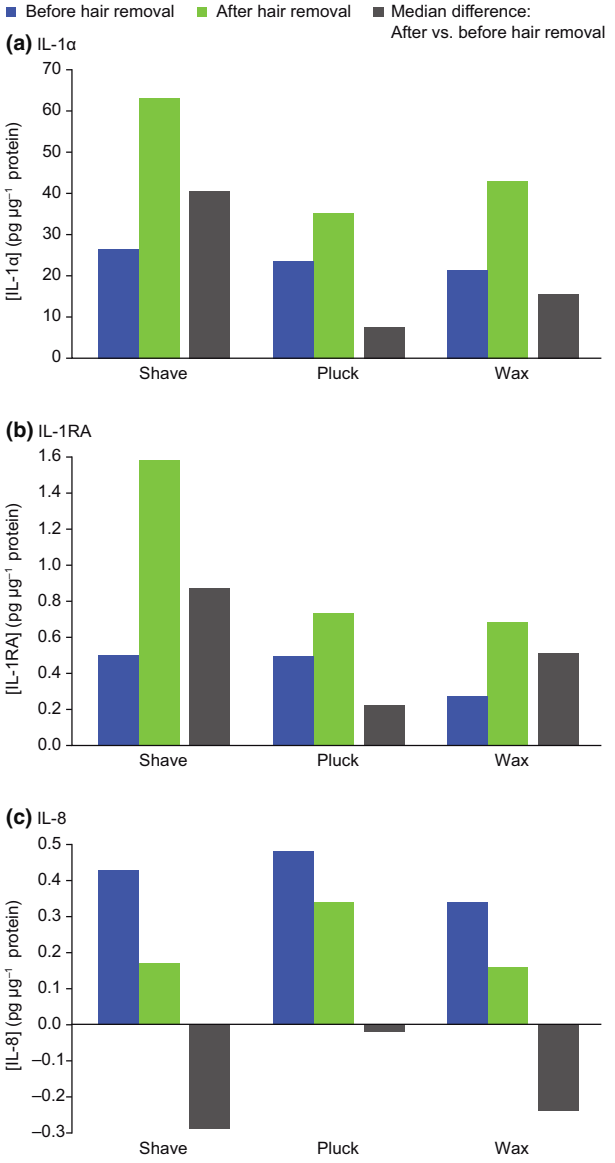

Figure 7 Normalized median concentrations of (a) IL-1α, (b) IL-1RA and (c) IL-8 in buffer scrub samples of whole axillae before and 30 min after hair removal. IL, interleukin; RA, receptor antagonist.

hair removal by each technique (Fig. 7c). The largest decreases compared with pre-hair removal were observed in shavers and waxers, followed by pluckers (Fig. 7c).

Discussion

Although a few studies have reported the effects of shaving and plucking on axillary skin appearance, little is known about the biophysical effects or inflammatory process associated with different hair-removal techniques. This study has provided novel insights into the differences in axillary skin reactions to shaving, plucking and waxing and has identified the principal cytokines associated with the inflammatory process triggered by axillary hair removal.

Visual assessments showed that hair removal by shaving, plucking and waxing leads to increases in axillary erythema and skin dryness scores. These results in Asian consumers are broadly consistent with earlier observations showing that hair removal results in axillary erythema and skin dryness in white subjects [4–6]. In the present study, shaving was associated with significantly less erythema but significantly greater skin dryness 30 min after hair removal compared with the other techniques. Across the whole study population, there was no evidence that a single hair-removal treatment via any of the methods examined in the study resulted in differences in axillary PIHP. Taken together, these results suggest that although single hair-removal events induce inflammation in the axillary skin of Asian consumers, this is not sufficient to induce PIHP. Thus, PIHP increases that individuals observe over time must result from cumulative effects. The data also show that none of the hair-removal techniques induced any biophysical changes in the quality of the axillary skin barrier, as measured by skin hydration, skin barrier integrity (TEWL) and skin surface roughness. In addition, none of these hair-removal habits stimulated changes in the mechanical (elastic) properties of the axillary vault and fossa 48 h after hair removal.

As far as we are aware, this is the first study to identify the principal cytokines associated with the inflammatory process triggered by axillary hair removal. This provides further details about the skin response step proposed in the axillary irritation cycle [6]. The results also suggest that buffer scrubs are a suitable technique (albeit with limitations – see below) for detecting some of the inflammatory response biomarkers associated with axillary irritation induced by hair removal. The results show that concentrations of both IL-1α and IL-1RA are elevated following hair removal by shaving, plucking or waxing, and that IL-8 concentrations are reduced following the same procedures. IL-1β, IL-4, IL-6 and histamine were detected occasionally, but generally at very low concentrations, and it was not possible to draw any statistically relevant conclusions regarding the level of involvement of these biomarkers with the hair removal-induced inflammatory cascade. Biomarker concentrations were generally lower in samples taken following hair plucking compared with the other techniques. One explanation for this would be that buffer scrubs primarily sample the surface of the skin, while inflammatory biomarkers released following irritation at the depth of the hair follicle (i.e. a few mm deep in the skin) would be less likely to be collected. Waxing also induces skin surface damage, which may explain why biomarker concentrations detected following waxing were similar to those following shaving.

The data imply that there is a degree of correlation between the increase in erythema observed in the biophysical study and the increase in IL-1α and IL-1RA concentrations seen following hair removal. Such a correlation would be expected, as erythema is known to result from the inflammatory response.

In terms of the broader understanding of the impact of hair removal on axillary skin, this study provides fresh insights. We can conclude that the principal inflammatory cytokines initially released as a consequence of shaving, plucking and waxing are IL-1α and IL-1RA. Changes in these cytokines are also seen at other skin sites. For example, scalp skin irritated by dandruff is characterized by a decrease in IL-1α concentration and an accompanying increase in IL-1RA concentration [13]. However, because of differences in the measurement techniques used in the two studies, it is not possible to compare axillary and scalp IL-1RA:IL-1α ratios and draw further conclusions concerning similarities or differences between the two skin sites. Both IL-1α and IL-1RA are known to be stored in large quantities in the keratinocytes located in the epidermis [14]. It is thus not surprising that these cytokines are released first in response to hair removal-induced irritation. However, an explanation for the observed decrease in the concentration of IL-8 (a neutrophil-targeting chemokine) following hair removal is less obvious. Neutrophils are typically located deep in the dermal tissue and are less likely to be associated with the epidermal–stratum corneum interface. The observed decrease in IL-8 concentrations seen across all hair-removal techniques could therefore be related to released IL-8 being targeted to neutrophil recruitment deeper in the dermis (to support secondary phase barrier recovery), thereby resulting in a decrease in buffer scrub sample concentrations. In the case of hair plucking, IL-8 may be retained deeper for longer (as damage from plucking is predominantly at the depth of the hair follicle), which might explain why there is a smaller post-event decrease in IL-8 concentration 30 min after plucking compared with shaving and waxing.

As a technique, buffer scrubs are clearly limited in their ability to reliably measure multiple biomarker responses. This is most likely due to the sampling technique being restricted to the skin surface. The opportunity clearly exists to investigate the characteristics of axillary inflammation further using novel approaches.

In summary, the biochemical data presented in this report provide new information on the inflammatory cascade initiated by hair-removal behaviours in typical Asian consumers. The underarms of individuals who shave, pluck and wax their axillary hair are characterized by similar inflammatory responses. However, based on the relative fold rises in IL-1α and IL-1RA in shavers (and to a lesser extent waxers) it could be argued that consumers who shave may benefit from higher concentrations of soothing skin care actives in their products than are needed by consumers who pluck their axillary hair.

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Conflicts of interest

Richard Evans, Robert Marriott and David Arnold are employees of Unilever Research and Development. Susan Bates was an employee of Unilever Research and Development at the time this study was completed.

References

1. Watkinson, A., Lee, R., Moore, A. *et al.* Identification of a barrier deficiency in the stratum corneum of the axilla. *J. Invest. Dermatol.* **117**, 415 (2001).
2. Watkinson, A., Lee, R.S., Moore, A.E. *et al.* Reduced barrier efficiency in axillary stratum corneum. *Int. J. Cosmet. Sci.* **24**, 151–161 (2002).
3. Wu, J.Q. and Kilpatrick-Liverman, L. Characterizing the composition of underarm and forearm skin using confocal raman spectroscopy. *Int. J. Cosmet. Sci.* **33**, 257–262 (2011).
4. Marti, V.P., Lee, R.S., Moore, A.E. *et al.* Effect of shaving on axillary stratum corneum. *Int J. Cosmet. Sci.* **25**, 193–198 (2003).
5. Turner, G.A., Moore, A.E., Marti, V.P. *et al.* Impact of shaving and anti-perspirant use on the axillary vault. *Int. J. Cosmet. Sci.* **29**, 31–38 (2007).
6. Evans, R.L., Marriott, R.E. and Harker, M. Axillary skin: biology and care. *Int. J. Cosmet. Sci.* **34**, 389–395 (2012).
7. James, A.G., Pople, J.E., Parish, W.E. *et al.* Histological evaluation of hyperpigmentation on female Filipino axillary skin. *Int. J. Cosmet. Sci.* **28**, 247–253 (2006).
8. Lacz, N.L., Vafaie, J., Kihiczak, N.I. *et al.* Postinflammatory hyperpigmentation: a common but troubling condition. *Int. J. Dermatol.* **43**, 362–365 (2004).
9. Taylor, S., Grimes, P., Lim, J. *et al.* Postinflammatory hyperpigmentation. *J. Cutan. Med. Surg.* **13**, 183–191 (2009).
10. Evans, R.L., Turner, G.A., Bates, S. *et al.* Human axillary skin condition is improved following incorporation of glycerol into the stratum corneum from an antiperspirant formulation. *Arch. Dermatol. Res.* **309**, 739–748 (2017).
11. Berardesca, E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin Res. Technol.* **3**, 126–132 (1997).
12. Rogiers, V. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol. Physiol.* **14**, 117–128 (2001).
13. Perkins, M.A., Cardin, C.W., Osterhues, M.A. *et al.* A non-invasive tape absorption method for recovery of inflammatory mediators to differentiate normal from compromised scalp conditions. *Skin Res. Technol.* **8**, 187–193 (2002).
14. Mee, J.B., Antonopoulos, C., Poole, S. *et al.* Counter-regulation of interleukin-1alpha (IL-1alpha) and IL-1 receptor antagonist in murine keratinocytes. *J. Invest. Dermatol.* **124**, 1267–1274 (2005).