doi:10.36849/JDD.6331

ORIGINAL ARTICLE

Journal of Drugs in Dermatology

Alteration to the Skin Ceramide Profile Following Broad-Spectrum UV Exposure

Rebecca Barresi, Hawasatu Dumbuya PhD, I-Chien Liao PhD, Ying Chen PhD, Xi Yan MD PhD, Janet Wangari-Olivero PhD, Nada Baalbaki PhD, Stephen Lynch PhD, Patricia Brieva PhD, Miao Wang, Qian Zheng MD PhD, Charbel Bouez PhD

L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

The epidermal *stratum corneum* (SC) lipid matrix, principally consisting of an equimolar ratio of ceramides, free fatty acids, and cholesterol, plays a crucial role in maintaining proper skin barrier function. Conditions which impair barrier integrity, such as in atopic dermatitis, correlate with the alternation of key ceramide subclasses and reduced chain length of acyl moieties. However, there is limited knowledge about the impact of unprotected repeat sun exposure on the skin lipid composition, especially ceramide profiles.

This study investigated the effects of ultraviolet (UV) radiation on the ceramide profile using both an *ex vivo* skin and a clinical model. Lipidomic analysis of UV-exposed skin showed shifts to the composition of ceramide subclasses essential in repairing and strengthening the SC barrier (including CER1[EOS], CER3[NP], and CER6[AP]) and reduced very long-chain acyl moieties. Gene expression analysis and immunohistochemical staining of key enzymes (aSMase, DES1, CerS5, CerS3) suggested that lipid alterations can be attributed to changes within the ceramide biosynthesis process. Topical application of ceramide-containing suncare products help maintain SC-essential ceramide subclasses and proper ceramide chain length, demonstrating the importance of proper photoprotection to maintain healthy skin barrier and ceramide quality during daily sun exposure.

J Drugs Dermatol. 2022;21(1):77-85. doi:10.36849/JDD.6331

INTRODUCTION

A healthy SC is critical in preventing water loss, maintaining relative barrier impermeability, and protecting against external aggressors.[1] One of the critical compositions of SC is the lipid matrix. This matrix contains an equimolar ratio of cholesterol (CHOL), free fatty acids (FFAs), and ceramides (CERs).[2] The molecular balance of the lipid matrix is strictly regulated by the lipid metabolism and acyl-chain trafficking in order to ensure the proper level of structural lipid synthesis.[3] Functionally, ceramides help regulate cellular processes and maintain the barrier integrity of the skin. Specifically, ceramides contribute to proliferation, differentiation, and apoptosis processes, as well as immunological activity.[4,5] Ceramides interact with other SC lipids to form multi-layered intercorneocyte matrix and control the water permeability of the skin. In skin disorders such as atopic dermatitis (AD), psoriasis, Netherton syndrome, and lamellar ichthyosis, the balance of the ceramide subclasses and the enzymes driving the lipid metabolism differ from the composition of normal skin.[4,6] These alterations in the ceramides, along with changes to FFA and CER chain lengths, lead to significant perturbation of the lipid organization and disruption of skin barrier function that often clinically manifest as dry, scaly, itching, erythematous skin with excessive trans-epidermal water loss (TEWL).[6–8]

The skin barrier is susceptible to various exogenous factors, such as solar UV radiation. UV was shown to disrupt skin barrier integrity by increasing TEWL, while decreasing skin hydration, promoting SC and epidermal thickness, and changing lipid and protein levels and structures in various human skin models.[9–11] Recently, we demonstrated that repeated UV exposures alter the expression of key barrier proteins and disrupt mechanical junctions.[12] These changes were prevented by the use of a ceramide-containing sunscreen.[12] In a recent clinical study, a physiological dose of UV (2MED) negatively impacted morphological organization and maturation of cells at the skin surface, which were prevented by application of a ceramide-containing sunscreen and moisturizing cream routine.[13] Nevertheless, despite the growing understanding of UV-induced skin barrier damage, there is limited knowledge on the impact of UV on SC lipid organization and how it relates to skin barrier function. Therefore, the objective of this study was to investigate the impact of UV on ceramide subclass and chain length following exposure to a single and repeated UV doses on fresh *ex vivo* human skin and on healthy volunteers.

EXPERIMENTAL DESIGN

Ex Vivo Tissue Model

Fresh post-abdominoplasty normal human skin samples (seven

donors total, 6 Caucasian and 1 Hispanic; 1 male and 6 females, age 29–51 years old) were acquired from BioIVT Inc. (Westbury, NY). The skin was freed from the subcutaneous tissue and a 12mm biopsy punch was used to form explants. The *ex vivo* skin samples were subjected to UV irradiation (96%UVA/4%UVB) using a solar simulator (Sol3A Class AAA Solar Simulator, Newport Corporation, Irvine, CA). Three different routines were employed to subject the skin to UV: (1) one-time exposure of 20J/cm^2, (2) five-time exposure of 20J/cm^2 each over 1 week (excluding weekends), and (3) one-time exposure of 100J/cm^2. The first two conditions were meant to mimic the effects of a single or 5 daily repeated sun exposures, respectively, as can be typically encountered in the temperate regions of the Northern hemisphere.[14] The third was a non-physiological UV dose corresponding to extreme conditions of exposure.[15] Prior to UV irradiation, designated tissue samples were treated with 4.42µL/cm^2 of broad-spectrum SPF 50 sunscreen in ceramide-containing multilamellar vesicular emulsion. The non-protected and unexposed samples served as controls.

After irradiation, skin explants were cleaned to remove products from the skin surface and maintained at air-liquid interface in Dulbecco's Modified Eagle's Medium (DMEM with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin) at 37°C and 5% CO^2. A subset of skin samples receiving repeated irradiations over 1 week also had topical application of the ceramide-enriched sunscreen prior to each exposure. Eight days after the initial exposure, explants from the repeat exposure (20J/cm^2 5x) and elevated exposure (100J/cm^2) conditions (3 explants per condition for each skin donor) were processed for lipidomic analyses.

In Vivo Clinical Evaluation

Study Participants

The study was monocentric, randomized, and double-blinded, and performed in accordance with Good Clinical Practices and the principles of the Declaration of Helsinki. The procedures used in this study were approved by Advarra institutional review board (Texas, USA). Before any study procedures, the subjects received the necessary information and provided informed consent. Eligibility was determined by physical examination and confirmation of all inclusion and exclusion criteria. Due to the impact of the SARS CoV-19 pandemic, the study was completed in two phases: the first phase was up to 14 days and included 7 subjects (Panel 1), while the second phase was completed in 7 days with 10 subjects (Panel 2). Both panels consisted of healthy men and women, aged 18–50 years with Fitzpatrick skin phototype III and an average individual typology angle (ITA°) of 35.1°.

UV Irradiation

UV exposure was performed in two consecutive procedures using a Newport ORIEL solar simulator, model 92292-1000 sn 115 (Irvine, CA) as previously described.[13] For both panels, the minimal erythematous dose of individual subjects (MEDi) was first determined during screening. Six areas of 2.25 cm^2 on the back of each subject were exposed to UV doses using a 1.25 geometrical progression. The starting UV dose was calculated according to the ITA° mean measured on the six areas. The MEDi of each subject was evaluated 24h after irradiation; the average MEDi was 0.06 J/cm^2. Secondly, at baseline (day 0), all test zones, excluding MED determination sites, were irradiated with a single dose of 2MED. Overall, a total of seventeen healthy subjects with skin Fitzpatrick phototype III were irradiated with 2MED on their back.

Test Materials

Test materials consisted of a currently marketed (1) ceramide-containing multilamellar vesicle emulsion with sunscreen SPF 25 and (2) a ceramide-containing moisturizing cream, which were applied at 4 mg/cm^2.

Clinical Study Design

At baseline, five test zones were delineated on the middle section of each subject's back (Supplementary Figure 1). The four irradiated zones, excluding the negative control, were exposed to 2MED. According to a randomization plan, out of the three product-treated and UV-irradiated zones, one received the sunscreen fifteen minutes before exposure at baseline; another received the moisturizer immediately after exposure at baseline, plus once a day for nine days for panel 1, or once a day for four days for panel 2. In both series, the third zone received both the sunscreen and moisturizer, as respectively described.

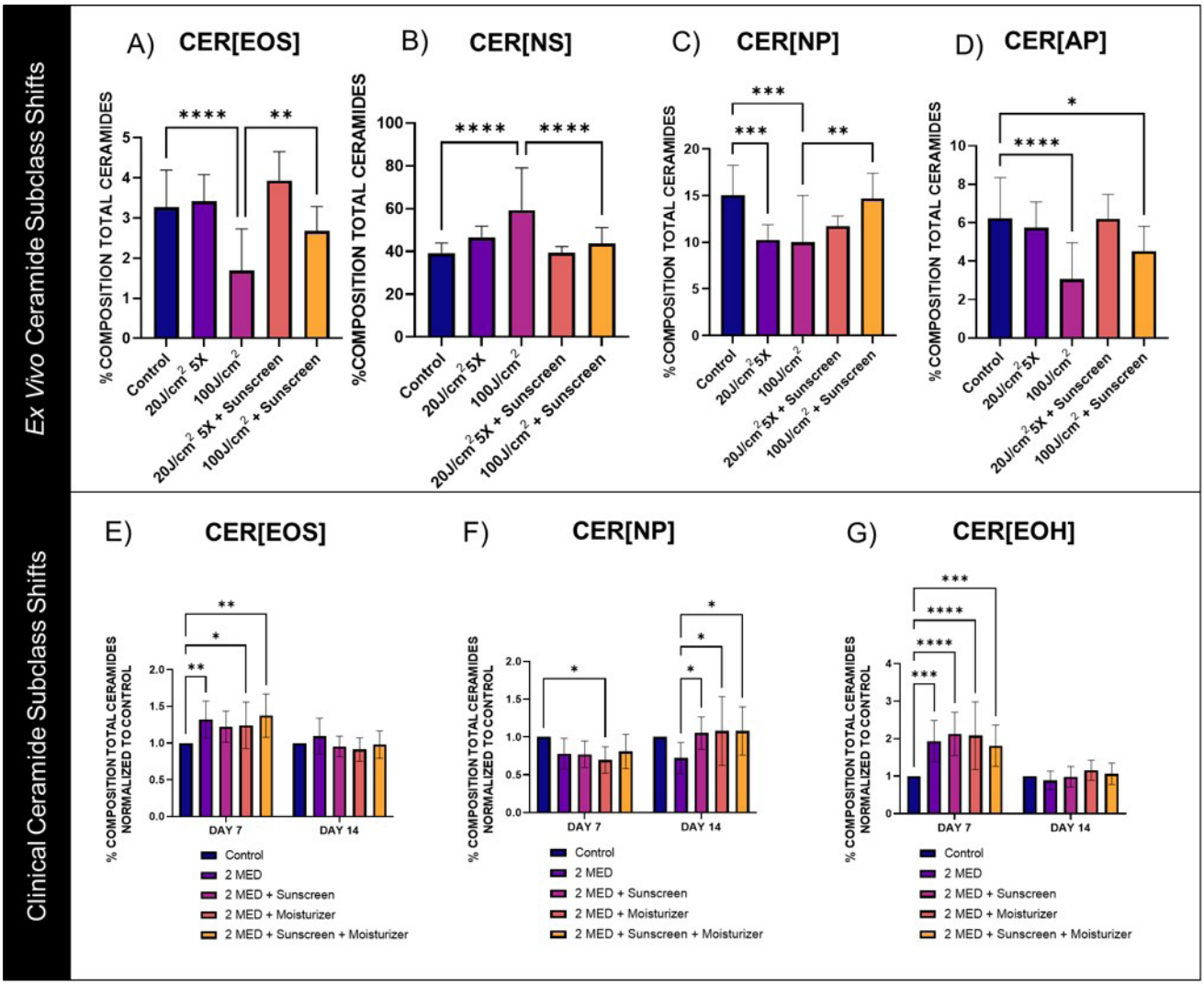
All procedures, including irradiations, tape-stripping and product applications, were conducted under controlled temperature (22°C) and relative humidity (40%) after subjects acclimated for at least 15 minutes as previously described.[13] Tape-stripping was performed using 22 mm D-Squame discs (CuDerm Corporation, USA). Six consecutive disks were placed onto cleaned test sites with even pressure using a pressure plunger, before being slowly removed with forceps. The first two strips were disregarded, and the four subsequent ones were collected from the same location, pooled together, and used for lipidomic analysis. Tape-stripping was performed at baseline (before application of products and UV irradiation) and day 14 for all 7 subjects in panel 1, and for all 10 subjects in panel 2 at baseline and day 7.

Analyses of the Epidermal Barrier Lipids and Enzymes Implicated in the Lipid Metabolism

Lipidomic Analysis

Lipidomic analysis was performed by Metabolon (Morrisville, NC). For *ex vivo* skin samples, epidermal layers were mechanically separated using a Thomas Stadie-Riggs Tissue Slicer to avoid contribution from the dermal tissue into analysis. For

FIGURE 1. UV exposure significantly modifies levels of human SC ceramide subclasses in *ex vivo* and clinical models. For *ex vivo* experiments (A-D), pretreatment with the ceramide-containing sunscreen protects the epidermal barrier integrity by preventing CER shifts. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. For clinical study (E-G), CER subclass shifts in subjects exposed to 2MED without sunscreen, with SPF25 sunscreen, moisturizer, or combination. Statistical significance compared with control: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$.



clinical samples, it was performed on the collected tape-strips. Briefly, the epidermal lipids were extracted with organic solvents and were analyzed on a Waters UPC2/Sciex QTrap 5500 Mass Spectrometer SFC-MS/MS system in MRM mode using characteristic parent-fragment mass transition for each analyte trace. In addition to detection of each lipid class, concentrations of each lipid species were calculated for individual analytes. The percent composition and ratios of individual ceramide subtypes, as well as the acyl and sphingoid base chain lengths, were calculated.

Immunohistochemical Staining of the Explants

Immunohistochemical staining was performed using a Leica Bond automated immunostainer (Reveal Biosciences, San Diego, CA). The *ex vivo* tissue explants were formalin-fixed, paraffin-embedded and sectioned. Prior to staining, heat-induced antigen retrieval was performed. Sections were then blocked for non-specific antibody binding and incubated with the primary murine monoclonal antibodies to β-glucocerebrosidase (GBA, ab55080, Abcam) and acid sphingomyelinase (aSMase/SPMD1, NBP2-45889, NovusBio), or with the rabbit anti-ceramide synthase 3 (CERS3, HPA024356, Sigma Aldrich) polyclonal antibody. The primary stainings were followed by rabbit anti-mouse IgG and labeled with horseradish peroxidase polymer-conjugated anti-rabbit antibody included in Bond Polymer Refine Detection kit (DS9800, Leica Biosystems, UK). All sections were then

counterstained with a hematoxylin nuclear stain and observed with an inverted microscope (Leica DM500, Wetzlar, Germany).

qRT-PCR of Human Neonatal Keratinocytes

Gene expression analysis of human primary neonatal keratinocytes was performed by Zen-Bio Inc. (Research Triangle Park, NC). Human primary neonatal keratinocytes were plated in a 12-well plate with Keratinocyte Growth Medium (KM-3, ZenBio) at $16x10^4$ cells per well. After adhering overnight, the cells were exposed to either $5mJ/cm^2$ or $10mJ/cm^2$ of UVB. Unexposed cultures constituted negative controls. 24 hours following irradiation, RNA was isolated using Qiagen RNeasy Mini Column Kit (Qiagen, Hilden, Germany). Isolated RNA quality and concentration were analyzed with the Nanodrop 1000 Spectrophotometer (Nanodrop, Wilmington, DE). Transcription to cDNA was performed with ABI High Capacity cDNA Reverse Transcription Kit (4368814, Thermo Fisher). 10ng of cDNA/cycle was used for gene expression analysis using the Quantstudio 12K Flex Real-Time PCR (Life Technologies, Wilmington, DE). The analysis was carried out using 25°C for 10 minutes, 37°C for 2 hours, and 85°C for 5-minute cycles. Five target genes (dihydroceramide desaturase 1 (DES1), acid sphingomyelinase (SMPD1), ceramide synthase 3 (CERS3), ceramide synthase 5 (CERS5), and stearoyl-CoA desaturase (SCD)) were analyzed with TATA-box-binding protein (TBP) housekeeping gene as the reference. Analysis was performed using Applied Biosystems Expression Suite Software v1.3 (Thermo Fisher, Waltham, MA).

Statistical Analysis

Lipidomic data were extracted using MATLAB R2020a and analyzed using a one-way analysis of variance (ANOVA) followed by the FDR method of Benjamini and Hochberg multiple comparisons test. The statistical analysis on the percent composition of total ceramides was conducted using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Alterations to Lipid Profile Following Broad Spectrum UV Exposure

Shift in Ceramide Subclass Distribution following UV Exposure

Evaluation of total lipid analytes (CER, CHOL, CHOL-S, FFAs) within the SC by lipidomic analysis in both *ex vivo* and clinical models demonstrated no significant differences between the different treatment groups (data not shown). These findings prompted further analysis of different ceramide subclasses. Irradiation of human skin with elevated UV doses of $100J/cm^2$ induced significant shifts in the ceramide subclass, accounting for proper barrier integrity. A decrease in CER1[EOS], CER3[NP], CER6[AP] and increase in CER2[NS] were observed when compared to the untreated control (Figure 1A-1D). Similarly, human skin exposed 5 times to $20J/cm^2$ showed statistically significant downward shift in CER3[NP] (Figure 1C). Application of the ceramide-containing sunscreen prior to exposure prevented or attenuated most of these UV-induced alterations. (Figure 1A–1D).

Exposure to both $20J/cm^2$ 5X and $100J/cm^2$ of broad-band UV provoked a decrease in CER3[NP]/CER2[NS] ratio, which was improved with the application of sunscreen (Supplementary Figure 2A). This suggests that UV can negatively impact epidermal differentiation and modify the integrity of epidermal barrier. Application of the ceramide-containing sunscreen mitigated such effects, particularly in the case of a single high dose UV exposure.

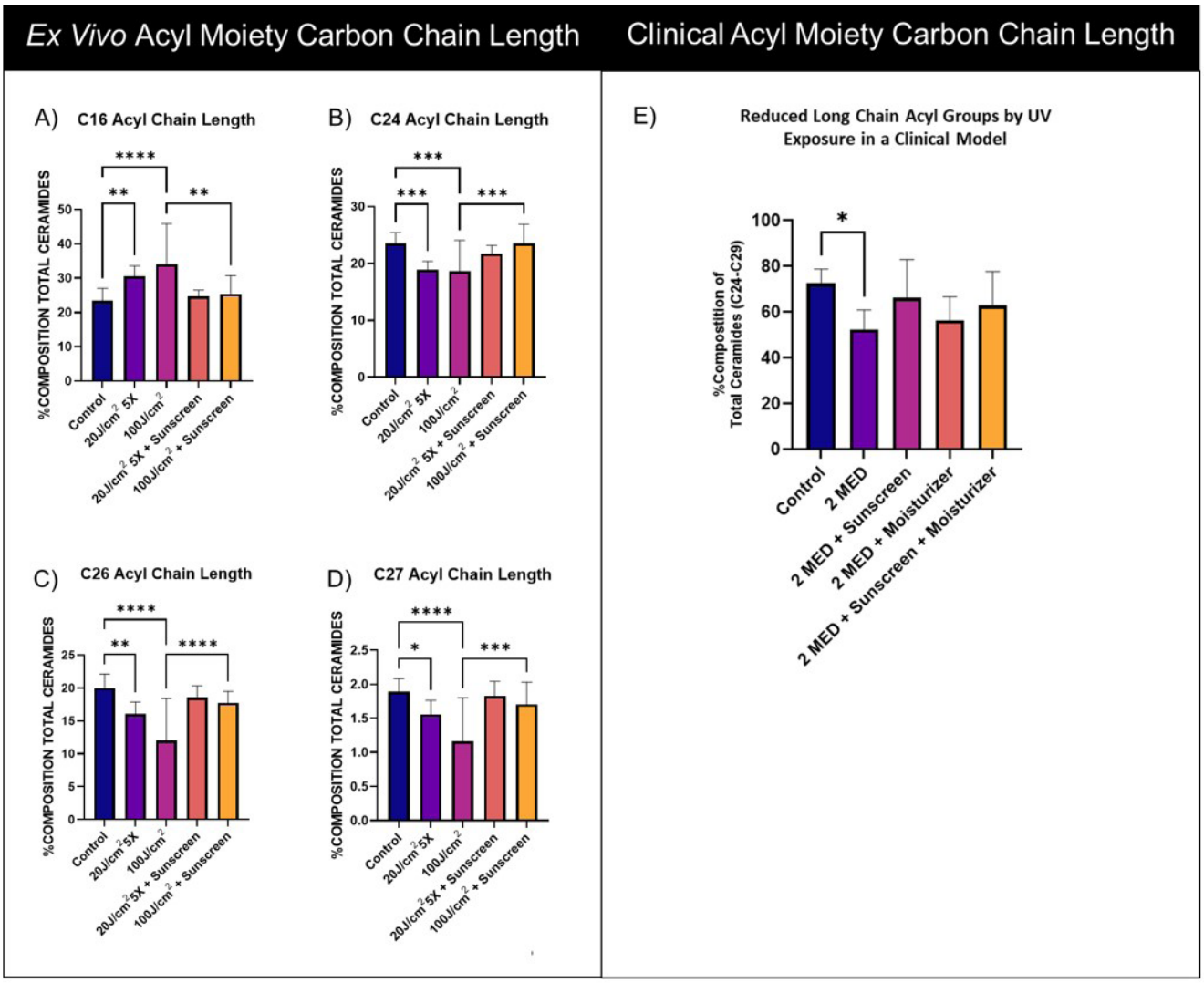
Under clinical conditions, alterations in specific ceramide subclasses were observed after UV exposure (Figure 1E–1G). Following 7 days after UV exposure, a significant increase in CER1[EOS] and CER4[EOH] were observed compared to control. Sunscreen alone or accompanied by moisturizer had no observable influence on UV-induced changes on these ceramide subclasses (Figures 1E, 1G). By day 14, a significant decrease in CER3[NP] was observed, which was prevented by ceramide-containing sunscreen alone or restored with the moisturizer (Figure 1F). CER3[NP] is shown to be elevated during keratinocyte differentiation.[16] The preservation of CER3[NP] by ceramide-containing products suggests that normal epidermal turnover and proper SC barrier function were supported in response to UV. In contrast to *ex vivo* results, there were no differences between groups for CER2[NS], CER6[AP], and CER8[NH] at days 7 or 14 (data not shown), suggesting that CER3[NP] could be sensitive to low UV dose, while other ceramide subclasses could be impacted when exposure levels are elevated or prolonged.

Interestingly, a physiological dose of UV (2MED) also caused significant decrease at day 14 for CER3[NP]/CER5[AS] (Supplementary Figure 2B), indicating that a small dose of daily UV on healthy subjects can also change the normal composition of ceramide subclass ratios to a phenotype seen in lesional skin of AD patients.[16]

Shift in Ceramide Chain Length Distribution Following Broad-band UV Exposure

Recently, there is growing evidence illustrating that a decrease in ceramide acyl chain length can detrimentally impact the skin permeability barrier by reducing the proportion of tight orthorhombic packing and inducing phase separation.[17–19] Lipidomic analysis also provided insight as to the shift of chain length distribution of both acyl and sphingoid moieties of ceramides following unprotected UV exposure. Figure 2 demonstrates the shift in select acyl carbon chain lengths that have been correlated with specific ceramide subclasses essential for barrier integrity. *Ex vivo* skin exposed to both the lower, repeated ($20J/cm^2$ 5x) and single elevated ($100J/cm^2$) UV doses demonstrated a significant decrease in the expression

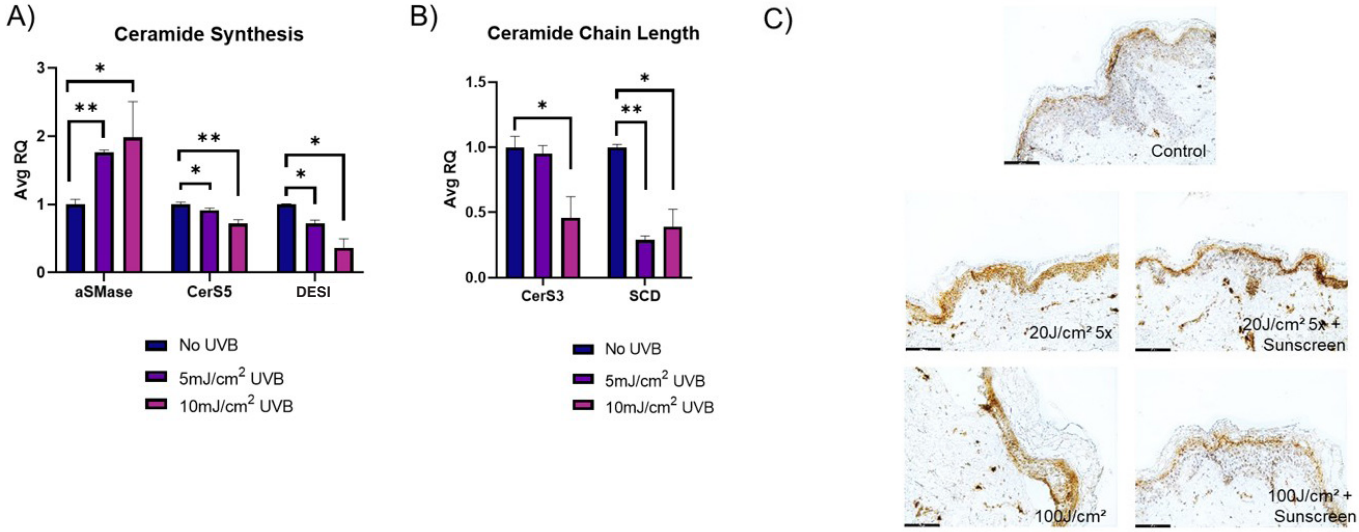
FIGURE 2. UV exposure induces changes to human SC ceramide acyl chain length in *ex vivo* and clinical models. For *ex vivo* (A-D), the relative abundance of ceramides with different lengths of acyl carbon chains, related to apoptosis (C16) and SC barrier function (C24, C26, C27), is reported as % of total ceramides found in the studied samples $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. (E) For clinical study, reduced proportions of representative ceramide species with very long acyl chains (C24 to C29) are observed in subjects exposed to 2MED without UV protection. Tape-stripped SC was analyzed at day 14 after exposure. Comparison with either ceramide-containing sunscreen, moisturizer, or combination of both.* $P<0.05$ compared to the non-exposed control.



of very long-chain acyl groups (C24, C26, and C27) associated with ceramide subclasses important for the SC barrier function (Figures 2B, 2C, 2D). Also, a significantly increased occurrence of C16 acyl chains, relating to augmented apoptosis, was observed (Figure 2A). The protective action of the ceramide-containing sunscreen was particularly obvious in explants exposed to the elevated UV dose. Shifts to the sphingoid moiety were also observed (data not shown).

Clinical evaluation of chain length distribution of the ceramide acyl moiety demonstrated that UV (2MED) induced a significant decrease in very long-chain acyl C24 to C29 following 14 days after exposure, whose distribution was maintained comparable to control level by ceramide-containing sunscreen alone or in combination with moisturizer (Figure 2E). As the length of the fatty acid residue also helps to determine the function of each ceramide, this result indicates that ceramide-containing products preserve the SC ceramide quality to promote their normal function in skin barrier health. Additionally, sphingoid components also demonstrated a decrease in chain length following irradiation, where similar trends to *ex vivo* findings were observed (data not shown).

FIGURE 3. Unprotected UV exposure alters key enzymes in lipid processing associated with ceramide biosynthesis pathways and acyl chain elongation. RT-PCR analysis demonstrates alteration to (A) ceramide biosynthesis pathway genes (aSMase, CerS5, DESI) and to (B) chain length gene targets (CerS3, SCD). Target genes are normalized to the TBP housekeeping gene expression. $^{*}P<0.05$; $^{**}P<0.01$, as compared with non-exposed controls. (C) Unprotected *ex vivo* skin exposed to 20J/cm² Sx and 100J/cm² demonstrates the increased expression of aSMase compared to control tissue. Application of a ceramide-containing SPF 50 sunscreen protects against such effects. Scale bar= l00µm.



Alteration to Ceramide Biosynthesis Pathway Following UV Exposure

The mechanism of UV-induced change was evaluated by studying the biosynthesis process related to ceramide subclass and chain length at the cellular level. Figure 3A demonstrates the alteration in expression of genes relating to ceramide biosynthesis and chain length formation in keratinocytes exposed to varying doses of UVB. Both 5mJ/cm² and 10mJ/cm² of UVB significantly increased acid sphingomyelinase (aSMase) and decreased ceramide synthase 5 (CerS5) and dihydroceramide desaturase-1 (DES1) gene expressions. For ceramide chain length, exposure to both UVB conditions decreased SCD expression, while elevated 10mJ/cm² UVB significantly decreased expression of CerS3 (Figure 3B).

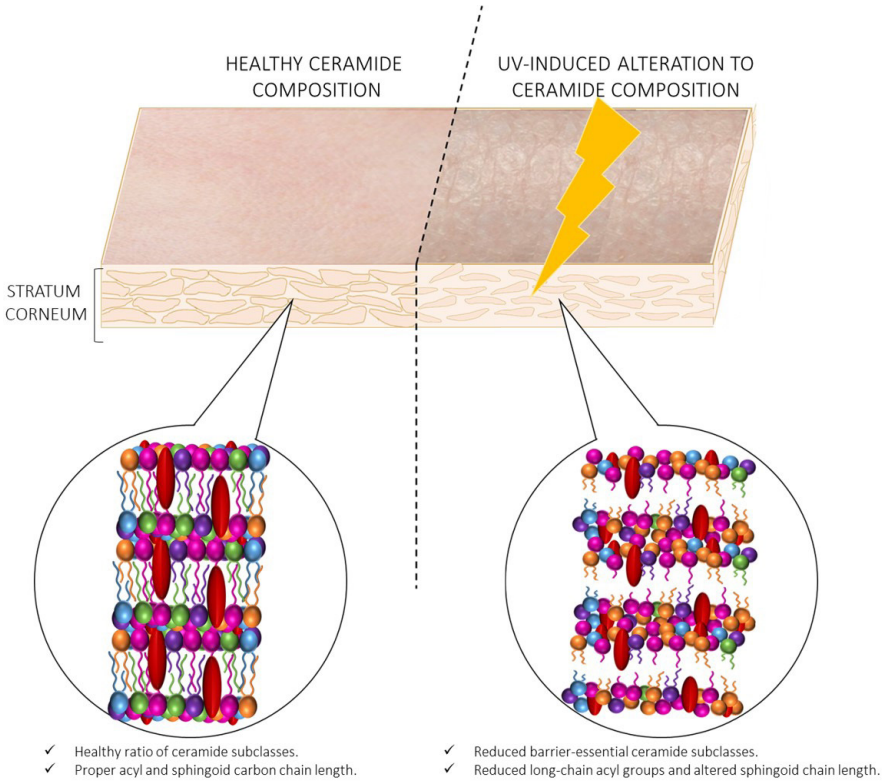
Next, the impact of UV was investigated on a tissue level, by performing immunohistochemical staining against enzymes crucial to the ceramide subclass distribution, such as aSMase and β-glucocerebrosidase (GBA), and for synthesis of ceramides with very long acyl chain, such as CerS3. A significant alteration of aSMase expression and localization was observed. The application of sunscreen at both 20J/cm² 5x and 100J/cm² conditions alleviated these effects (Figure 3C). No significant change in GBA and CerS3 under the same conditions was detected (data not shown). Altogether, these observations at mRNA and protein levels suggest UV exposure impacts enzymes involved in lipid metabolism, similarly to what has been previously observed in pathological skin conditions characterized by reduced acyl chain lengths or abnormal lipid processing.

DISCUSSION

In this study, we identified the effect of both daily and elevated UV exposures on the SC ceramide subclass distribution and chain lengths. UV exposure demonstrated a clear negative impact on the level of essential CER1[EOS], CER3[NP] and CER6[AP] subclasses. Under a low physiological UV dose (2MED), CER3[NP] subclass appeared to be more sensitive and susceptible to the irradiation, which might relate to the previously observed disruption of corneocyte maturation process resulting in altered surface morphology after real life sun exposure.[13] A significant decrease *in vivo* at day 14 of the CER3[NP]/CER5[AS] ratio (Supplementary Figure 2B), indicates that even a small dose of daily UV on healthy subjects can result in a cumulative change of the SC ceramide profile, resembling one seen in the lesional skin of AD patients.[16]

The observed shifts in the abundance of various ceramide subclasses were associated with the overall reduction of ceramide acyl and sphingoid chains, suggesting that UV can affect SC ceramide proportions and quality, indicated by proper chain length. These findings suggest that the quality and functionality of multiple ceramide subclasses including essential ones, such as CER1[EOS], CER3[NP], and CER6[AP], could be impaired by acyl chain length reduction under physiological UV conditions. Alterations to ceramide subclass and chain length distribution have been observed in skin disorders, where both barrier integrity and function are impaired. For instance, patients suffering from atopic and contact eczema and psoriasis experience significant decrease in CER1[EOS], CER3[NP] and CER6[AP], as well as significant increase in CER2[NS] in the

FIGURE 4. Pictorial representation of the proposed mechanism of UV impact on skin ceramide composition and quality.



lesional epidermis; additionally, AD patients also have shown reduced CER8[NH] and CER4[EOH].[4,20,21]

Studies on lipid mixture models have demonstrated that the sphingoid chain length acts as a driving factor in chain matching within the lipid multi-layers, whereas truncated acyl chains cause altered phase behavior.[22,23] Interestingly, shifts in both acyl and sphingoid base chain moieties were observed in our *ex vivo* and clinical studies, where an overall reduction of ceramide chain length was observed. As acyl components with longer carbon chains are known to produce stronger Van der Waals forces to form tight lateral molecular packing, an overwhelming decrease in very long acyl and sphingoid base chain length following UV exposure should result in loosened lipid packing.[24] In turn, this would modify the biophysical properties of the extracellular lipid matrix and compromise barrier impermeability. These findings also suggest the need for replenishing skin with multiple essential ceramides daily, in order to compensate the continuous damage occurring under real life sun exposure.

Changes observed in ceramide subclass and chain length distribution could be attributed to multiple factors, including biosynthesis, metabolism, and degradation. In our study, the ceramide biosynthesis pathways were investigated. The increase in aSMase mRNA by keratinocytes exposed to UVB and the UV-dependent overexpression of aSMase in the *ex vivo* skin suggest that UV irradiation impacts lipid enzymatic activity. Increased CER2[NS] and CER5[AS] levels after UV exposure in *ex vivo* skin could thus result from an increased hydrolysis of sphingomyelin precursors, because proper ceramide ratios are typically maintained by a balance of β-glucocerebrosidase (GBA), aSMase and ceramidase.[24] In addition to the conversion of sphingomyelin to CER[NS] and CER[AS], aSMase also plays a role in the formation of C16 acyl chain lengths, that also showed increase in the present study.[4] C16 chain length is pro-apoptotic and has been related to incomplete maturation processes within keratinocytes, followed by incomplete lipid barrier formation and inflammatory responses.[25] The triggering of C16 formation can also play a role in blocking the synthesis of very long-chain ceramides and can cause the degradation of very long-chain ceramides by lysosomal enzymes such as aSMase.[25] Acyl groups with a chain length greater than C20 play an important role in the skin barrier and, interestingly, similar shifts in these longer acyl chain lengths were observed in our *ex vivo* and clinical studies. It is plausible that the shortening of acyl chains results from the UV radiation-induced oxidative stress impacting *de novo* lipid synthesis pathways, likely due to insufficient NADPH levels.[25] In parallel to the up-regulation of aSMase, we found that UV irradiation negatively impacted ceramide synthesis, as demonstrated by a decrease in CerS3 and SCD expression. These enzymes are involved in the synthesis of sphingolipids with very long-chain acyl moieties and catalyze conversion of saturated

FFAs to unsaturated ones.[26] Altogether, the UV-induced impact on enzymes involved in lipid metabolism draws parallelism with several pathological skin conditions in which reduced length of lipid acyl chains and/or abnormal lipid processing have been reported, such as AD, Netherton syndrome, and autosomal recessive congenital ichthyosis.[7,8,27,28]

Treatment with ceramide-containing sunscreen or in combination with moisturizer maintained normal distribution of the majority of ceramide chain lengths and some ceramide subclasses following UV exposure. It has also been speculated that the generation of free radicals following UV exposure may influence skin lipid composition via oxidation.[29] The protective effect provided by the ceramide-containing sunscreen is likely due to its UV-filter capability, as the photoprotective efficacy of sunscreens against UV-induced cutaneous responses are well documented.[30] However, the ceramide content of the sunscreen formulation may also be integrated into the native lamellar lipid matrix, further fortifying the skin barrier.[31] We previously demonstrated that the ceramide-containing moisturizer alone provides long-lasting skin hydration and was sufficient to maintain normal superficial skin cells morphology and turnover in response to UV.[13] Our new results indicate that the ceramide-containing sunscreen serves as a protective shield against UV rays, while the added benefits of the moisturizer are mostly to retain and repair barrier integrity after UV.

Although in this study we were unable to distinguish between endogenous and topically applied lipids, growing evidence indicates that topical application of lipid-formulations, particularly ceramides, can penetrate into the human skin *ex vivo* and accumulate mostly in the SC.[32] While the exact *in vitro* mechanism awaits further investigation, *in vitro* studies suggest that these exogenous lipid molecules have the ability to improve skin barrier function by restoring lamellar structures through an increase of rate-limiting enzyme activity that is involved in sphingolipid synthesis.[33,34] Future studies are needed to expand on these premises and determine the mechanism of ceramides and other exogenous lipids uptake within the skin barrier, and added benefits when combined with UV filters and other ingredients for maintaining skin barrier health in response to UV.

CONCLUSION

Collectively, our *ex vivo* and clinical results demonstrate that physiological UV conditions shift the composition of epidermal ceramide subclasses, reduce the prevalence of the long/very long-chain acyl moieties, and induce changes to the lipid biosynthesis process. The understanding of how UV impact skin ceramide content permits to explain one of the mechanisms by which the SC barrier alteration may occur following UV. Such changes align with disturbances to the ceramide composition in skin conditions that lead to barrier impairment. Treatment with products containing multiple essential ceramides not only help preserve healthy SC ceramide subclass profile, but also maintain ceramide quality by preserving key acyl chain lengths that are essential for proper barrier integrity in response to UV.

DISCLOSURES

The authors declare no conflict of interest. This research is sponsored by L'Oreal Research & Innovation.

ACKNOWLEDGMENT

We thank Gladys Osis and her team, especially Elouise Whyte (Eurofins CRL), for supporting the clinical study; L'Oreal Data Management & Quality, plus Scientific Computing teams, especially Christine Criqui, Marie Bertoncello, Lise Vriet, Aline Van Der Lee, and Hussein Jouni, Valentine Laizet, Olivia Dufour, and Cloe Hernandez for data processing and statistical analysis guidance; Dr. Marek Haftek, Dr. Yaxian Zhen, Dr. Daniel Roy for critical reading of the manuscript and fruitful discussions.

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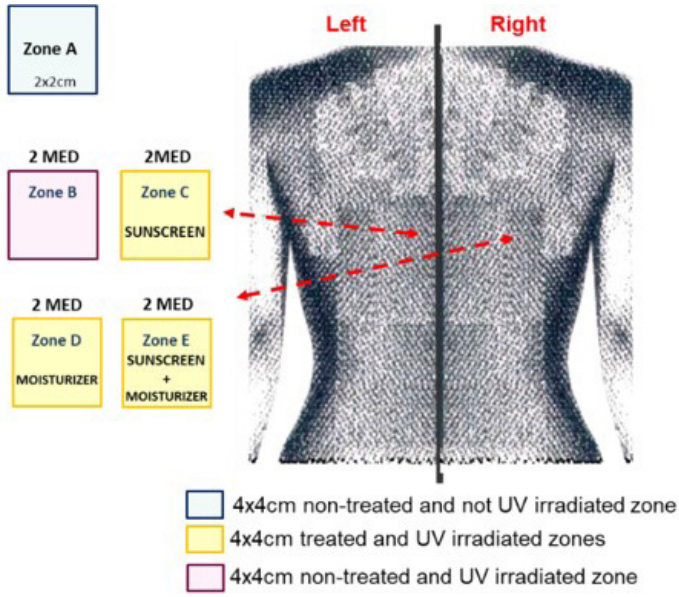
AUTHOR CORRESPONDENCE

Rebecca Barresi

E-mail:..... rebecca.barresi@rd.loreal.com

SUPPLEMENTAL FIGURE 1. Schematic of investigational zones on subjects back. Zones were randomized to either left or right side of the back.



SUPPLEMENTAL FIGURE 2. Physiological doses of UV induce changes in the subclass ratios of SC ceramides related to epidermal differentiation. (A) *Ex vivo* skin exposure to 20J/cm² SX and 100J/cm² broad-band UV significantly reduces subclass ratio of CER[NP]/CER[NS] ceramides. Pretreatment with the ceramide-containing sunscreen largely prevents this shift. (B) Ratio of CER[NP]/CER[AS] in tape-stripped SC of subjects (at day 14) exposed to 2MED without sunscreen, with SPF25 sunscreen, moisturizer, or combination thereof were compared to unexposed controls. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$.