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Lesional Psoriasis is Associated With Alterations in the Stratum Corneum Ceramide Profile and Concomitant Decreases in Barrier Function

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ABSTRACT

Psoriasis is an inflammatory skin disease associated with an impaired skin barrier. The skin barrier function is dependent on the extracellular lipid matrix which surrounds the corneocytes in the stratum corneum. Ceramides comprise essential components of this matrix. Alterations in the stratum corneum ceramide profile have been directly linked to barrier dysfunction and might be an underlying factor of the barrier impairment in psoriasis. In this study, we investigated the ceramide profile and barrier function in psoriasis. Lesional and non-lesional skin of 26 patients and 10 healthy controls were analysed using in-depth ceramide lipidomics by liquid chromatography-mass spectrometry. Barrier function was assessed by measuring transepidermal water loss. Lesional skin showed a significant decrease in the abundance of total ceramides with significant alterations in the ceramide subclass composition compared to control and non-lesional skin. Additionally, the percentage of monounsaturated ceramides was significantly increased, and the average ceramide chain length significantly decreased in lesional skin. Altogether, this resulted in a markedly different profile compared to controls for lesional skin, but not for non-lesional skin. Importantly, the reduced barrier function in lesional psoriasis correlated to alterations in the ceramide profile, highlighting their interdependence. By assessing the parameters 2 weeks apart, we are able to highlight the reproducibility of these findings, which further affirms this connection. To conclude, we show that changes in the ceramide profile and barrier impairment are observed in, and limited to, lesional psoriatic skin. Their direct correlation provides a further mechanistic basis for the concomitantly observed impairment of barrier dysfunction.

Abbreviations: AD, atopic dermatitis; CCL, ceramide chain length; LC–MS, liquid chromatography-mass spectrometry; LSS, lesion severity score; PASI, Psoriasis Area and Severity Index; SC, stratum corneum; sqv, squamescan value; TEWL, transepidermal water loss.

The Collaborators name have been in Appendix.

Jannik Rousel and Catherine Mergen have contributed equally.

1 | Introduction

Psoriasis is a chronic, immune-mediated, inflammatory skin disease with a worldwide prevalence of approximately 2%–3% [1]. The most common type, plaque psoriasis, is characterised by scaly, erythematous and clearly demarcated plaques [2]. The pathophysiology is complex and involves multiple genetic, immunological and environmental factors, resulting in a dysfunctional immune response [3]. The abnormal differentiation and hyperproliferation of keratinocytes leads to the development of skin lesions with a markedly impaired skin barrier [4]. Additionally, inflammation can lead to barrier dysfunction, as demonstrated in in vitro studies [5, 6]. These barrier perturbations allow exogenous compounds to cross the skin barrier, which might further enhance epidermal dysregulation [7, 8].

The skin barrier function is mainly provided by the stratum corneum (SC), the outermost layer of the epidermis, where terminally differentiated keratinocytes are embedded in a complex lipid matrix [9]. This extracellular lipid matrix is essential for proper barrier function, as it represents the only continuous pathway for exogenous compounds through the skin. The lipid matrix primarily contains cholesterol, free fatty acids and ceramides [10]. Ceramides are structurally highly diverse lipids that consist of an acyl chain covalently linked to a sphingoid base [11]. Based on the specific combination of acyl chain and sphingoid base, ceramides can be categorised into different subclasses (Figure S1) [12]. Around 25 ceramide subclasses have been identified in human SC [13]. Within each subclass, ceramides can vary in their degree of unsaturation and ceramide chain length (CCL), defined as the total number of carbon atoms in a ceramide. The CCL generally ranges from 42 to 54 carbons in the Cer[N] and Cer[A] (Cer[N, A]) classes and up to 72 carbons in the Cer[EO] class [10, 11]. The Cer[EO] class consists of ceramides with a very long ω-hydroxy acyl chain, also referred to as Cer[O], which are esterified with primarily linoleic acid. This linoleic acid moiety is required for covalent binding of ceramides to the corneocytes, anchoring the lipid matrix to the cornified envelope [14–16].

The specific composition of the SC ceramide profile plays an important role in maintaining a functional skin barrier and has been shown to be altered in many inflammatory dermatoses [6, 12, 17–22]. In atopic dermatitis (AD) and seborrheic dermatitis, alterations in the ceramide profile have been correlated to the impaired skin barrier, establishing barrier dysfunction as a key factor in their pathogenesis [22–27]. A recent meta-analysis has shown a high degree of similarity in the SC ceramide profile between AD and psoriasis [28]. As in AD, a reduced barrier function has been linked to lesional psoriatic skin [6, 17–19, 29–34]. However, direct correlation between the composition of the complete ceramide profile and barrier impairment has been neglected. Several studies have shown that the ceramide profile is altered in psoriasis, but they were often limited by methodology that did not allow for separation and detection of individual ceramides, nor did provide information on chain length distribution and degree of unsaturation [12, 20, 21]. Studies using more sensitive and higher resolution methods, such as liquid chromatography-mass spectrometry (LC–MS), used a more targeted approach that focused on specific ceramides rather than the full profile [6, 18, 22, 29]. Furthermore, previous studies only included a relatively small sample population without taking into account disease and lesion severity [6, 17–19, 22, 29]. Most importantly, the association between ceramide composition and barrier function was only marginally reported in psoriasis and might provide further insights into its pathogenesis. Only one previous study correlated the ratio of Cer[NP]:Cer[NS] to barrier dysfunction, and those correlations were, however, limited by the relatively small sample size without consideration of disease severity [22].

Therefore, we performed an in-depth characterisation of the full SC ceramide profile in lesional and non-lesional psoriatic skin in a cohort of 26 patients and 10 matched healthy controls. We evaluated the relationship between ceramide profile and barrier impairment by correlating single ceramide characteristics to barrier function. The different parameters were assessed in a 2-week interval to confirm reproducibility.

2 | Materials and Methods

2.1 | Chemicals

HPLC grade chloroform (Honeywell, Charlotte, North Carolina, United Statesmo), UPLC grade methanol (Biosolve, Valkenswaard, the Netherlands), UPLC grade heptane (LiChrosolv, Merck, Darmstadt, Germany), UPLC grade isopropyl alcohol (Biosolve, Valkenswaard, the Netherlands) and UPLC grade ethanol (Biosolve, Valkenswaard, the Netherlands) were used as solvents. Ultrapure water was obtained from a Milli-Q Advantage A10 system (Merck, Darmstadt, Germany), and reagent-grade potassium chloride was from Sigma-Aldrich (St. Louis, Missouri, United States). Deuterated internal standard and ceramide standards for calibration curves were purchased from Avanti Polar Lipids (Alabaster, Alabama, United States) or received from Evonik (Essen, Germany).

2.2 | Study Design and Population

The study was conducted at the Centre for Human Drug Research (Leiden, the Netherlands) in accordance with the principles of the Declaration of Helsinki. The trial was approved by the medical research ethics committees Brabant (Tilburg, the Netherlands) and registered under NCT04394936. In total, 26 patients with plaque psoriasis and 10 healthy volunteers participated in the study after giving written informed consent. An overview of the in- and exclusion criteria for the study is provided in the Supporting Information. Included patients had mild or moderate-to-severe plaque psoriasis as defined by a PASI (Psoriasis Area and Severity Index) score of ≤ 5 or ≥ 10, respectively [35]. Furthermore, all patients had at least one lesion of adequate size for sampling with a lesion severity score (LSS) of ≥ 6, determined by summing erythema, scaling and induration on a 5-point scale [36]. Patients received no active treatment and had a wash-out depending on their prior treatment (4 weeks topical, 3 months systemic). Assessments were performed at two time points 2 weeks apart at the same lesional and non-lesional site. Controls were healthy volunteers without dermatological conditions matched for age and sex. Sampling sites of the control group were matched to the sampling sites of lesional and

non-lesional skin of the patients, and are referred to as controls (lesional site) and controls (non-lesional site).

2.3 | Transepidermal Water Loss

Transepidermal water loss (TEWL) constitutes a widely used measure for skin barrier integrity [37]. TEWL was measured using an AquaFlux AF200 (Biox Systems Ltd., London, United Kingdom) after acclimatisation for at least 15 min to controlled conditions (humidity < 60%; temperature 22 ± 2°C).

2.4 | Tape Stripping

Tape stripping for the collection of SC lipids was performed after TEWL measurements at the same location. Eight consecutive polyphenylene sulfide tapes (Nichiban, Tokyo, Japan) were applied to the skin with standardised pressure using a D500 D-Squame Pressure Instrument (CuDerm, Dallas, Texas, United States). One blank tape strip was collected per site. As a measure for the total amount of SC removed by tape stripping, protein content was determined using a SquameScan 850A (Heiland Electronic, Wetzlar, Germany), which is a frequently used and reliable method for protein quantification on tape strips [16, 38–42]. The squamescan value (sqv) was then calculated by subtracting the values of two blank tapes [43]. Subsequently, a 16 mm diameter circle was punched out of each tape strip, which was stored in chloroform: methanol (2:1) at −20°C prior to extraction.

2.5 | Lipid Extraction

A validated lipid extraction protocol was used as described previously [41]. In short, tape strips 5–8 were shaken on a rotary shaker at 120 rounds per minute at 40°C for 1 h in chloroform: methanol (2:1) and the solvent was subsequently collected and pooled. This procedure was repeated with 1 mL of chloroform:-methanol:water (1:2:0.5), chloroform: methanol (1:1) and heptane:isopropylalcohol (1:1). In total, 4 mL of water and 160 μL of 0.25 M potassium chloride were added to the combined extracts for liquid–liquid extraction and left overnight for phase separation. The organic layer was collected, and the aqueous phase washed with 4 mL chloroform. The pooled organic layers were filtered through a 0.45 μm PVDF syringe filter (Grace, Deerfield, Illinois, United States), and the filtrate was evaporated and reconstituted in chloroform:methanol (2:1). No internal standards were added before lipid extraction, as this method allows for an adequate recovery of all ceramide subclasses [41].

2.6 | Lipid Analysis

A validated LC–MS platform was used for ceramide analysis [41]. A sample aliquot was evaporated and reconstituted in 60 μL heptane:chloroform:methanol (95:2.5:2.5). The samples were analysed using an Acquity UPLC H-class (Waters, Milford, Massachusetts, United States) with a normal phase PVA-silica column (5 μm particles, 100 × 2.1 mm i.d.) (YMC, Kyoto, Japan) connected to a XEVO TQ-S mass spectrometer (Waters, Milford, Massachusetts, United States) operating in positive ion mode with atmospheric pressure chemical ionisation. The samples were analysed in three randomised runs, each including quality control samples and calibration curves. The quality control samples were prepared from a mixture of ex vivo SC lipid extract at 0.24 mg/mL and the calibration curves consisted of a mixture of synthetic ceramide standards, listed in Table S1. Deuterated CER N(24deu)S [18] was added as internal standard to all samples at 3 pmol per injection, and 5 μL of each sample was injected. The data were processed and analysed using MassLynx and TargetLynx V4.1 (Waters, Milford, Massachusetts, United States). Two out of 144 samples showed insufficient responses below the limit of detection and were excluded from analysis. Responses were determined for all individual ceramides within the 16 ceramide subclasses. The analysed chain lengths, defined as the combined number of carbons in the acyl chain and sphingoid base moieties, ranged from 32 to 56 carbons for Cer[N,A], 62–78 carbons for C[EO] and 42–58 carbons for Cer[O]. Peaks were integrated at the monoisotopic mass of the most abundant ion of the specific ceramide. Due to the limited availability of ceramide standards and to avoid the use of deuterated standards for every ceramide class, a response model was used to predict the response factor for each specific ceramide based on subclass and chain length [41]. First, the area under the curve was corrected for the internal standard. Subsequently, quality control samples were used to correct for adduct formation and water loss by integrating the adducts of the most abundant peak. The ceramide standards of the calibration curves were used to correct for increasing response by mass. Finally, this was corrected with the theoretic Carbon-13 isotope distribution. Absolute ceramide abundances were calculated by correcting the amount of ceramides with the average sqv of the four tapes within a sample as a measure for the amount of SC removed by tape stripping [16, 40, 41, 43].

2.7 | Statistical Analysis

Data visualisation and statistical analysis were performed using Prism 9.0 (GraphPad Software, Boston, Massachusetts, United States). The ceramide profiles of controls, non-lesional psoriasis and lesional psoriasis were compared using one or two-way analysis of variance (ANOVA). Multiple comparisons were conducted with Tukey's test. Absolute quantification using the sqv resulted in three lesional samples and two non-lesional samples which were identified as outliers (ROUT method) and excluded from analysis. Principal component analysis (PCA) was performed using the relative abundance of individual ceramides as a percentage of all detected saturated ceramides. Statistical significance is indicated as not significant: ns, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

3 | Results

In total, 26 patients with plaque psoriasis and 10 healthy controls were included in the study. All patients had mild or moderate-to-severe plaque psoriasis as defined by a PASI score of ≤ 5 or ≥ 10, respectively. The lesions were all of moderate severity with LSS ≥ 6. Controls were matched for age and sex. Demographics of the study population are listed in Table S2.

3.1 | The Ceramide Profile Is Altered in Lesional Psoriatic Skin

The total amount of saturated ceramides (ceramides without an additional unsaturation present in the N-acyl chain or sphingoid base moiety) was significantly reduced in lesional skin compared to both controls and non-lesional skin (76.1 ± 45.6 pmol/sqv vs. 172.1 ± 79.2 pmol/sqv ($p < 0.05$) and vs. 218.9 ± 113.2 pmol/sqv ($p < 0.001$), Figure 1a). There was no significant difference in the abundance of total ceramides between non-lesional skin and controls.

The absolute abundance was determined for all 16 major ceramide classes (Figure 1b, Table S3 lists all values). Lesional skin showed a significant decrease in Cer[NdS], Cer[NP], Cer[NH], Cer[AP], Cer[OS] and Cer[OH] compared to controls. Compared to non-lesional skin, lesional skin was also characterised by a decrease in Cer[NdS], Cer[NP], Cer[NH], Cer[AP], Cer[OS] and Cer[OH] and an additional decrease in Cer[AdS] and Cer[AH]. In contrast to lesional skin, the level of Cer[NP] was increased in non-lesional skin compared to controls. Apart from that, no significant differences were present in the absolute ceramide profile of non-lesional skin and controls. There were no significant differences in the Cer[EO] fraction between lesional, non-lesional skin and controls.

The relative ceramide profile shows the different ceramide classes as a percentage of total detected ceramides (Figure 1c, Table S3 lists all values). Here, lesional skin exhibited reduced Cer[NdS], Cer[NP], Cer[NH], Cer[AdS], Cer[AP], Cer[OS], Cer[OH] and Cer[EOS] abundances compared to controls. Compared to non-lesional skin, lesional skin also showed a significant decrease in Cer[NP], Cer[NH], Cer[AdS], Cer[AP], Cer[OS], Cer[OH] and Cer[EOS]. When comparing non-lesional skin and controls, the relative abundance of Cer[NdS] and Cer[OS] was reduced. In contrast to the absolute profile, the relative abundance of Cer[NS] and Cer[AS] in lesional skin was significantly increased compared to both non-lesional skin ($p < 0.001$) and controls ($p < 0.001$). The relative abundance of Cer[AH] was significantly increased in lesional ($p < 0.05$) and in non-lesional skin ($p < 0.05$) compared to controls.

The ratio of Cer[NS] to Cer[NP] was calculated as an indicator for skewing of ceramide subclass synthesis. Indeed, the average Cer[NS]:Cer[NP] ratio was significantly higher in lesional skin compared to both non-lesional skin and controls (3.96 vs. 0.45 vs. 0.55, $p < 0.001$, Figure 2a).

The amount of ceramides with an additional unsaturation in the acyl chain or sphingoid base in the Cer[NS] subclass was determined, referred to as monounsaturated ceramides (MUCer[NS]). As the abundance of Cer[NS] remains high in controls and in lesional and non-lesional skin, the degree of monounsaturation within Cer[NS] can be used as an indicator for the degree of monounsaturation across the entire ceramide profile [25]. Although not determined in this study, it might be assumed that the increased degree of monounsaturation as observed in Cer[NS] is present throughout all ceramide subclasses

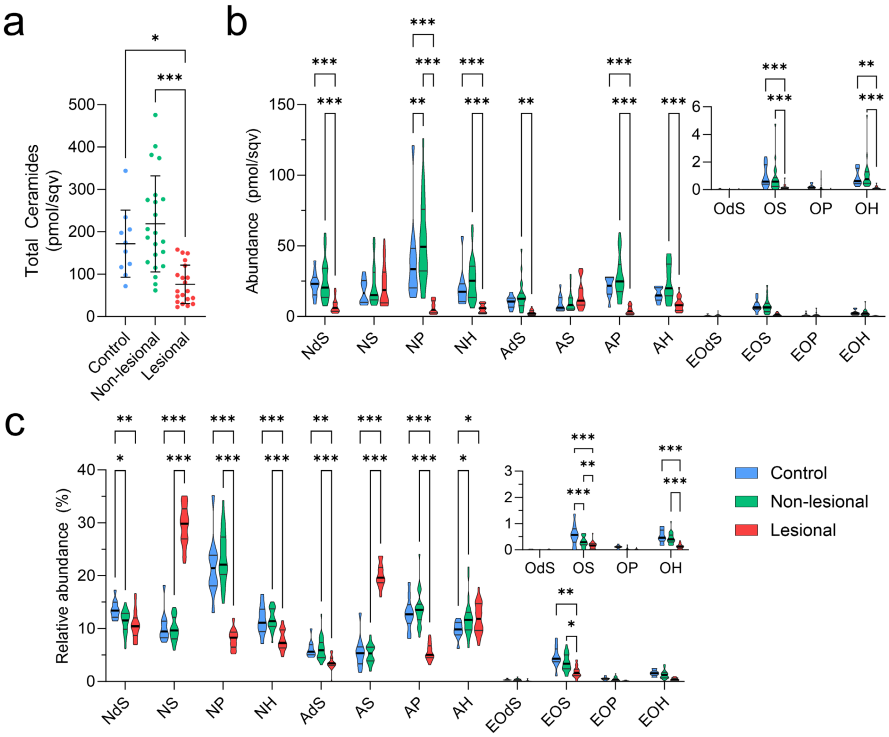

FIGURE 1 | The ceramide profile of the stratum corneum in controls, non-lesional psoriasis and lesional psoriasis. The absolute abundance of total ceramides was calculated by correction with the squamescan value (sqv) in pmol/sqv. Bars represent the mean ± SD, and statistical analysis was performed using one-way ANOVA (a). The absolute abundance of ceramides per subclass, calculated in pmol/sqv (b), and the relative ceramide subclass profile, indicated as the percentage of total detected ceramides in mol% (c), are shown as violin plots with median and interquartile range to provide better visualisation of the data distribution. Statistical analysis was performed using two-way ANOVA. Statistical significance is indicated as *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

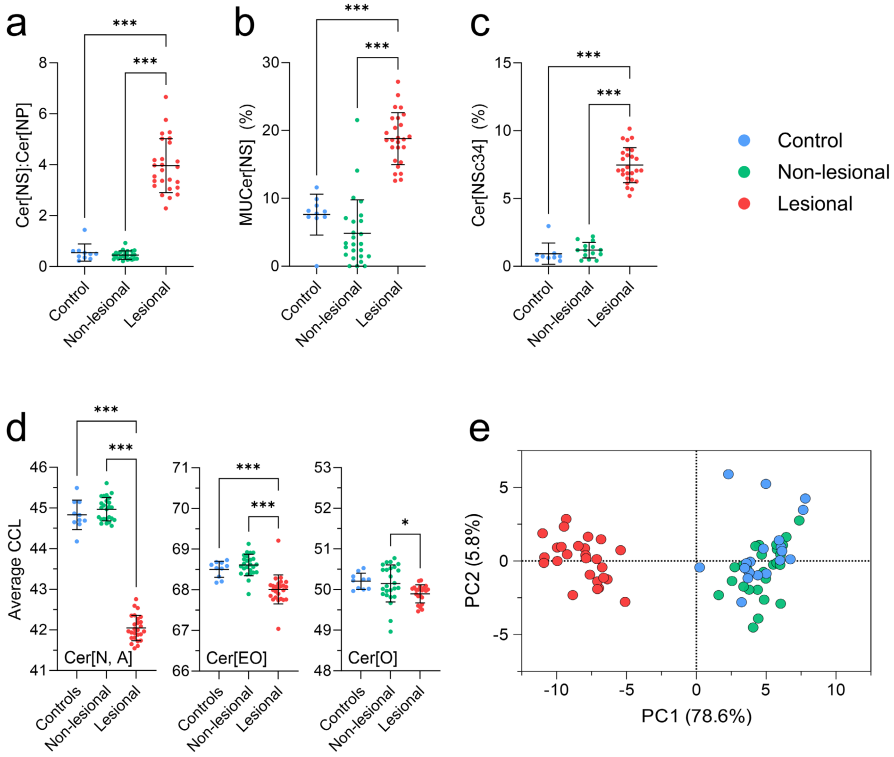

FIGURE 2 | Characteristics of the stratum corneum ceramide profile in controls, non-lesional psoriasis and lesional psoriasis. The molar ratio of Cer[NS]:Cer[NP] was calculated as an indicator for ceramide subclass skewing (a). The percentage of monounsaturated ceramides in the Cer[NS] subclass (MUCer[NS]) serves as an indicator for monounsaturation throughout all ceramide subclasses (b). The percentage of very-short-chain Cer[NSc34] of Cer[NS] was plotted (c). The average ceramide chain length (CCL) is shown per Cer[N] + Cer[A] (Cer[N, A]), Cer[EO] and Cer[O] (d). Bars represent the mean ± SD, and statistical analysis was performed using one-way ANOVA (a–d). The principal component analysis was performed with the relative abundance of all individually detected saturated ceramides, with the amount of variance explained by the respective components on either axis (e). Statistical significance is indicated as $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

based on the fact that unsaturation is introduced in fatty acids prior to incorporation into ceramides [44]. In lesional skin, the percentage of monounsaturated ceramides MUCer[NS] was significantly higher than in non-lesional skin and controls (18.8% vs. 4.8% vs. 7.6%, $p < 0.001$, Figure 2b).

For calculating the average CCL, expressed in number of total carbon atoms of acyl chain and sphingoid base, the ceramide subclasses were divided into three groups: Cer[N,A], Cer[EO] and Cer[O] (Figure 2d). Lesional skin showed a significant reduction in average CCL in the Cer[N,A] and Cer[EO] fractions compared to non-lesional skin and controls (42.1 vs. 45.0 vs. 44.8, $p < 0.001$, and 68.0 vs. 68.6 vs. 68.5, $p < 0.001$). Additionally, the average CCL in the Cer[O] fraction was reduced in lesional skin, but only compared to non-lesional skin (49.9 vs. 50.2, $p = 0.04$) and not compared to control. In particular, the percentage of Cer[NSc34], a very short-chain Cer[NS] with a total chain length of 34 carbons, was significantly higher in lesional skin compared to non-lesional skin and controls (7.5% vs. 1.2% vs. 0.9%, $p < 0.001$, Figure 2c). The average CCL per subclass indicates that the decrease in chain length was seen throughout all subclasses, except for Cer[EOP], Cer[EOH] and Cer[OH] (Figure S2). In the Cer[EOdS] subclass, the average CCL was increased in lesional skin.

Dimension reduction analysis by PCA was used to visualise the (dis)similarity of the different ceramide profiles (Figure 2e). The PCA plot illustrates how lesional skin forms a separate cluster compared to the overlapping datapoints of non-lesional and control skin, indicating a high degree of difference between the ceramide profile of lesional skin and that of non-lesional and controls. It also shows the high degree of similarity between non-lesional and control skin. The PCA loadings (Table S4) indicate that Cer[NP], Cer[AP], Cer[NS], Cer[NdS] and Cer[NH] with a CCL of 42 to 50 carbons contribute most to the distribution of data points. Of note, stratification of patients into mild and moderate-to-severe disease severity based on a PASI score of ≤ 5 or ≥ 10, respectively, revealed no evident differences in the ceramide profile (Figures S3 and S4).

3.2 | Skin Barrier Dysfunction Is Only Observed in Lesional Psoriasis and Correlates With Ceramide Characteristics

TEWL was measured as a surrogate marker for skin barrier integrity and was significantly higher in lesional skin compared to both non-lesional skin and controls (38.0 ± 16.4 g/m^2/h vs. 12.7 ± 5.6 g/m^2/h ($p < 0.001$) and 15.5 ± 7.6 g/m^2/h ($p < 0.001$), Figure 3a). There was no significant difference in TEWL between non-lesional skin and controls. To illustrate the relationship between ceramide composition and barrier function, selected ceramide parameters were correlated to TEWL. TEWL correlated positively with the ratio

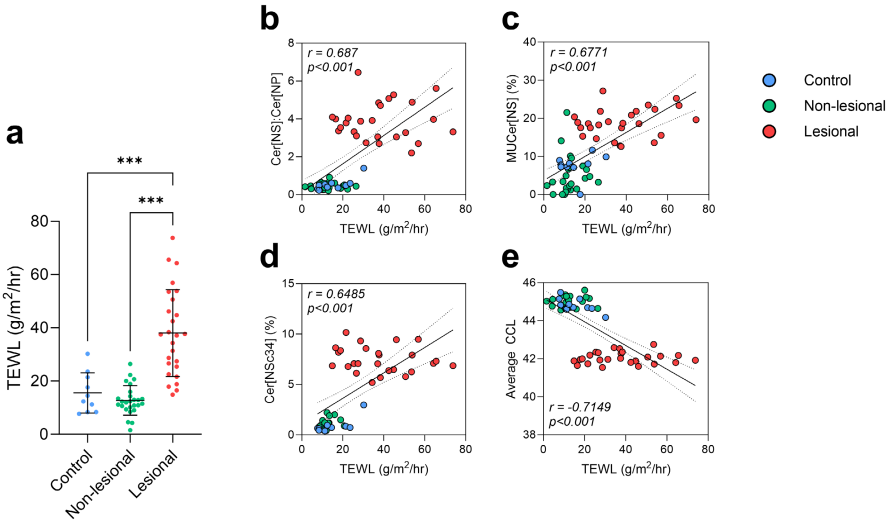

FIGURE 3 | Transepidermal water loss in controls, non-lesional psoriasis and lesional psoriasis, and correlation to ceramide parameters. Skin permeability was assessed by measuring transepidermal water loss (TEWL) in controls, non-lesional and lesional psoriatic skin. Bars represent the mean ± SD, and statistical analysis was performed using one-way ANOVA (a). The relationship between TEWL and Cer[NS]:Cer[NP] ratio (b), percentage of MUCer[NS] (c), percentage of Cer[NSc34] (d) and average CCL of Cer[N,A] (e) are demonstrated with a line representing the best fit of a linear regression through all data points with its 95% confidence intervals shown together with the Pearson correlation coefficient and associated *p*-value. ***$p < 0.001$.

of Cer[NS]:Cer[NP] ($r = 0.0687$; Figure 3b), the fraction of MUCer[NS] ($r = 0.6771$; Figure 3c) and the abundance of Cer[NSc34] ($r = 0.6485$; Figure 3d). A negative correlation was observed between TEWL and the average CCL of Cer[N,A] ($r = -0.7149$; Figure 3e). However, overall linearity appears limited as lesional skin is markedly different to control and non-lesional skin in terms of both TEWL and ceramide composition, resulting in two distinct populations.

3.3 | Barrier Characteristics Are Constant Over 2 Weeks

To assess the stability of the ceramide profile and repeatability of the results, the measurements were performed at a second timepoint 2 weeks later (Figure 4). No significant differences were found between the two timepoints (Figure 4a–d), except a small, but significant increase in the relative abundance of Cer[NdS] and Cer[NS] in the control group (13.6% vs. 19.5% and 10.4% vs. 15.4%, respectively, $p < 0.001$). No differences were observed in the barrier function (Figure 4e), the ratio of Cer[NS]:Cer[NP] (Figure 4f), the percentage of monounsaturation in Cer[NS] (Figure 4g), the average CCL in Cer[N,A] (Figure 4h), nor the abundance of Cer[NSc34] (Figure 4i). There were also no differences in the average CCL in the Cer[EO] and Cer[O] fraction (Figure S5).

4 | Discussion

In this study, we expand the knowledge on the SC ceramide profile in psoriasis by the in-depth profiling of lesional and non-lesional skin in a well-defined cohort of 26 patients and contrast this with healthy controls. We demonstrate a relationship between the altered ceramide composition and barrier impairment in lesional skin. The findings are substantiated by similar ceramide profiles obtained in a 2-week interval.

4.1 | Lesional Psoriasis Is Characterised by an Altered Ceramide Subclass Composition, Increased Unsaturation and Decreased Average Chain Length

Lesional skin showed a significant reduction in total SC ceramides compared to non-lesional skin and controls. Although some studies reported no significant difference in total ceramide content in psoriasis [12, 17], decreased abundances align with observations from the lesional skin of other dermatoses with an impaired barrier, such as AD [24, 27, 45, 46] and ichthyosis [47–50]. The lesional ceramide subclass composition showed strong decreases in the abundance of Cer[NP] and Cer[AP], countered by increased Cer[NS] and Cer[AS] abundances. These changes result in a skewing of the Cer[S] to Cer[P] ratio in lesional skin [22]. The alterations are in agreement with previously published work [12, 17–19] and align with other dermatoses [28]. However, the molar amount of ceramide per sqv indicates a major reduction in Cer[P] without a change in the amount of Cer[S]. Reflecting on the ceramide composition of lesional skin in AD, which has been described more extensively, increased levels of Cer[S] were mostly observed in relative profiles [24, 51], rather than in absolute profiles [27, 46, 52]. Presumably, increased relative Cer[S] abundances might merely reflect the interdependence of compositional data resulting from reductions in absolute Cer[dS], Cer[P] and Cer[H] abundances. Although previous studies on psoriasis reported a reduction in Cer[EO] [12, 17, 20], no evident changes in the Cer[EO] subclass abundance were observed in this study.

The role of alterations in ceramide processing enzymes and their effect on ceramide composition in psoriasis remains unclear. The expression of serine palmitoyltransferase, catalysing the first step in de novo ceramide biosynthesis, has been shown to be reduced in psoriasis and knocking serine palmitoyltransferase out in mice resulted in the development of psoriatic-like skin lesions [53, 54]. While this could explain

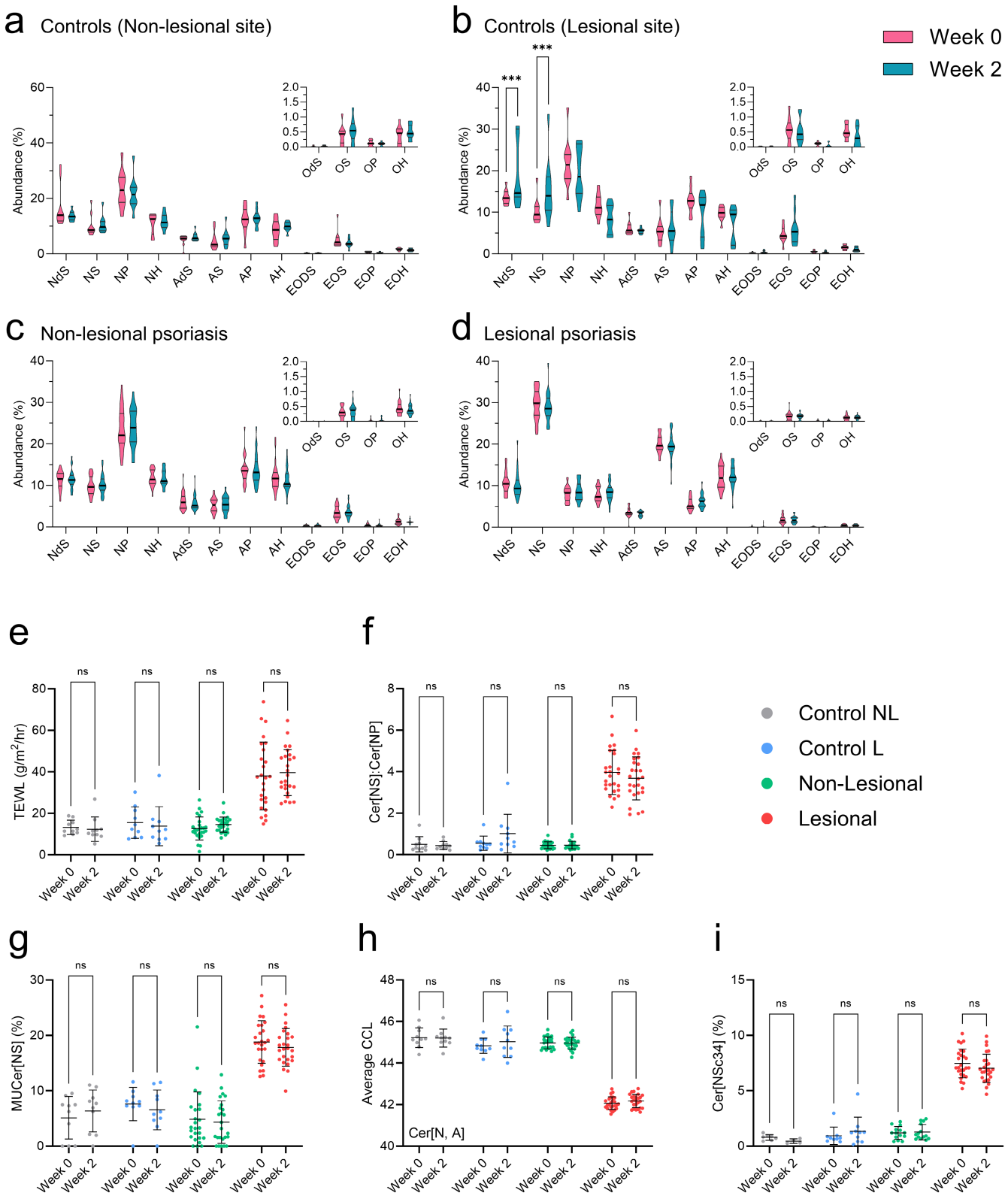

FIGURE 4 | Stability of the relative ceramide profile, barrier function and selected ceramide characteristics over 2 weeks. The relative ceramide profile was determined 2 weeks apart for controls harvested at the same site as used in case of non-lesional site (a) and lesional site (b), and for psoriasis at non-lesional site (c) and lesional site (d). The violin plots show median and interquartile range, and statistical testing was performed using two-way ANOVA (a–d). The barrier function was monitored using transepidermal water loss (TEWL) (e). The molar ratio between the abundance of Cer[NS] and Cer[NP] (f) and the percentage of monounsaturated MUCer[NS] of the entire Cer[NS] fraction (g) were calculated. Specifics on the average ceramide chain length (CCL) are shown of the Cer[N] + Cer[A] fraction (Cer[N,A]) (h) and the percentage of Cer[NSc34] of total Cer[NS] (i). Bars represent the mean ± SD, and statistical analysis was performed using two-way ANOVA (e–i). Statistical significance is indicated as not significant: ns; ***$p < 0.001$.

the overall reduction in ceramides, the changes in ceramide subclass composition indicate other enzymes might also be affected. In AD, lesional skin has been shown to exhibit an altered expression of β-glucocerebrosidase and acid sphingomyelinase, which are responsible for converting glucosylceramide and sphingomyelin into ceramides [24, 51, 55]. Additionally, dihydroceramide desaturase 2, responsible for the conversion of Cer[dS] to Cer[P], has also been shown to be downregulated in lesional AD [11, 56] and might explain the skewed Cer[S]:Cer[P] ratio in psoriasis. However, the decrease in Cer[dS], which is a precursor of Cer[S] and Cer[P], and the reduction in total ceramides might indicate a more upstream inhibition in the ceramide synthetic pathway as other enzymes appear to differentially contribute to Cer[P] synthesis, as Cer[P] was still present in a psoriatic murine model after knocking out dihydroceramide desaturase 2 [57]. As we show similar alterations in the ceramide profile in psoriasis as in AD, this might indicate comparable enzymatic changes.

No previous studies have investigated the degree of monounsaturation in psoriasis. Here, we demonstrate an increase in monounsaturation in lesional psoriatic skin, determined as percentage of monounsaturated ceramides within Cer[NS] (MUCer[NS]). This higher degree of monounsaturation might be linked to an increased activity of stearoyl-CoA-9 desaturase-1 [58–60], as altered expression of stearoyl-CoA-9 desaturase-1 has been reported in AD [51]. An increase in monounsaturated ceramides has also been reported in AD, Netherton syndrome and seborrheic dermatitis [25, 40, 47].

The reduction in average CCL in lesional psoriatic skin is in line with previous work where an increase in the level of short-chain ceramides and a decrease in the level of long-chain ceramides have been described [6, 17, 18]. An impaired chain elongation has also been extensively reported in AD [24, 27, 45, 46, 61, 62] and in skin models after inducing inflammation [51]. The activity of enzymes involved in ceramide chain length elongation might be affected by the increased expression of interferon-γ (IFN-γ) in psoriasis. IFN-γ was shown to reduce the amount of Cer[NS] with long-chain fatty acids in skin models and decreased the expression of fatty acid elongases ELOVL1, ELOVL4, ELOVL5 and ceramide synthase CerS3 and CerS6 in vitro and in murine models [6, 18, 63]. Especially very-short-chain ceramides Cer[NSc34] were significantly higher in lesional skin, which is in agreement with previous studies [17, 19]. As these ceramides might be an indicator of cellular stress, higher levels of Cer[NSc34] may more appropriately indicate a more dysregulated homeostasis rather than impairment of lipid chain elongation [64, 65]. However, this might be exaggerated due to the higher overall synthesis of ceramides in the SC. The average CCL in the Cer[EO] subclass showed less variation compared to other subclasses. It could be hypothesised that this is due to the fact that the esterification of Cer[O] with linoleic acid to Cer[EO] only occurs when Cer[O] have already been elongated to a certain chain length.

These similar changes in the ceramide profile in psoriasis and other skin conditions might indicate that these alterations are not specifically related to distinct pathogenic mechanisms but rather reflect a more general state of epidermal dysregulation driven by inflammation. In fact, pro-inflammatory cytokines that are increased in psoriasis due to immunological dysfunction affect the expression of ceramide processing enzymes [6, 18, 63, 66].

4.2 | Similar Ceramide Profile of Non-Lesional Psoriasis and Controls

The integration of the complete saturated ceramide profile through PCA highlights the difference of lesional skin compared to the high similarity of non-lesional and control skin. In contrast to lesional skin, non-lesional skin is very similar to control skin in terms of subclass composition, degree of monounsaturation and average CCL. Previous studies on uninvolved skin in psoriasis report either a similar profile [21] or less pronounced changes in subclass composition and chain length distribution compared to controls [18, 19, 29]. The high similarity of non-lesional psoriatic skin compared to controls reiterates the non-inflammatory phenotype of uninvolved psoriasis. This differentiates psoriasis from AD, where non-lesional skin is clearly associated with inflammation [67] and concurrently exhibits significant alterations in the SC ceramide profile [24, 45, 68]. These alterations could be detected even before disease onset [69].

In AD, changes in ceramide composition have been correlated to disease severity [23]. Since the aim of our study was to limit variability by including patients based on validated clinical scores, the lesions assessed in this study are all of similar severity, as defined by LSS ≥ 6, which hampers a correlation. The similarity of the lesions is substantiated by stratifying the patients into a mild and moderate-to-severe cohort, which revealed no evident changes in the ceramide profile and in target lesion severity between both cohorts. This is in line with pathomechanistic studies demonstrating a high degree of similarity in the lesional transcriptomic profiles between plaques of these groups [70, 71] and might suggest that local severity provides a more accurate reflection of the disease stage in psoriasis.

4.3 | The Ceramide Profile Is Stable Over 2 Weeks

In order to confirm our findings, a longitudinal component was added to the study. The robustness of the method is substantiated by the consistency of the ceramide profile when measured at two different timepoints. While the SC turnover time in healthy skin ranges from 14 days [72, 73] up to 20–30 days [74], the SC turnover in psoriatic plaques is much faster with only 2 days [73]. In this study, the assessments were performed 2 weeks apart, therefore exceeding the SC turnover time in psoriasis. The consistency in the ceramide profile between the two timepoints highlights the stability of the ceramide profile in psoriasis and the reliability of the findings.

The SC ceramide profile can vary due to factors such as age, ethnicity, seasonal variations and body site [68, 75–80]. In this study, the variability between groups has been limited by including controls matched for age and sex, recruiting throughout the

seasons and performing assessments on controls on the same location as patients.

4.4 | Barrier Dysfunction Can Be Correlated to Alterations in the Ceramide Profile, Chain Length and Unsaturation

Barrier impairment has been clearly linked to lesional psoriasis [30–34] and has also been observed in psoriatic mouse models [53, 81, 82]. Here, we confirm that the reduced barrier function is restricted to lesional skin, where changes in the ceramide profile are concomitantly observed. Inflammation, abnormal keratinocyte differentiation and reduced expression of epidermal proteins are all factors that contribute to the dysfunctional skin barrier in psoriasis [4]. Previous studies on lipid model systems have demonstrated association between changes in ceramide composition and barrier function by correlating the ratio of Cer[NS]:Cer[NP] [83, 84], average CCL [85, 86] and degree of unsaturation [87] to permeability. Barrier impairment of in vitro cultured human skin equivalents has also been linked to changes in the ceramide composition [88, 89]. In AD and seborrheic dermatitis, similar changes in the ceramide profile have been correlated to reduced barrier function [22–27].

It is known that the composition of ceramides is highly important for the SC lipid organisation, and thus essential for a functional barrier; [10] however, the association between ceramide profile and barrier function in psoriasis is not yet established. Therefore, we correlated single parameters of the ceramide subclass composition, degree of unsaturation and average CCL with barrier function and show that linearity between ceramide alterations and barrier dysfunction within lesional psoriatic skin appears limited. This might indicate co-occurrence rather than correlation and differentiates psoriasis from AD and seborrheic dermatitis, where alterations in the ceramide profile and barrier function follow a more gradual transition from lesional to non-lesional skin [22, 24, 25, 27]. However, this study included patients based on lesion severity, resulting in patients with lesions of comparable and moderate severity. While this allows for proper association between severity and barrier parameters, the inclusion of more diverse lesions might show a more nuanced transition instead of a strict dichotomy and might further affirm this relationship.

In contrast to psoriasis, barrier dysfunction in AD is not limited to lesional skin. These non-lesional barrier defects in AD are hypothesised to instigate inflammation and have been clearly linked to AD pathogenesis [67, 90]. In psoriasis, however, the absence of such alterations and barrier impairment in non-lesional skin indicate that chronic barrier dysfunction may not a causative factor for psoriatic lesions but rather a consequence of inflammation caused by immunological dysregulation. Ultimately, the skin barrier disruption might further aggravate inflammation. Therefore, treatments aimed at reducing inflammation may also normalise the SC ceramide profile and barrier function in psoriasis [90].

5 | Conclusion

In this study, we demonstrate that the SC ceramide profile in the lesional skin of psoriasis patients is characterised by an altered subclass composition, increased unsaturation and decreased chain lengths compared to both non-lesional psoriatic skin and healthy controls. These changes can be correlated to a reduction in skin barrier function, which underlines their interdependence. The alterations in the ceramide profile and barrier impairment are limited to the lesional skin of psoriasis patients. By adding a longitudinal component to the study, we confirm the reproducibility of the results.

Author Contributions

Conceptualisation and Design: J.R., C.M., M.B.A.D., J.A.B. and R.R. Data acquisition: J.R., C.M. and M.E.B. Data analysis and interpretation: J.R. and C.M. Supervision: N.B.K., T.N.K., M.B.A.D., J.A.B. and R.R. Writing – original draft: J.R. and C.M. Writing – review and editing: J.R., C.M., M.E.B., N.B.K., T.N.K., M.B.A.D., J.A.B. and R.R. All authors have read and approved the final manuscript.

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Ethics Statement

The clinical trial was approved by the medical research ethics committees Brabant (Tilburg, the Netherlands) under IRB approval number P1926 and registered on ClinicalTrials.gov. under NCT04394936.

Consent

All participants gave written informed consent.

Conflicts of Interest

M.B.A.D has received consulting fees or honorarium from Novartis, AbbVie, Pfizer, LEO pharma, Sanofi, Lilly, Janssen and Celgene and grant and payment for lectures including service on speakers bureaus from Novartis, Sanofi and Janssen outside the submitted work. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Appendix A

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