

## INVESTIGATIVE REPORT

# Skin-identical Lipids Versus Petrolatum in the Treatment of Tape-stripped and Detergent-perturbed Human Skin

MARIE LODÉN and EBBA BÁRÁNY

ACO Hud AB, Upplands Väsby, Sweden

**The cutaneous permeability barrier is localized to the stratum corneum interstices and is mediated by lamellar bilayers enriched in cholesterol, free fatty acids and ceramides. Topically applied lipids may interfere with the skin barrier function and formulations containing “skin-identical lipids” have been suggested to facilitate normalization of damaged skin. The aim of the present study was to compare the ability of “skin-identical lipids” in a petrolatum-rich cream base and pure petrolatum to facilitate barrier repair in detergent- and tape-stripped-perturbed human skin. Barrier recovery and inflammation were instrumentally monitored for 14 days as transepidermal water loss and skin blood flow, using an Evaporimeter and a laser Doppler flowmeter, respectively. Treatment with the 2 different products gave no indication that “skin-identical lipids” in a cream base are more efficient than pure petrolatum at promoting normalization in either of the 2 experimentally perturbed areas. This finding may support the hypothesis that different types of skin abnormality should be treated according to the underlying damage. Key words: permeability barrier; transepidermal water loss; skin blood flow; emollients; moisturizers.**

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M. Lodén, ACO Hud AB, Box 622, SE-194 26, Upplands Väsby, Sweden. E-mail: marie.loden@acohud.se

The epidermal permeability barrier resides in the stratum corneum and is mediated by lamellar bilayers enriched in cholesterol, free fatty acids and ceramides. A disturbance of the epidermal barrier function induces a rapid response from the keratinocytes to restore cutaneous homeostasis. The mRNA coding for pro-inflammatory cytokines, adhesion molecules and growth factors is upregulated (1). Likewise there is an increase in DNA synthesis, leading to epidermal hyperplasia, and in lipid synthesis (2–7). The synthesis activity involves unsaponifiable lipids (3, 6, 7), fatty acids (3) and sphingolipids (8). Sterols and fatty acids are synthesized immediately after barrier disruption, whereas the increase in sphingolipid synthesis is somewhat delayed (8). Over time, the content of lipids in the stratum corneum is restored to the normal level in parallel with the return of barrier function (3–8).

Topically applied lipids have previously been considered to exert their effects on the skin solely by forming an inert, epicutaneous, occlusive membrane. Lipids have, therefore, been incorporated into formulations on the basis of their technical and sensory properties rather than on their possible impact on the epidermis. However, recent findings indicate that petrolatum is absorbed into the outer layer of delipidized stratum corneum (9) and that more physiological lipids

penetrate the skin (10–13) and modify endogenous epidermal lipids (11, 14) and the rate of barrier recovery (15–18).

Increased knowledge concerning the interactions between topically applied lipids and skin barrier homeostasis is expected to improve treatment of dry skin and inflammatory skin disorders (19, 20). Skin-identical lipids (SIL) (cholesterol, ceramide 3, oleic acid and palmitic acid) have also been incorporated in a cream base, and the aim of the present study was to compare the effect of this commercially available product on barrier recovery with that of pure petrolatum in disrupted human skin. The skin was damaged by an irritant (sodium lauryl sulphate, SLS) (17, 21, 22) and by mechanical removal of the outer stratum corneum by tape stripping (17, 23, 24). Barrier recovery and inflammation were instrumentally monitored for 14 days as transepidermal water loss (TEWL) and skin blood flow, using an Evaporimeter and a laser Doppler flowmeter, respectively (21, 24, 25).

## MATERIAL AND METHODS

### *Experimental design*

In a double-blind, bilateral, randomized study, 15 healthy volunteers (9 females, 6 males) treated the volar aspect of their forearms twice daily for 14 days with a test product after barrier abrogation. One of the arms was treated with a cream containing skin lipids and the other with pure petrolatum. Both products were supplied in white coded tubes. Informed consent was obtained from all volunteers and the study was approved by the local ethics committee, Uppsala University, Sweden. During the test period, the subjects were allowed to wash normally but were not allowed to use any other skin-care products on their arms. The test subjects were recruited among persons with no apparent knowledge about the test products. The study was carried out from March to April 1999.

One area in the middle of the volar aspect of each forearm was abrogated by exposure to SLS (22) and an adjacent area closer to the hand was stripped with adhesive tape (for details see below). Immediately after tape stripping these 2 areas and a non-treated control area were instrumentally evaluated (see below) and then treated with the cream. The next evaluations were performed after 4 h, and then after 1, 4, 8 and 14 days. To ensure that no cream residue was left on the skin to influence the results, the subjects were asked to wash their forearms on the morning of Days 1, 4, 8 and 14 and then not to apply any product before the measurement. Measurements were obtained within 1 day of Days 4, 8 and 14.

### *Test products*

Locobase<sup>®</sup> Repair (Yamanochi Pharma AB, Sweden) contains a SIL mixture consisting of ceramide-3, cholesterol and the fatty acids oleic acid and palmitic acid in a fatty cream base with water, petrolatum, paraffin, paraffin liquidum, carnauba, sorbitan oleate, carbomer, tromethamine and glycerine as excipients. Petrolatum (Wilburine;

Witco Corporation, conforming to USP) consists of hydrocarbons of different chain lengths.

#### Barrier abrogation

The skin on the volar aspect of both forearms was exposed to 2% aqueous solutions of SLS (99% purity; Sigma-Aldrich). A 50  $\mu$ l aliquot of the solution was pipetted onto 1 layer of filter paper placed in 12 mm diameter aluminium chambers (Finn chambers; Epitest OY, Finland). The chambers were fixed to the skin for 24 h with adhesive tape (Scanpore; Norgeplaster, Oslo, Norway). Upon removal of the patches, the skin was gently rinsed with water and allowed to dry; 1 h later each site was examined instrumentally, followed by treatment with the creams.

An adjacent area on the volar aspect (below the SLS patch) was successively stripped 40 times with adhesive tape (Scotch Tape 500). The test areas were marked with a skin compatible and water-proof pen.

#### Instrumental evaluation

All measurements were performed without knowledge of prior cream treatments. A control area above the SLS patch was also measured prior to any treatment (0 h). TEWL was recorded first, followed by skin blood flow. TEWL was quantified using an Evaporimeter EP1 (Servomed, Kinna, Sweden). After applying the probe to the skin, TEWL values were automatically transferred to a computer during the following 70 s. The mean values of the last 30 s of measurements were recorded and used for further calculations. The cutaneous blood flow was measured with a laser Doppler flowmeter (Periflux Pfl; Perimed, Stockholm, Sweden) equipped with a special multifibre probe (PF 113 integrating probe, Perimed) which had 7 fibre triplets instead of 1, 1 in the centre and 6 forming a circle 8 mm in diameter around it. Thus, each blood flow value is the mean of the 7 spots, which reduces variation due to spotty erythema. The probe was attached to the skin with a standard probe holder without pressure and using double-sided adhesive tape. The output signals were recorded on a chart strip recorder and the value at equilibrium was used for the calculations.

#### Statistics

The results are presented using box plots. The bottom line of the box is the first quartile (Q1), and the top is at the third quartile (Q3) value. A line is drawn across the box at the median. The whiskers are the lines that extend from the top and bottom of the box to the lowest

and highest observations that are still inside the region defined by the following limits:

1. Lower limit:  $Q1 - 1.5 (Q3 - Q1)$
2. Upper limit:  $Q3 + 1.5 (Q3 - Q1)$

Outliers are points outside the lower and upper limits and are plotted with asterisks.

The Wilcoxon signed rank test on paired data was used to test the differences between the SIL- and petrolatum-treated areas. Correction of multiplicity was performed according to Holm (26) to obtain the overall significance level of  $p \leq 0.05$ . Minitab<sup>®</sup> statistical software, Release 12 for Windows, was used for calculations and plots.

## RESULTS

In the control skin TEWL was 6.4  $\text{g}/\text{m}^2/\text{h}$  (interquartile range 5.3–7.8) and skin blood flow 30 AU (interquartile range 27–43).

Barrier abrogation with SLS increased TEWL to 20–30  $\text{g}/\text{m}^2/\text{h}$  and skin blood flow to 100–150 AU (Fig. 1) ( $p < 0.001$ ). During the following 2 weeks these values decreased, but no statistically significant differences between the treatments were found (Fig. 1). Normalization of TEWL and skin blood flow followed a similar pattern.

Tape stripping of skin increased TEWL to 10–12  $\text{g}/\text{m}^2/\text{h}$  ( $p = 0.003$ ) and skin blood flow to 30–40 AU ( $p = \text{NS}$ ) before the first application of the creams (Fig. 2). During the next 2 weeks, no significant differences between the 2 areas were found, except on day 4 where a significantly higher skin blood flow was noted in the area treated with SIL ( $p = 0.007$ ).

## DISCUSSION

Dermatologists have always realised that emollients have a steroid-sparing effect and are important treatment adjuncts in inflammatory skin disorders; however, their mechanism of action has not been fully understood (27). Apart from hydrating the stratum corneum, topically applied lipid mixtures (16, 18) and commercially available creams have been shown to accelerate skin barrier recovery in experimentally perturbed human skin (25, 28, 29). Normalization of barrier function is considered to be of benefit in diseased skin, as it has been suggested to prevent persistent dermatitis by

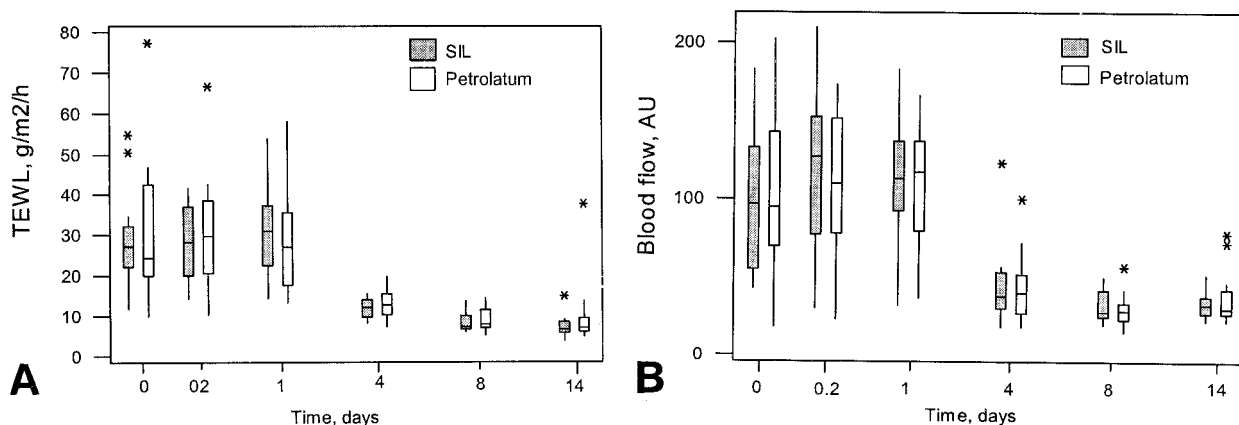


Fig. 1. Transepidermal water loss (TEWL) ( $\text{g}/\text{m}^2/\text{h}$ ) (A) and skin blood flow (AU) (B) in human skin exposed to SLS and treated with "skin-identical lipids" (SIL) and pure petrolatum for 14 days. The results are presented as box plots with the median value as a line across the box and the first quartile value at the bottom and the third at the top. The whiskers are the line that extend from the top and bottom of the box to the lowest and highest observation within a defined region, with outliers plotted as asterisks outside this region ( $n = 15$ ).

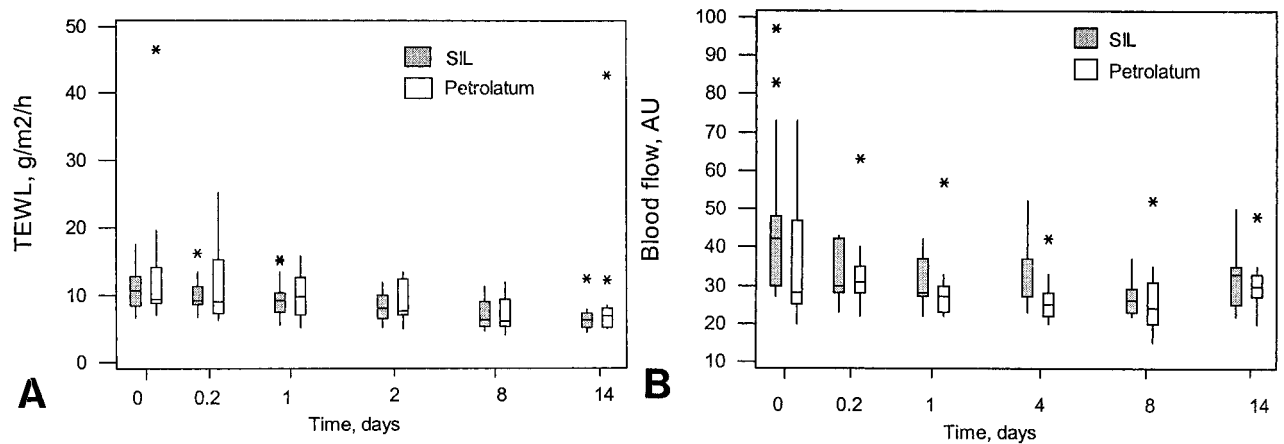


Fig. 2. Transepidermal water loss (TEWL) ( $\text{g}/\text{m}^2/\text{h}$ ) (A) and skin blood flow (AU) (B) in tape-stripped human skin and skin treated with "skin-identical lipids" (SIL) and petrolatum for 14 days. The results are presented as box plots with the median value as a line across the box and the first quartile value at the bottom and the third at the top. The whiskers are the line that extend from the top and bottom of the box to the lowest and highest observation within a defined region, with outliers plotted as asterisks outside this region ( $n=15$ ).

mitigation of the cytokine cascade (30). Thus, it is tempting to assume that SIL are superior to pure petrolatum in the treatment of barrier-abrogated skin. In the present study, 2 models for barrier deterioration were used. One reflects irritant contact dermatitis, caused by exposure to an aqueous solution of a surfactant (21). In the other model TEWL is increased by mechanical removal of the outer layers of the stratum corneum by stripping the skin with adhesive tape. Stripping the skin with tape has been used to assess the ability of the layer to withstand, or recover from, insults to the epidermal permeability barrier in both subjects of different race, sex and skin type (24) and in patients with atopic dermatitis (23). Adding measurements of skin blood flow to the TEWL evaluations provides additional data on the normalization of the skin, as TEWL may be depressed by physical blocking of the surface with occlusive material without any true decrease in skin damage, such as inflammation.

Treatment with the 2 different creams gave no indication that SIL in a cream base are more efficient than pure petrolatum to promote normalization in either of the 2 perturbed areas. SIL did not reduce either TEWL or skin blood flow more efficiently than petrolatum in the 2 experimental models used. However, both formulations may well have accelerated barrier recovery compared to no treatment at all. Previous studies have shown that the composition of the applied lipids is crucial to the rate of barrier recovery (15, 18). Application of an equimolar mixture of ceramide, fatty acid and cholesterol, or pure cholesterol is reported to allow normal barrier recovery in mice (15), whereas a lipid mixture with cholesterol as the dominant lipid has been found to accelerate barrier recovery in aged human skin (16). Other compositions of the lipid mixtures may delay barrier recovery and certain fatty acids, such as oleic acid, are also known to increase skin permeability, probably by inducing a less ordered, more heterogeneous state of the stratum corneum lipids (31). Thus, the presence of oleic acid in SIL or a suboptimal lipid mixture may explain its non-superiority compared to petrolatum. Replacement of oleic acid with linoleic acid (an essential fatty acid) in a patented

lipid mixture is claimed to be effective in damaged human skin (18). Thus, the composition of the applied formulations seems important, emphasizing the need for more comparative studies on different formulations in human skin. Different compositions may well have different efficacies depending on the type of skin defect (17, 19).

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