N-acyl ethanolamide and eicosanoid involvement in irritant dermatitis

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What is already known about this topic?

- Bioactive lipid mediators are emerging as important players in cutaneous homeostasis and inflammation.
- Irritant dermatitis is a considerable problem in dermatology/occupational health, and would benefit from greater understanding of the role of lipids in skin's response to irritants.

What does this study add?

- Specific eicosanoid and endocannabinoid mediators contribute to the inflammatory response to common irritant SLS, but not to UVR-challenge matched to generate comparable erythema.
- Findings provide insights into pathways involved in irritant dermatitis, with potential translation to novel treatments and new means for assessing contact irritants.

Abbreviations:

AA, arachidonic acid; AEA, arachidonoyl ethanolamide; CB, cannabinoid receptor; COX, cyclooxygenase; DGLA, dihomogamma-linolenic acid; DHA, docosahexaenoic acid; EA, ethanolamide; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HFA, hydroxy fatty acid; HODE, hydroxyoctadecadienoic acid; ICD, irritant contact dermatitis; LA, linoleic acid; LC/ESI-MS/MS, liquid chromatography coupled to electrospray ionisation tandem mass spectrometry; LOX, lipoxygenase; MED, minimum erythema dose; NAE, N-acyl ethanolamide; OEA, oleoyl ethanolamide; PEA, palmitoyl ethanolamide; PG, prostaglandin; PUFA, polyunsaturated fatty acids; SEA, stearoyl ethanolamide; SLS, sodium lauryl sulfate; SPE, solid phase extraction; TEWL, transepidermal water loss; TRPV1,

transient receptor potential cation channel subfamily V member 1; TX, thromboxane; UVR, ultraviolet radiation.

Summary

Background: Sodium lauryl sulfate (SLS) and ultraviolet radiation (UVR) represent two commonly-encountered cutaneous inflammatory stimuli. Differing histopathological and clinical features implicate involvement of alternative inflammatory pathways; bioactive lipid mediators, including eicosanoids, endocannabinoids and sphingolipids, are likely candidates for regulation of the divergent inflammatory responses.

Objectives: Perform a comprehensive assessment of bioactive lipid involvement in SLS- and UVR-induced inflammatory responses, to provide a better understanding of bioactive lipid mediator pathways in irritant inflammation.

Methods: Buttock skin in 10 healthy volunteers was treated with two minimal erythema doses of UVR (275-380nm, peak 305nm), or an SLS dose optimised for each individual to produce a comparable, moderate erythema. Punch biopsies were taken 24h post-challenge and from untreated skin, and separated into dermis and epidermis. Lipids (including 15 prostanoids, 15 hydroxy fatty acids (HFA), 9 endocannabinoids and related N-acyl ethanolamides (NAE), and 21 sphingolipids) were extracted and quantified using liquid chromatography coupled to tandem mass spectrometry.

Results: We observed increased epidermal NAE and HFA expression in response to SLS, but not the UVR-induced low level inflammation. Significant changes following SLS treatment included augmented levels of NAE, possessing pro-inflammatory and some reported anti-inflammatory properties, with 3.7-fold ($P=0.025$) and 3-fold ($P=0.009$) expression of palmitoyl and stearoyl ethanolamides, respectively, in addition to 1.9-fold ($P=0.017$) expression of the chemoattractant 12- hydroxyeicosatetraenoic acid (12-HETE).

Conclusion: The differential bioactive lipid upregulation implicates their involvement in skin irritant responses, potentially reflecting roles in inflammatory cell recruitment and subsequent

resolution of inflammation and giving scope for new treatment approaches in irritant dermatitis.

Introduction

Skin provides a protective barrier that regulates water loss, immune and inflammatory reactions. Dysregulation of cutaneous inflammation contributes to pathological conditions impacting on quality of life and inflicting societal economic burden^{1,2}. Two commonly-encountered environmental stressors are sodium lauryl sulfate (SLS) and ultraviolet radiation (UVR). SLS is an anionic surfactant that deposits on human skin³, which may result in irritant reactions in high dose or long-term exposure. At high levels (up to 20% w/v), SLS is a well-studied positive control commonly used *in vitro* and *in vivo* to study irritant inflammation⁴⁻⁶. Irritant contact dermatitis (ICD) is a significant issue in dermatology and occupational health. People in certain occupations, such as cleaners, hairdressers, and chemical workers, are particularly susceptible due to regular exposure to high concentrations of irritants such as SLS. This is additionally of major concern in healthcare workers, since its presence in some soaps can discourage people from performing effective and regular hand-washing, and can cause skin barrier breakdown, together leading to increased bacterial colonisation, and transfer to patients⁷. Examination of the differential involvement of bioactive lipids in the inflammatory responses to UVR and SLS may assist identification of molecular pathways underlying ICD, which may both suggest biomarkers to aid identification of potential irritants at sub-clinical doses, and reveal specific therapeutic targets for ICD.

Cutaneous inflammation and immunity may involve various lipid mediators including eicosanoids, endocannabinoids and ceramides, expressed in both epidermis and dermis^{8,9}. Eicosanoids are derivatives of 20-carbon polyunsaturated fatty acids (PUFA) arachidonic acid (AA), eicosapentaenoic acid (EPA) and dihomo-gamma-linolenic acid (DGLA); cyclooxygenase (COX)-mediated reactions produce prostanoids while lipoxygenases (LOX) and cytochrome P450 monooxygenases (CYP) yield hydroxy fatty acids (HFA). Octadecanoids derived from 18-carbon linoleic acid (LA) and docosanoids from 22-carbon

docosahexaenoic acid (DHA) are also produced by the same enzymes¹⁰. Properties of the eicosanoid prostaglandin (PG) E₂ include vasodilation, keratinocyte and fibroblast growth, differentiation and migration⁸, while 12-hydroxyeicosatetraenoic acid (HETE) is a potent leukocyte chemoattractant¹¹, and 9- and 13-hydroxyoctadecadienoic acid (HODE) promote inflammatory cytokine production and differentiation in keratinocytes^{12,13}.

The endocannabinoids arachidonoyl ethanolamide (anandamide; AEA) and 2-arachidonoyl glycerol (2-AG) are also AA derivatives, while other fatty acid ethanolamides (N-acyl ethanolamides; NAE) also exhibit endocannabinoid-like properties⁸. Agonists of the endocannabinoid receptor (CB) CB1 inhibit keratinocyte proliferation and keratin production¹⁴, AEA and 2-AG promote sebum production¹⁵, and linoleoyl-EA (LEA) can reduce contact dermatitis and inflammatory cytokine expression¹⁶. Finally, skin expresses a range of sphingolipids including ceramides, sphingoid bases and phosphorylated species^{9,17}. Whilst ceramides are crucial for the epidermal barrier¹⁸, phosphorylated ceramides and sphingoid bases play signalling roles¹⁹.

Although there is growing evidence for the contribution of lipid mediators in irritant- and UVR-induced inflammation, there are no comparative studies. SLS in high doses (5% w/v) can cause loss of the dermo-epidermal junction, damage of cutaneous proteins and disruption of the epidermal barrier²⁰. The latter can activate the innate immune system, with concomitant production of inflammatory cytokines and immune cell recruitment²¹. While some prostanoids have been measured in SLS-treated skin²², the role of endocannabinoids and NAE in ICD remains unclear. Interestingly, falcarinol, a pesticide causing ICD, has been shown to covalently bind the CB1 receptor²³. Ceramides are important in skin barrier function, and whilst SLS irritation increases transepidermal water loss (TEWL), this effect is reduced in subjects with high ceramide levels^{24,25}. Phosphorylated sphingolipids such as ceramide-1-phosphate (C1P) and sphingosine-1-phosphate (S1P) activate PLA₂ and COX-2,

respectively, potentially promoting eicosanoid production and activation of mast cells¹⁹. Little else is known about the involvement of other sphingolipids in irritant reactions.

Several eicosanoid species including PGE₂ and 12-HETE have been shown to be involved in the acute inflammatory response of human skin *in vivo*, to high dose (four times the minimum erythema dose (4xMED)) UVR²⁶⁻²⁹. Additionally, NAE expression was augmented in response to very high level UVR (~12xMED) in an epidermal cell line³⁰, but less is known concerning effects of lower more physiological UVR doses. Repeated UVR exposure appears to improve barrier function although it does not change the ratio of stratum corneum ceramides³¹.

In this study, *in vivo* responses of three families of bioactive lipids to two common inflammatory stimuli in human skin were concurrently assessed. Both sunburn and ICD reactions demonstrate erythema as a central component, while features of sunburn can also include oedema and pain, and irritant reactions may involve oedema, dryness, scaling, itching and burning. By generating comparable erythema using topical SLS and a moderate dose (2xMED) of UVR, and using lipidomics³², we sought to understand similarities and differences in the lipid mediator response to these stressors. Distinct lipid responses were generated in the irritant-challenged skin; identification of specific lipid biomarkers of inflammation highlights potential future interventions in ICD.

Materials and Methods

Participants

Ten healthy volunteers were recruited (seven female, three male; median age 22, range 20-59yrs; skin type II (n=1) or III (n=9)³³). Volunteers were excluded if pregnant or breastfeeding, had a history of skin cancer or photosensitivity, or were taking photoactive or anti-inflammatory medication or nutritional supplements. Ethical approval was granted by The University of Manchester research ethics committee (project 12087). Written informed consent was obtained before inclusion. The study was performed in line with the Declaration of Helsinki, at Salford Royal NHS Foundation Hospital, Manchester, UK.

Sodium lauryl sulfate irritant inflammation

On their first visit volunteers received a patch test series of five SLS doses (0.3, 0.6, 1.2, 2.5, 5% w/v in distilled water; ACS reagent $\geq 99\%$, Sigma-Aldrich, Poole, UK), to determine the subject-specific dose to be used. SLS solutions (20 μ l) were pipetted onto filter paper discs inside 8mm Finn Chambers (Bio-diagnostics Ltd, Worcestershire, UK). The chambers were applied to photoprotected upper buttock skin and remained in place for 24h. Irritant response was assessed 24h after patch removal, using the European Society of Contact Dermatitis (ESCD) guidelines on clinical scoring of irritant reactions (scoring system based on visual inspection)³⁴. The dose that induced a moderate and confluent erythematous response, comparable with the erythema produced by 2xMED of UVR, was selected and applied to two further sites. Erythema was measured in triplicate at both treated and untreated sites using a spectrophotometer (CM 600d, Konica Minolta Sensing Europe BV, Warrington, UK) which provided a Haemoglobin (Hb) Index, prior to skin sampling.

UVR-induced inflammation

The MED for each individual was determined as previously described²⁸, applying a geometric series of ten doses (7-86mJ/cm²) of erythemally-weighted UVR to photoprotected upper buttock skin (TL20W/12 broadband UVB lamp; 270-400nm, peak 310nm; Philips GmbH, Hamburg, Germany). To provoke a moderate confluent erythematous response comparable to the moderate irritant inflammation induced by the individualised SLS dose, a dose of 2xMED of UVR was given to two distinct 10mm diameter areas of photoprotected upper buttock skin. Erythematous response was measured by spectrophotometry (as described above for SLS) 24h later, prior to skin sampling.

Skin sampling

Skin punch biopsies (6mm, Militec Inc. York, USA) were taken using lignocaine local anaesthetic (1%; Antigen Pharmaceuticals Ltd., Tipperary, Ireland), in duplicate, from: 1) SLS-treated skin (24h post-patch removal), 2) UVR-exposed skin (24h post-irradiation) and 3) control untreated skin. Samples were bisected, snap-frozen and stored at -80°C. Prior to analysis, skin was separated into dermis and epidermis by scalpel on ice to avoid degradation of lipid mediators, as described previously⁹; separation of the skin layers was confirmed by visual inspection at 40X magnification. While precise separation was not achieved, and epidermal samples demonstrated minor contamination with dermal tissue, there was no contamination of dermal tissue with epidermal tissue. Although 10 volunteers were recruited, loss of SLS patches resulted in n=9 complete datasets.

LC-MS/MS analysis of eicosanoids

Analysis was performed as described previously⁹. Briefly, skin samples (5-20mg) were homogenised in ice-cold methanol (15% v/v) with internal standards added to each sample

(40ng each of 12-HETE-*d*8 and PGB₂-*d*4; Cayman Chemicals, Ann Arbor, MI, USA). Homogenates were semi-purified by solid phase extraction (SPE; C18-E; Phenomenex, Macclesfield, UK) and eicosanoids analysed by LC/ESI-MS/MS using a triple quadrupole mass spectrometer with electrospray probe coupled to liquid chromatography (Quattro Ultima, Waters, Elstree, Hertfordshire, UK). Data are expressed as pg/mg protein.

LC-MS/MS of endocannabinoids and N-acyl ethanolamides

Analysis was performed as described previously⁹. Skin samples (5-20mg) were homogenised in ice-cold 2:1 (v/v) chloroform/methanol (3ml per sample) with internal standards added (40ng each of AEA-*d*8 and 2-AG-*d*8; Cayman). Water was then added (500µl per sample), the organic extracts separated by centrifugation (1500xg, 4°C, 5min), and analysed by LC/ESI-MS/MS. Data are expressed as pg/mg protein.

LC-MS/MS analysis of sphingolipids

Analysis was performed as described previously⁹. Skin samples (5-20mg) were homogenised in ice-cold isopropanol:water:ethyl acetate (30:10:60; v/v/v; 4ml per sample), internal standards (Ceramide/Sphingoid Internal Standard Mixture I; 200pmol per standard; Avanti Polar Lipids, Alabaster, Alabama, USA) were added and protein precipitates were removed by centrifugation (1500xg, 4°C, 10min). The lipid extract was analysed by LC/ESI-MS/MS. Data are expressed as pmol/mg protein.

Protein content

During lipid extractions, protein pellets were retained for analysis of protein content using a standard Bradford protein assay kit (Bio-Rad Protein Assay, Bio-Rad, Hemel Hempstead, UK)³⁵.

Statistical analysis

Statistical analyses were performed using repeated measures ANOVAs with Greenhouse-Geisser corrections and Bonferroni post-hoc tests. Analyses were conducted using SPSS 20 software and $P < 0.05$ was considered significant.

Results

Optimisation of SLS and UVR dose

In order to allow direct comparison between SLS- and UVR-induced clinical inflammation, comparable erythema for the two conditions were generated. Since individual responses vary^{26,34}, erythema dose-response testing was performed to both SLS and UVR (**Fig.1a-d**). Doses of SLS and UVR were selected such that the overall levels of inflammation, as assessed by erythema, were the same for all volunteers and both treatments (**Fig.1e**).

Effect of SLS and UVR on cutaneous production of eicosanoids and related species

Fifteen prostanoids and fifteen HFA were quantified in the dermis and epidermis at baseline and following challenge with SLS and UVR (**Fig.2**). SLS did not affect PG production but increased epidermal TXB₂ expression to 1.7-fold that of control (P=0.005) (**Fig.2b**). Although the SLS challenge induced an overall increase in epidermal HFA, only the proinflammatory chemoattractant 12-HETE reached a statistically-significant increase, to 1.9-fold that of unstimulated expression (P=0.017) (**Fig.2d**). The UVR challenge used in this study did not have any measurable effect on cutaneous prostanoid and HFA species.

Effect of SLS and UVR on cutaneous endocannabinoids and N-acyl ethanolamides.

The endocannabinoids AEA and 2-AG, and seven other NAE, were quantified in the dermis and epidermis (**Fig.3**). Epidermal AEA, 2-AG and NAE expression was much higher than dermal. Most epidermal mediators demonstrated increased expression in response to SLS, with statistically-significant increases for stearoyl-EA (SEA), oleoyl-EA (OEA), LEA and palmitoyl-EA (PEA) at levels that reached 3-fold (P=0.009), 5.6-fold (P=0.032), 4.2-fold (P=0.031) and 3.7-fold (P=0.025), respectively, that of untreated skin. There was no significant change in 2-AG expression in response to either SLS or UVR exposure. Although

a similar trend was observed in the dermis, the SLS-induced changes did not reach statistical significance (**Fig.3a**). The UVR treatment had no effect on dermal or epidermal endocannabinoids and NAEs.

Effect of SLS and UVR on cutaneous sphingolipids

Two sphingoid bases and their phosphorylated forms, thirteen ceramides and four phosphorylated ceramides were quantified in dermal and epidermal tissue. Whilst epidermis exhibited higher levels of all these sphingolipids compared to dermis, neither SLS nor 2xMED UVR treatment affected the levels of these cutaneous mediators (**Supplementary Fig.1-2**).

Discussion

Bioactive lipids are emerging as important players in cutaneous inflammation⁸. We investigated the response of three lipid mediator families to two commonly-encountered inflammatory stimuli, the irritant surfactant SLS and environmental stressor UVR, to gain a better understanding of their involvement in skin health and disease. This study identifies a number of bioactive lipids involved in the dermal and epidermal responses to SLS. Importantly, induction of inflammation of a similar magnitude (as assessed by comparable erythema), by UVR, did not induce the same lipid response. This indicates the specific roles of lipid mediators in different cutaneous inflammatory responses, and highlights potential targets in the treatment of ICD.

Changes in both eicosanoids and endocannabinoids were observed in response to SLS. Two potent leukocyte chemoattractants³⁶⁻³⁸, thromboxane A₂ (TXA₂; measured as its stable derivative TXB₂) and 12-HETE, were significantly upregulated in response to SLS, suggesting their potential contribution to immune cell recruitment, a major feature of SLS-induced irritancy²¹. This is supported by previous observations of 12-HETE release in skin organ culture in response to higher concentrations of SLS³⁹. Additionally, 12-HETE acts as a vasodilator and could contribute to SLS-induced erythema⁴⁰.

Although 12-HETE was the only HFA significantly upregulated following SLS treatment, a similar trend among other HFAs indicates that SLS stimulated LOX pathways and HFA production to a greater extent than COX-mediated prostanoid production. SLS has been reported to increase PGE₂ production in suction blister fluid²², while low levels (0.075%) showed no effect on COX-2 mRNA expression in reconstructed human epidermis⁴¹. Recent proteomic analysis of SLS-induced skin changes found no change in COX-1 expression, although this was after only 4h of treatment⁶. Interestingly, LEA, OEA and PEA, all found significantly upregulated in response to SLS (**Fig.3**), are known

suppressors of COX-2 activation and prostanoid production, and may have negated any SLS-induced COX expression^{16,42,43}. Overall, it is possible the SLS dose used in the present study was below the threshold to significantly upregulate eicosanoid production. While it would be interesting to determine the effect of higher doses, our study protocol is more relevant to moderate skin irritancy, and the early course of irritant responses where specific interventions may be most effectively targeted.

Our data suggest that SLS may specifically activate cutaneous LOX isoforms; although a similar LOX-specific effect has previously been seen in platelets in response to cigarette smoke⁴⁴, there are no similar reports in skin in response to irritants. Another possibility is that endocannabinoid-mediated inhibition of COX-2 may direct fatty acid substrates to the LOX pathway, resulting in increased HFA rather than prostanoid production; this hypothesis requires further exploration.

The increased expression of NAE species in response to SLS indicates increased activity of the enzymes involved in this pathway. NAE congeners of endocannabinoids are believed to operate through an entourage effect, boosting the activity of AEA through enhanced transient receptor potential cation channel subfamily V member 1 (TRPV1) binding or impaired AEA catabolism^{45,46}. Exogenous SEA has been shown to reduce allergic skin inflammation via TRPV1⁴⁷, while both SEA and PEA have been implicated in pain, although not in skin^{48,49}. Topical application of SLS modulates pain responses in the skin and our finding suggests the potential involvement of NAE⁵⁰. Also recently linked to inflammatory pain are the endogenous TRPV1 ligands 9- and 13-HODE⁵¹, which showed non-significant upregulation following SLS treatment. Pruritus is a more prominent symptom of SLS irritation than pain, and PEA is known to have anti-pruritic effects, with increased production thought to represent an attempt to quench the irritant-induced itch⁵². Finally, OEA and AEA have been implicated in vasodilation, and could be partially responsible for the erythema

observed, alongside 12-HETE^{53,54}. Overall, such a marked lipid response to an SLS concentration inducing only a moderate erythema suggests that sub-erythematous doses may also alter NAE metabolism. This should be further explored, since erythema is currently often used as a marker for inflammation, and NAE expression may act as a subclinical indicator for assessing irritants⁵⁵⁻⁵⁷.

Whether the NAEs mediate the pathogenesis of ICD, or their increased expression indicates the skin's attempt to moderate the inflammation, requires further investigation. It has been shown that the NAE pathway can be manipulated using receptor agonists/antagonists, or by applying enzyme inhibitors to alter NAE metabolism, for example inhibitors of fatty acid amide hydrolase (FAAH) may be a useful treatment for dry skin⁵⁸. Further, topical treatments including PEA have proven an effective eczema treatment, and merit investigation in ICD^{59,60}.

SLS irritation impairs epidermal barrier function and can alter mRNA expression of enzymes involved in ceramide synthesis^{61,62}. However, no changes in expression of non-hydroxylated medium-chain ceramides and sphingoid bases examined here were observed. These sphingolipids are not specific to the stratum corneum and can be found in epidermal and dermal cellular membranes, where they mediate regulation of apoptosis and inflammation⁶³. UVR has been shown to increase ceramide production by keratinocytes *in vitro* although the effect of low-dose UVR was short lived, and by 24h levels had returned to normal⁶⁴. It is possible that changes in sphingolipid expression could be observed *in vivo* at different time-points, but none appeared to contribute to SLS- or UVR-induced inflammation 24h post-treatment.

Despite UVR and SLS generating similar levels of erythema, different lipid responses were observed. The modest UVR dose used in the present study could explain the absence of the upregulated eicosanoid production previously seen with higher UVR doses²⁸. This

suggests the lower level inflammation induced in the present study did not reach the threshold for detectable COX/LOX activation, and that the erythema was induced by eicosanoid-independent mechanisms, which may include nitric oxide²⁶. Indeed, nitric oxide mediates the erythema generated in human skin by low dose UVR, with prostanoids making a greater contribution at higher UVR doses⁶⁵. Similarly, it is possible that different pain pathways are involved in SLS- and UVR-induced inflammation, which could explain why NAEs were upregulated in response to SLS but not UVR.

We have profiled a range of lipid mediators following cutaneous challenge with SLS and moderate UVR, and highlight the differential roles of bioactive lipids in the different types of inflammation. SLS itself is of great relevance to skin health as it is a prevalent ingredient in cleaning and industrial products, and one of the most common inducers of ICD, often affecting the hands and with employment repercussions. By identifying mediators involved in initiation of SLS-induced irritancy, and directly in human skin *in vivo* as opposed to experimental models, we gain an understanding of the early events in the pathogenesis of ICD. Endocannabinoids and their NAE congeners may mediate ICD or be involved in the host response, and the impact of manipulating the NAE pathway with receptor agonists/antagonists and enzyme inhibitors warrants investigation. A successful intervention might prevent unavoidable occupational exposure to certain chemicals from developing into ICD.

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Figure Legends

Figure 1. Optimisation of SLS and UVR treatments. Panels **a-d** represent one individual's dose optimisation, performed to identify the doses that initiated matching moderate erythema (2.5 % SLS (**a** and **b**) and 58 mJ/cm² UVR (2x the MED of 29 mJ/cm²; **c** and **d**)). Treated skin was compared with untreated skin in all individuals to confirm consistent erythema induction (**e**). Data are expressed as Hb index (representing the haemoglobin measurement in the treated area of skin), mean $\pm$ SE, n=3 technical repeats for parts **a-d**, n=9 volunteers for part **e**.

Figure 2. Prostanoids and hydroxy fatty acids were measured by LC/ESI-MS/MS in the dermis (**a** and **c**, respectively) and epidermis (**b** and **d**, respectively) under control conditions and following treatment with SLS or UVR. Data are expressed as mean $\pm$ SE, n=9 volunteers. *P<0.05, **P<0.01 vs control.

Figure 3. N-acyl ethanolamides and arachidonoyl glycerol were measured by LC/ESI-MS/MS in the dermis (**a**) and epidermis (**b**) under control conditions and following treatment with SLS or UVR. Data are expressed as mean $\pm$ SE, n=9 volunteers. *P<0.05, **P<0.01 vs control.

Figure 1

Figure 2

Figure 3

Supplementary Information

SUPPLEMENTARY FIGURE 1

Supplementary Figure 1. Sphingolipids were measured by LC/ESI-MS/MS in the dermis and epidermis under control conditions and following treatment with SLS or UVR. Lipid species are grouped into sphingoid bases and phosphorylated sphingoid bases (**a** – dermis, **b** - epidermis), phosphorylated ceramides (**c** – dermis, **d** - epidermis). Data are expressed as mean $\pm$ SE, n=9 volunteers.

SUPPLEMENTARY FIGURE 2

Supplementary Figure 2. Ceramides were measured by LC/ESI-MS/MS in the dermis (a) and epidermis (b) under control conditions and following treatment with SLS or UVR. Data are expressed as mean $\pm$ SE, n=9 volunteers.