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REVIEW ARTICLE

The role of ceramides in skin barrier function and the importance of their correct formulation for skincare applications

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Abstract

Ceramides are a family of lipids constituted by a sphingoid base and a fatty acid. In the skin, they are mainly present in the stratum corneum where, with cholesterol and free fatty acids, they constitute the inter-corneocyte lipids. With the other lipid groups, they play a key role in the formation of dense lamellar structures between adjacent corneocytes, collectively ensuring the vital efficient barrier to water evaporation and protection from foreign agents´ penetration. Changes in ceramide level and relative composition, with potential impairment of lipid arrangement, have been evidenced in different skin conditions and skin diseases. Therefore, use of suitably formulated ceramides has been proposed for topical treatment to help re-structure damaged lipid arrangement and repair impaired skin barrier function. Nonetheless, the formulation of ceramides in products necessitates specific processes such as heating to high temperature before their introduction in the final formula. In this review on the structure, the role and the potential of ceramides for skincare, we point out the necessity of rigorous process when formulating ceramides into the final product. We demonstrate the counterproductive effects of undissolved ceramides on skin barrier repair capacity of the formulas, when assessed in different in vitro models of disrupted skin barrier.

KEYWORDS

ceramides, emulsion, formulation, skin barrier, skin physiology/structure, topical application

Résumé

Les céramides sont une famille de lipides constituée d'une base sphingoïde et d'un acide gras. Dans la peau, ils sont principalement présents dans la couche cornée où, avec le cholestérol et les acides gras libres, ils constituent les lipides inter-cornéocytes. Avec les autres groupes de lipides, ils jouent un rôle clé dans la formation de structures lamellaires denses entre les cornéocytes adjacents, assurant collectivement la barrière efficace vitale contre l'évaporation de l'eau

et la protection contre la pénétration des agents étrangers. Des modifications du taux de céramides et de la composition relative, avec une altération potentielle de l'arrangement lipidique, ont été observées dans différentes affections cutanées et maladies cutanées. Par conséquent, l'utilisation de céramides formulés de manière appropriée a été proposée pour un traitement topique afin d'aider à restructurer la disposition des lipides endommagés et à réparer la fonction de barrière cutanée altérée. Néanmoins, la formulation des céramides dans les produits nécessite des processus spécifiques tels que le chauffage à température élevée avant leur introduction dans la formule finale. Dans cette revue sur la structure, le rôle et le potentiel des céramides pour les soins de la peau, nous soulignons la nécessité d'un processus rigoureux lors de la formulation des céramides dans le produit final. Nous démontrons les effets contre-productifs des céramides non dissous sur la capacité de réparation de la barrière cutanée des formules, lorsqu'ils sont évalués dans différents modèles in vitro de barrière cutanée perturbée.

INTRODUCTION

Skin is a vital organ with two major roles. It is a barrier preventing evaporation of internal water and aggressions from the external environment. It is also a sensory organ at the interface between the internal and external world [1]. The skin barrier possesses numerous exceptional functions. The *stratum corneum* (SC) forms the physical barrier integrating various levels of intricate protective mechanisms including the acidic buffering capacity protecting us from chemical aggressions and the skin microbiome that controls over the proliferation of pathogens. This outermost barrier is completed by other functions of the viable epidermis with the detoxification and antioxidant capacity of the keratinocytes and the skin local immune system that prevents microbial infection [2-4]. Moreover, the skin neuronal terminations detect and prevent abnormal situations on the skin surface and the subcutaneous layer with the sweat glands help to regulate the body temperature.

The SC is constituted of two major components, namely the corneocytes (anucleated skin keratinocytes) and intercellular lipids. The renewal of the SC is ensured by the continuous proliferation and differentiation of keratinocytes and its structuration and maintenance by multiple enzymatic processes that still take place in this part of the body constituted of dead cells [5]. The architecture of SC resembles a 'brick and mortar' model which ensures a very thin and flexible but still very efficient physical barrier [6,7]. The intercellular lipids are mainly composed of three lipid classes, cholesterol, free fatty acids (FFA) and ceramides with an approximate 1/1/1 molar ratio [8,9]. The SC lipids arrange themselves into specific lamellar structures to build efficient barrier to the diffusion of water and other molecules. Within the SC lipids, ceramides are by far the most specific and the most diverse type. Decades of research have led to the identification of the SC ceramides and the understanding of their arrangement [10]. If the vital architecture of SC lipid matrix encounters a discrepancy in its composition, the barrier function is diminished and together with other pathophysiological processes, they give rise to various levels of skin impairments [11]. In the light of the need for effective skin restoration agents, this article focuses on the structure and the role of ceramides within the intercellular lipids, their potential benefits in skincare and the challenges associated with their formulation in products for topical application.

CERAMIDE BIOLOGY

Ceramide structure

Ceramides are a family of lipids characterized by the association of a sphingoid base and a fatty acid through an amide bond. The fatty acid carbon chain varies in length and is, in most cases, saturated. Ceramides are amphipathic molecules with hydrophilic and hydrophobic regions. The hydrophilic region is constituted mostly by hydroxyl groups on the sphingoid base and the amide bond, and the hydrophobic region by the two carbon chains of the sphingoid base and of the fatty acid. This amphipathic nature is of importance for the creation of the lamellar structures found in the intercellular cement of the stratum corneum (Figure 1).

Ceramide classification

Ceramides are grouped into several classes based on their molecular structure [12]. The classes of ceramides are denoted by a combination of letters that describes the type

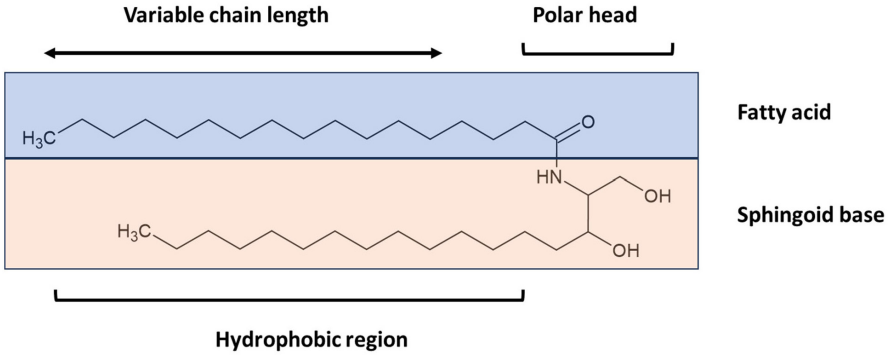


FIGURE 1 Ceramide structure. All SC ceramides consist of a sphingoid base and an acyl chain from a fatty acid linked by an amide bond. The sphingoid base is constituted by the association of a palmitic acid and a serine. Variation of the acyl length, degree of saturation, hydroxyl α or ω position on the acyl chain or saturation or presence of hydroxyl on the sphingoid carbon chain define the different types of ceramides which are further described in Table 1 (adapted from Bouwstra et al. [10]).

of fatty acid for the first letter and the type of sphingoid base for the second letter. This classification proposed by Motta et al. [13] can be further refined by giving the number of carbons and the level of unsaturation of the fatty acid chain to describe a single ceramide [14,15]. Most common components of skin ceramides are, for the fatty acid moiety: non-hydroxy (N), α-hydroxy (A), ester-linked ω-hydroxy acid (EO) and to a lesser extent ω-hydroxy (O) fatty acids and for the sphingoid base: phytosphingosine (P), sphingosine (S), 6-hydroxy-sphingosine (H), dihydrosphingosine (dS) and at a lower level 4, 14-sphingadiene (SD). For example, CER NP is a non-hydroxy acyl chain linked to a phytosphingosine, CER AS is an α-hydroxy acyl chain linked to a sphingosine base and CER EOH is a linolenic chain esterified to an ω-hydroxy acyl chain linked to a 6-hydroxysphingosine. The subclasses with the esterified ω-hydroxy acyl chain (EO), that is, CER EOS, CER EOH, CER EOP and CER EOdS, are referred to as CER EO. The subclasses with the ω-hydroxy acyl chain are referred to as CER O.

Table 1 describes the classes of ceramides so far found in the stratum corneum [10].

Ceramide biosynthesis

The generation of ceramides can occur through different pathways: the hydrolysis of complex sphingolipids (e.g. sphingomyelin) or glucosyl ceramides and the de novo synthesis from basic building blocks that involves a series of enzymatic reactions in cells [16,17]. The latter process typically occurs in the endoplasmic reticulum with the following major steps:

- condensation of two fundamental building blocks, serine and palmitoyl-CoA, by the enzyme serine palmitoyltransferase (SPT) to create the 3-keto-dihydro-sphinganine.
- reduction of the ketone by the keto-dihydro-sphinganine reductase (KDHR) to generate the dihydro-sphingosine.
- condensation of the dihydro-sphingosine with an acetyl-CoA activated fatty acid to create dihydroceramides. The step is catalysed by a group of six enzymes called Ceramide Synthase (CerS) 1–6 that have different affinities for different types of fatty acids and thus generate different dihydroceramides [18].
- dihydroceramides modification by dihydroceramide desaturases. These enzymes introduce a double bond into the dihydroceramide, converting it into a ceramide. A summary of the de novo bio synthesis of ceramides is described in Figure 2.

Ceramides are then transported from the endoplasmic reticulum to the Golgi apparatus, and can undergo additional modifications, such as glycosylation (addition of sugar groups) and sulfation (addition of sulfate groups). These processes result in the formation of complex sphingolipids, including sphingomyelin and glycosphingolipids.

The structural diversity of ceramides arises from the variety of sphingoid bases and fatty acids used during the synthesis, and from the differential expression of ceramide synthases. Different types of ceramides can be distinguished based on the length of the fatty acid chain, with ceramides having long chains (LC) (C14–C18), very long chain (VLC) (C20–26) and ultra-long chain (ULC) (>C26) [18,19]. In human stratum corneum fatty acid length ranges from C18 to C36 with a majority of ceramide with C24–C28 fatty acids moiety [12,20].

ULC ceramides can be further processed with the introduction of a hydroxyl group on the ω-carbon by cytochrome P450 enzymes followed by esterification with linoleic acid to generate the ω-esterified ULC ceramide or ester-linked ω-hydroxy acids ceramides (EO series). Although those ceramides are not the most abundant in the inter-corneocyte

lipid matrix, they play a specific role in the formation of the corneocyte lipid envelope [21-23].

Composition of ceramides in the stratum corneum

Ceramide composition of normal (healthy) stratum corneum lipid matrix in humans has been explored in different studies; thanks to lipidomic techniques based on mass spectrometry. It was found that most of the ceramides belong to the non-hydroxy family (NP, NH, NS and NDS) representing about 55% of the total free ceramides seconded by the α-hydroxy ceramides (AH, AP and AS) with about 35% of the total unbound ceramides. At a lower level, but always present, the ω-esterified ceramides (EOS, EOH and EOP) represent about 10% of the total ceramide mass [24]. Those numbers are approximate, and the mean measured content from different studies and more details are shown in Table 2 [12,14,25,26].

The relative abundance of ceramides also varies among body sites. Some ceramides such as CER NH, CER NP or CER AH are highly variable in content among body location whereas CER EOS or CER EOP level is fairly constant. Based on this and with the repartition of the FFA chain length, a clustering of the different body sites (with similar composition of the lipids) shows that the stratum corneum lipids composition is different on the face, abdomen, back and arm, the hand and lower leg and finally the plantar heel each with a very specific lipid composition [27].

CERAMIDES AND SKIN BARRIER FUNCTION

Together with cholesterol and free fatty acids, ceramides are the major components of the inter-corneocyte lipids and play a major role in the skin barrier function preventing internal water evaporation and penetration of external aggressors. The efficacy of the lipid to constitute an efficient barrier is intimately linked with their relative composition, structure and arrangement, with all parameters being also linked together.

Looking at the overall stratum corneum (SC) organization, the SC lipids are located between corneocytes filling the space that separates the cells. Zooming in on this inter-corneocyte space, images from electron microscopy reveal that the lipids are organized in a succession of regular lamellas that are approximately parallel to the corneocyte and the skin surface [28]. It also shows the existence of a specific sheet covering the surface of the corneocytes, called the corneocyte lipid envelope (CLE), that constitutes the interface between the hydrophilic surface of the corneocytes and the inter-corneocyte lipids [22].

The corneocyte lipid envelope (CLE)

The CLE is made of ω-hydroxylated ceramides and in a lesser amount with ω-hydroxylated free fatty acids covalently bound to the corneocyte protein envelope (Figure 3). This structure results from an enzymatic processing of the esterified ω-CER, mainly CER EOS. The terminal linolenic acid bound is oxygenated by the 12R-lipoxygenase (12R-LOX) and the epidermal lipoxygenase 3 (eLOX3) to generate a CER EO-epoxy-alcohol. This alcoholic compound can either be further oxidized by the short-chain dehydrogenase/reductase 9C7 (SDR9C7) into a ketone allowing a potential non-enzymatic covalent bonding on the ɣ-amino functional group of the glutamine from the corneocyte envelope proteins. The same CER EO-epoxy-alcohol compound can be also processed by the epoxyhydrolase 3 (EPHX3) and esterase to generate the CER O (ω-hydroxylated ceramide) that can be bound to the same protein residues by the transglutaminase 1 (TGM1) [29,30].

CLE creates an interface between the corneocyte surface and the intercellular lipid cement and is important in the barrier function. Impairment of the ω-hydroxylation of ceramides [31] or loss of function by mutation of 12R-LOX [32] and eLOX3 [33] generates severe ichthyosis in human and a deficient barrier in mice. The suggested role of CLE is to serve as a scaffold for the organization of the lipid lamellar bilayers and for their orientation parallel to the corneocyte surface. It may also serve as semipermeable membrane that allows the free passage of water from corneocytes while retaining the larger hygroscopic molecules such as filaggrin breakdown. Attack of bound CER O by bacterial ceramidases that process bound ceramides into ω-hydroxylated free fatty acid could decrease this semipermeable barrier function and could be one mechanism contributing to atopic dermatitis [22].

Organization of the lipids in the inter-corneocyte space

As stated above, intercellular lipids are organized in lamellar structures with an alternance of planar sheets of hydrophobic (constituted by the non-polar region of the cholesterol and the carbon chain of the sphingoid base and the fatty acids) and hydrophilic (corresponding to the polar head of the ceramide and of the cholesterol and the acid termination of the FFA) regions. The comprehension of these structures and their impact

on the barrier efficacy has been studied during the last four decades by different groups using different models and different methods. Models can be, for example, SC from human, pig or mouse, reconstituted epidermis, or human skin explant and in vitro lipid models. Methodologies to explore the lipid organization include microscopy, infra-red or Raman spectroscopy and diffraction of electron, neutron or X-ray coupled with the use of deuterated lipids [10].

Lipid organization can be described in two dimensions: the spacing of the lipid lamellae and the lateral packing of the lipids. Lipid lamellae can arrange themselves into repeated units of sheets exhibiting two different lengths: either a repeated unit of 6 nm also called the short periodicity phase (SPP) [34,35], or a repeated unit of 13 nm called the long periodicity phase (LPP) [36]. Orthogonally to the lamellar plane axis, lipids can form very dense orthorhombic packing, a less dense hexagonal packing or a more disorganized fluid packing (Figure 4) [26]. LPP organization as well as the orthorhombic packing show a higher barrier function [37,38] when compared to SPP and hexagonal and fluid lateral packing respectively [39-41]. In the human SC, the LPP and orthorhombic structures are mainly found in the central part of the SC where the barrier function is also the highest. The lipids show more disorganized structure in the basal part and near the surface of the stratum corneum. The reason of this disorganization is not completely understood but could be the consequence of the ceramides' biochemical maturation and an uncomplete structuration of the lipids in the basal part of the stratum corneum. On the outer side, there can be an impact of sebum lipids, xenobiotics, microbiota and other external effects [42].

TABLE 1 Classification of stratum corneum ceramides (adapted from Bouwstra et al. [10]).

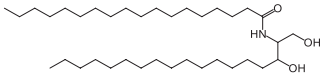
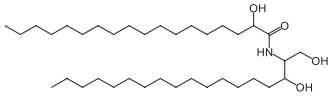
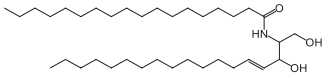
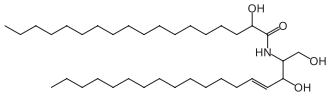
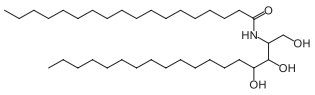
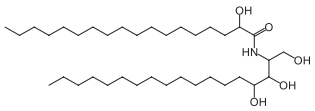
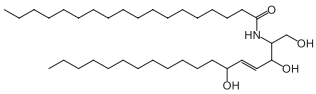
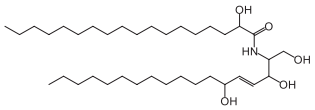
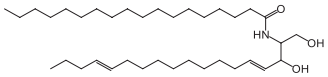
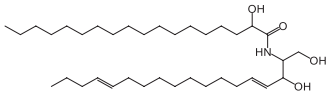
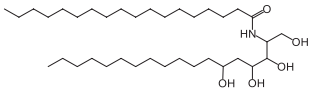
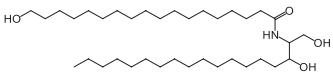
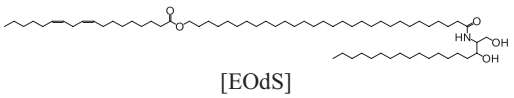
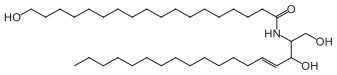
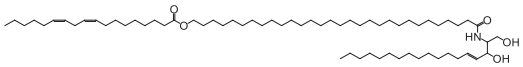
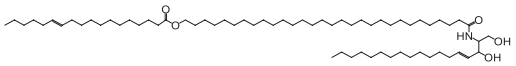
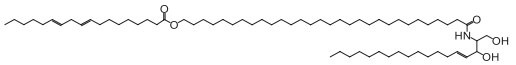
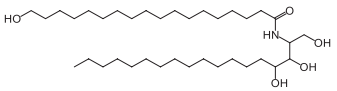
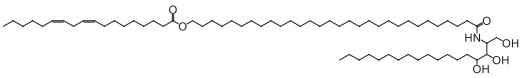
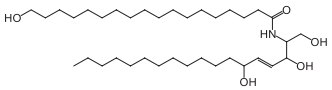
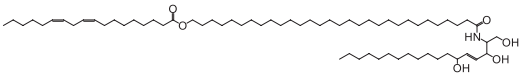
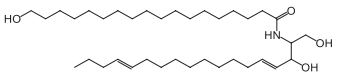
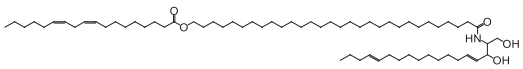
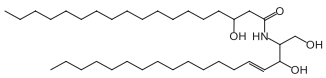
	Non-hydroxy fatty acid [N]	α-hydroxy fatty acid [A]
Dihydrosphingosine [dS]	 [NdS]	 [AdS]
Sphingosine [S]	 [NS]	 [AS]
Phytosphingosine [P]	 [NP]	 [AP]
6-hydroxysphingosine [H]	 [NH]	 [AH]
4,14-sphingadiene [SD]	 [NSD]	 [ASD]
Dihydroxy- dihydrosphingosine [T]	 [NT]	
	ω-hydroxy fatty acid [O]	**Esterified ω-hydroxy fatty acid [EO]**
Dihydrosphingosine [dS]	 [OdS]	 [EOdS]

TABLE 1 (Continued)

	ω-hydroxy fatty acid [O]	Esterified ω-hydroxy fatty acid [EO]
Sphingosine [S]	 [OS]	 [EOS]
		 [EO - 18:1]
		 [EO - 18:2]
Phytosphingosine [P]	 [OP]	 [EOP]
6-hydroxysphingosine [H]	 [OH]	 [EOH]
4,14-sphingadiene [SD]	 [OSD]	 [EOSD]

	ß-hydroxy fatty acid [B]
Sphingosine [S]	

Note: The table describes the most common ceramides of the stratum corneum according to the nomenclature introduced by Motta et al. [13]. The acyl chains and the sphingoid bases are indicated by one or two letters [13]. The acyl chains are either the non-hydroxy (N), α-hydroxy (A), β-hydroxy (B), ω-hydroxy (O) or linoleic acid esterified to an ω-hydroxy acyl chain (EO). The sphingoid base is either dihydrosphingosine (dS), sphingosine (S), phytosphingosine (P), 6-hydroxysphingosine (H) or 4, 14 sphingadiene (SD) and dihydro dihydrosphingosine (T). In each ceramide subclass, there is a distribution of acyl chain lengths and sphingoid base chain length. In general, the acyl chain length distribution is much broader than the sphingoid base chain length distribution. Linoleate, and less often oleate are linked to the ω-hydroxy acyl chain of the CER EO.

Using the lipid model systems, different parameters have been identified for the formation of the different lamellar structures. First, each of the lipid classes plays a role in the organization of the SC lipids: non-CER EO are the building blocks of these lamellar structures as they can arrange themselves in parallel sheets with the SPP structure. However, to obtain the LPP, the presence of cholesterol is important [43,44] as it allows a better mixture of ceramides and the FFAs. Also, the presence of the specific structure of the ultra-long chain (ULC) CER EO is important to form and stabilize the LPP units with CER EOS having the highest potential [38,45,46,47,48]. The presence of FFA with very long chains (at least C24) is determinant for the orthorhombic packing and consequently the barrier function of lipids mixtures. The presence of FFA at the same ratio but with shorter chain can have a detrimental impact on barrier function of these models [43].

Besides the mandatory presence of CER EO for the creation of the LPP structure, the composition of the 'standard' ceramides has a lower impact. Nonetheless, phytosphingosine ceramides (CER 'X'P) show a higher lamellar ordering but because of their more polar and more steric head they tend to form more hexagonal lateral packing than the sphingosine ceramides (CER 'X'S) [40,41]. Overall, lipid organization is more stable when different types of ceramides are used to build the lipid models compared to those where only one ceramide type is used [10].

The molecular arrangement of the lipids within the lamellae is still under discussion. Using deuterated CER EOS and cholesterol in different position of the molecules Mojumdar et al. [49] were able to demonstrate that LPP could be made of three symmetric units of 4.4, 4.2 and 4.4 nm with CER EOS located at the boundaries pointing acyl or sphingosine chains towards either the centre of

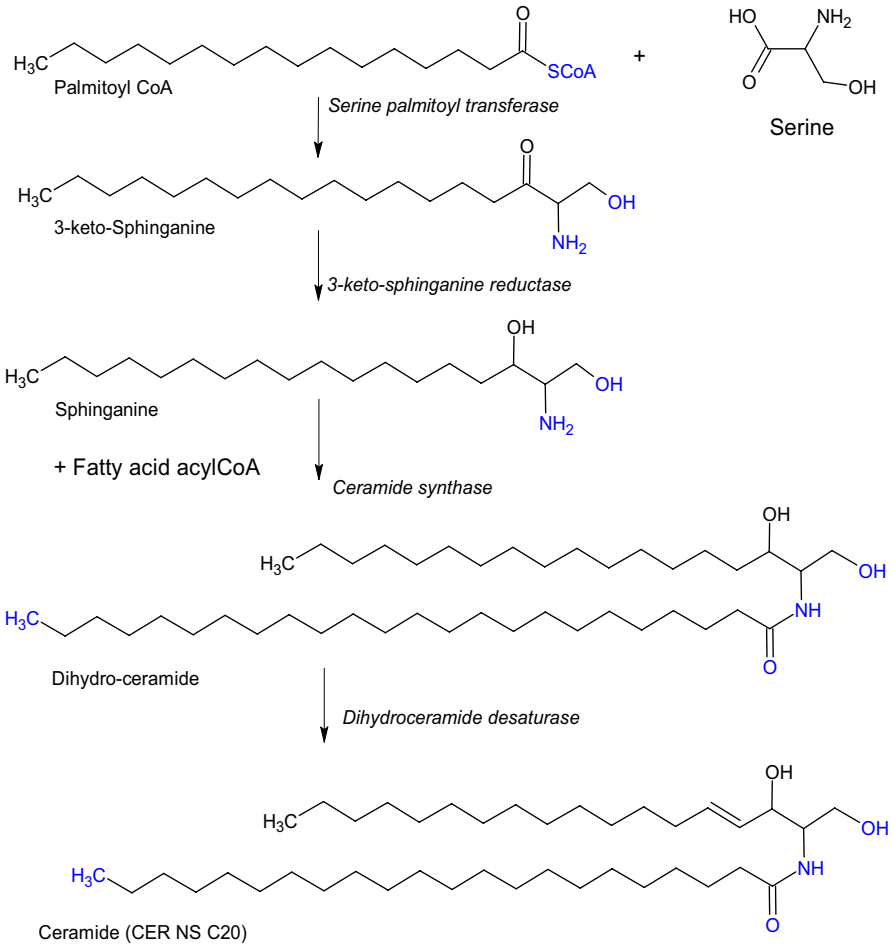


FIGURE 2 De novo biosynthesis of ceramides. The first and rate limiting step is the condensation of amino acid serine with saturated fatty acid palmitate which is catalysed by serine palmitoyl transferase (SPT). Subsequent reduction to sphinganine is catalysed by 3-ketosphinganine reductase (KDSR). A variety of ceramide synthases (CerS1–6) catalyse the conjugation of a second acyl-CoA of variable chain length (C14-C32) leading to the formation of dihydroceramide. Dihydroceramide desaturase converts the intermediaries into proper ceramides. Ceramides are synthesized de novo in the endoplasmic reticulum.

TABLE 2 Composition of unbound intercellular ceramides.

	N non-hydroxy FA	A α-hydroxy FA	O ω-hydroxy FA	EO esterified ω-hydroxy FA	B β-hydroxy FA
DS Dihydrosphingosine	NDS 6.1	ADS 1.3	ODS 0.1	EODS 0.6	
S Sphingosine	NS 6.4	AS 5.3	OS 0.7	EOS 4.6	BS 0.S2
P Phytosphingosine	NP 23.4	AP 11.9	OP 0.3	EOP 1.4	
H 6-Hydroxyshingosine	NH 18.3	AH 14.2	OH 0.5	EOH 3.8	
SD 4-14-Sphingodiene	NSD 0.1	ASD 0.2	OSD 0.0	EOSD 0.0	
T Dihydroxy- Dihydrosphingosine	NT 1.7				

Note: The table compiled data on ceramide content in the stratum corneum from the literature as indicated in the following references: t'Kindt et al. [12], van Smeden et al. [25,26], Masukawa et al. [12] and Kawana et al. [14]. Values represent the average percentage of the ceramide subclass reported to the total ceramides. For the calculation, a null value was adopted when the ceramide subclass was not detected.

the unit or to an adjacent unit, whereas C24 fatty acid and cholesterol are located inside the unit with the hydroxyl of the cholesterol interacting with the ester function of the acyl chain of the CER EOS. In this model, the conformation of the ceramides could be either in hairpin or extended conformation.

Another model was recently proposed by Norlén et al. [42]. The authors used cryo-electron microscopy and molecular design to propose lipid arrangement models. They described the changes in the pattern of lipids observed within the five first layers of corneocytes corresponding to the maturation of the ceramides as well as re-arrangement of the molecules. In this model, the ceramides shift from a mixed hairpin and linear conformation to a fully extended conformation. The model also predicts the formation of 12 nm repeated unit with no interdigitation of the ceramides except for the CER EOS. In this model, the FFAs interact preferentially with acyl chains of the ceramide and cholesterol with the sphingoid chain (Figure 5) [42].

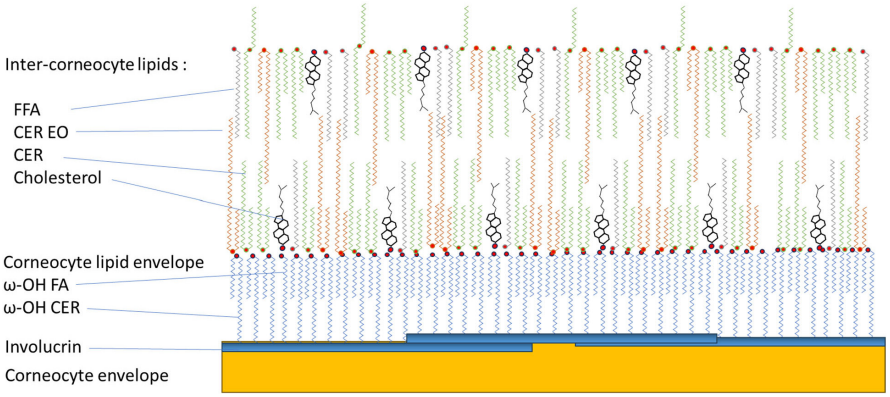


FIGURE 3 Model of the corneocyte lipid envelop (CLE). ω-OH ceramide and ω-OH FFAs (represented with blain chains) are covalently linked to the corneocyte envelope proteins (involucrin) and serve as a frame to orient lamellar structure of the inter-corneocyte lipids.

SKIN CONDITIONS AND CERAMIDE

Ceramide relative quantity, ceramide classes distribution and acyl chain length have been associated with the quality of the barrier function in lipid models [10]. Given the importance of the barrier constituted by the SC in the cutaneous physiology, research and clinical teams have investigated potential changes in the level and arrangement of inter-corneocyte lipids in normal skin and when skin is exposed to different external or internal stressors such as cold, UV light, ageing or during chronic inflammatory skin diseases like atopic dermatitis and psoriasis conditions where skin barrier is often compromised.

In normal human volunteers, Ishikawa et al. [50] measured the level of different ceramide classes in the SC of different body parts and negatively correlated them with the barrier function assessed by trans-epidermal water loss (TEWL) and positively with SC hydration. Interestingly, in the same study, TEWL was also very nicely correlated with acyl chain length of non-EO ceramides.

Seasonal variation

In the same study [50], the total level of ceramides and their acyl chain length were explored in the same volunteers over 1 year and variations were evidenced with less ceramide level and lower carbon number of acyl chains in winter season on some exposed body sites. Even if the number of volunteers included ($n = 10$) was too low to reach statistical significance, this study confirmed the results of a previous one showing that the level of total ceramides decreased in winter [51]. In a more recent study, Fujiwara et al. [51] described a lower level of saturated and unsaturated ceramides in autumn and winter compared to spring and summer in the tape strip done on the volar forearm of 99 volunteers. Season was also described to have an impact on ceramide levels in patients with acne with notable reductions in CER NH and CER AH as well as the acyl-ceramides, namely CER EOS and CER EOH in autumn and winter. These differences correlated with an increase in TEWL [53].

Effect of ageing

Study of the evolution of the skin barrier function as a function of age has been a topic of interest during the last decades. In 1995, Ghadially et al. [54] described that in aged subjects, epidermis displays a diminution in secreted lamellar body-derived contents, and a failure of these contents to form a continuous series of multilamellar bilayers. A year later, Rogers et al. [51] described that levels of all major lipid species, in particular ceramides, decreased with age including ceramide 1 linoleate (CER EOS linoleate) while CER EOS oleate increased with age. The previously cited study of Fujiwara et al. [52] also showed that, if the level of total ceramide was similar in young and aged donors during autumn and winter, this level significantly decreased in the aged group (>50 years) during spring and summer.

Also, studies that focused on the effect of menopause showed a significantly decreased level of total ceramides in the skin of post-menopausal women (vs pre-menopausal group) [55]. This result was confirmed in a larger number of volunteers [56] where reduction of the ceramide level together with lower carbon content on the sphingoid base and increase of TEWL were reported. Interestingly, the level of sphingomyelins was higher in post-menopausal skin suggesting an impairment of the pathway producing ceramides from sphingomyelin precursors. In the two cited studies, a hormone replacement therapy can counteract most of the changes observed [56]. Overall, although the ceramide levels and structure differ in the aged skin, the underlying physiological mechanisms are not yet clear.

Finally, one of the major drivers of skin ageing is the exposition to sunlight and especially the effect of ultraviolet radiation (UVR) on the skin leading to an accelerated

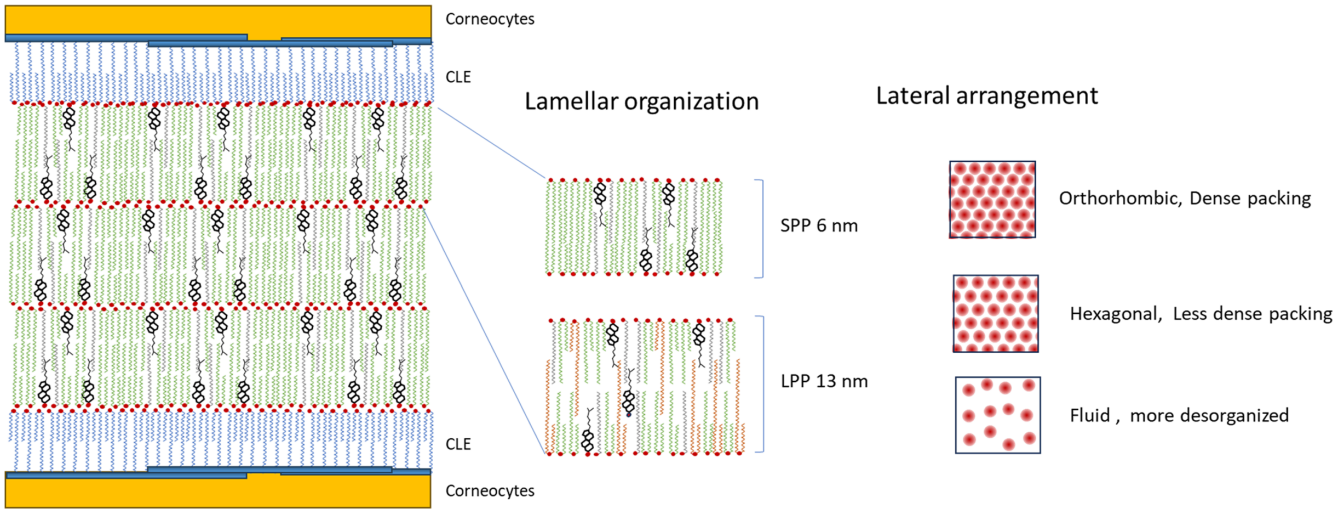


FIGURE 4 Inter-corneocyte lipid organization in the stratum corneum. The stratum corneum is the outermost layer of the epidermis (1). The SC consists of dead cells (corneocytes) embedded in a lipid matrix (2). The inter-corneocyte lipids are arranged in lamellae (3), with two coexisting lamellar phases with either a repeat distance of 6 nm (referred to as the SPP) or 13 nm (referred to as the LPP). The lateral organization in the plane perpendicular to the direction of the lamellar organization exhibits three possible arrangements of the lipids: a very dense, ordered orthorhombic packing; a less dense, ordered hexagonal packing and a disordered liquid lipid packing (adapted from van Smeden et al. [26]).

ageing of the skin also called photo-ageing. Exposure of the skin to UVR appears to impair the expression of the enzymes involved in CER EO bonding (12R-LOX and SDR9C7) to corneocytes in mouse skin and in human skin. The consequences are reduction of covalently bound CER EO levels and an increase in the free ω-acyl-ceramides for which an impairment in the barrier function in lipid models was observed when introduced at too high concentrations [5,57].

Skin diseases

Atopic dermatitis (AD)

Atopic dermatitis is a relatively frequent skin condition characterized by a chronic inflammation of the skin with recurrent flares affecting different parts of the body and face [58]. The aetiology of the disease is now associated with barrier defect allowing the penetration of antigens that could trigger the Th2 response of the immune system. This response leads to local skin inflammation with the creation of Th2 memory cells that can be reactivated chronically. The impairment of the barrier function has motivated scientists to explore different components of the skin barrier and led to identify the role of loss-of-function mutations in the filaggrin genes that increase the risk of atopy in the mutation carriers [59,60]. Changes in the inter-corneocyte lipids were also investigated and multiple observations were made by different authors. It is not the purpose to perform an extensive review of the skin lipid changes accompanying AD. Nonetheless, AD is often associated by an increase in CER AS, AH, ADS and NS and a decrease in CER AP, NP, NH and EOs classes together with a decrease of ultra-long chain ($C > 44$) ceramides and a concomitant increase in long and very long chain ($C > 34$) ceramides [61-64]. This later phenomenon is also observed for FFAs with an increase in long chain ($C < 18$) and decrease of very long chain FFAs ($C > 24$) with also an increase in monounsaturated fatty acids [64]. In AD skin, the protein-bound ceramide also showed a reduction in the length of acyl chains for ceramide and FFA and an increase in the level of monounsaturated ceramides with a reduction of the chain length especially in lesional versus non-lesional skin [65].

Those changes can be correlated with variations of the expression level of enzymes involved in ceramide and fatty acid synthesis observed in atopic skin: the increase in CerS4, that catalyses the acylation of ceramide with short chain fatty acids [66], the decrease of CerS3 expression that catalyses the acylation of ceramides with long fatty acids ($C > 26$), an increase in lesional skin of the stearoyl-CoA desaturase1 (SCD1) that transform the saturated fatty acids into monounsaturated ones [66] and a decrease in the expression of elongase 1 (ELOVL1) responsible of the elongation of fatty acids from C20 to C26 [67].

At the lipid organization level, shorter repeat distance, and lower presence of LPP is observed in atopic skin together with a smaller fraction of the lipids adopting a packed orthorhombic structure that correlated with a higher TEWL [68-70]. Finally, using a meta-analysis tool, a correlation between the expression of

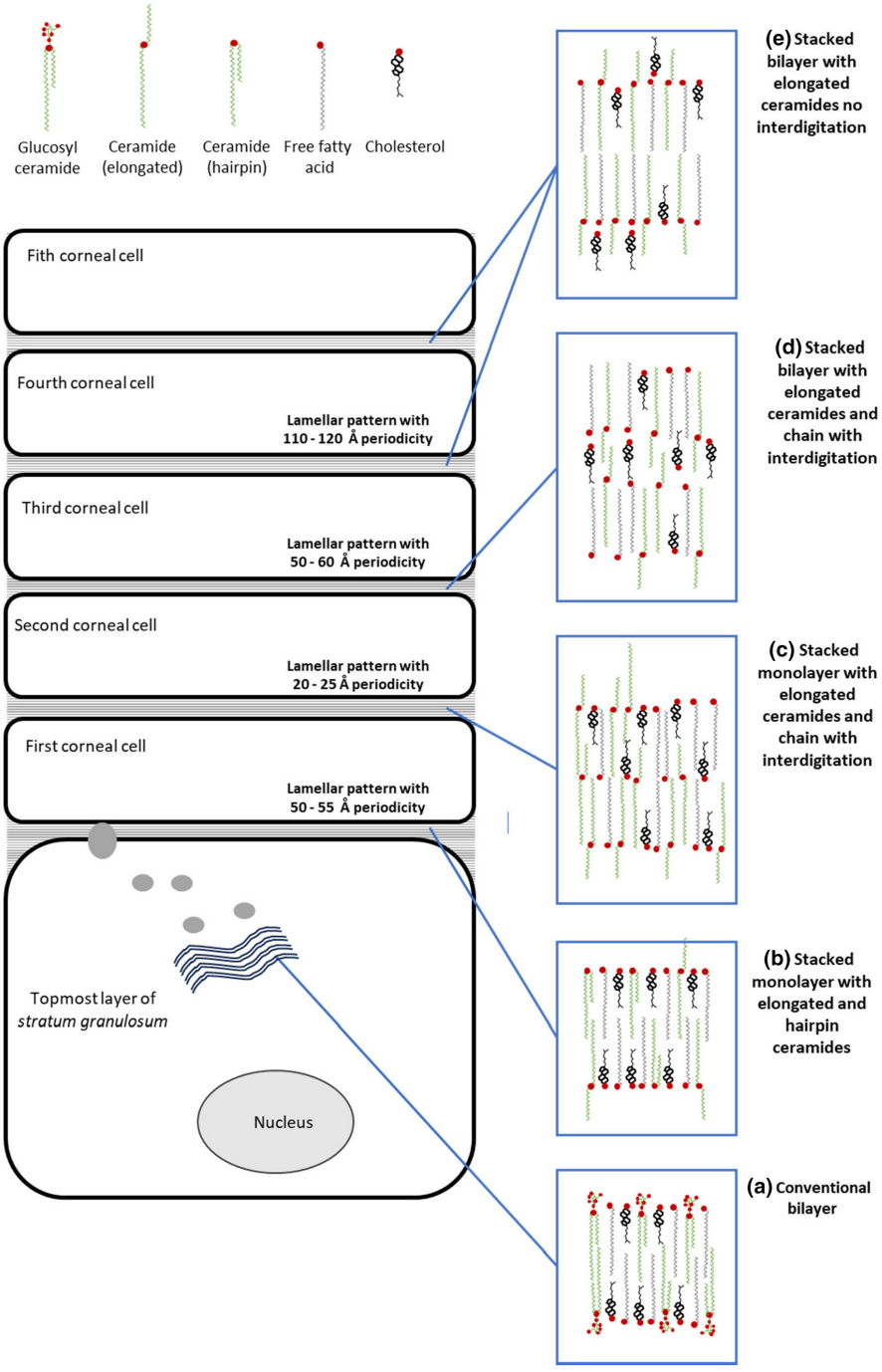


FIGURE 5 Model of the stages of stratum corneum lipid arrangement within the first bottom layers of the stratum corneum (adapted from Norlén et al. [42]). Based on cryo-electron microscopy and molecular modelling, the model proposes five stages of arrangement of the intercellular lipids: (a) Highly folded and highly hydrated glucosyl-ceramide-based conventional lipid bilayer. (b) Stacked monolayer with mixed hairpin and splayed-chain ceramides with a periodicity of 50–55 nm. (c) Stacked monolayer with splayed-chain ceramides and chain interdigitation with a periodicity of 20–25 nm. (d) Stacked bilayer with splayed ceramides and chain interdigitation with a periodicity of 55–60 nm. (e) Stacked bilayer with splayed-chain ceramides without interdigitation with a periodicity of 110–120 nm.

Th2 activation genes is seen with the decrease in genes coding enzymes involved in lipid metabolism such as elongase 3 (ELOVL3), fatty acid 2-hydroxylase FA2H and fatty acyl-CoA reductase 2 (FAR2), thus linking the inflammatory response to the disorder in skin lipid metabolism [71].

Psoriasis

Psoriasis is a chronic inflammatory skin disorder characterized by the major symptom of 'plaques.' Plaques are raised, red, scaly patches of skin caused by an overactive immune response, which leads to an accelerated skin cell growth cycle. These plaques can appear anywhere on the body and are often accompanied by itching and discomfort. Th17 lymphocytes activation and its related cytokines play a crucial role in the immune response and the development of this condition. A perturbation of the skin barrier is also observed with the marked elevation of TEWL in involved skin [72].

Changes in the ceramide profile have been described as a significant decrease in CER EOS, CER NP and CER AH and an increase in CER NS together with a reduction in

acyl fatty acid chain length [13,73,74,75]. The knockout of serine palmitoyl transferase (SPT) genes in mice induced a decrease in ceramide content, the development of psoriasiform lesions and the expression linked to a Th17 response [76]. In this disease, the role of interferon-ɣ (IFNɣ) has also been documented as it decreases the expression of several elongases of long chain fatty acids and redistributes the expression of the different CerS (CerS 1–6) [77].

POTENTIAL USE OF CERAMIDES IN SKINCARE

The change in the composition of ceramides is associated with a deficit in skin barrier. Regardless of the disruption origin, the healthy, fully functional skin should be able to initiate the restoring processes and by the synthesis of appropriate lipids reverse the damage. However, owing to the number of underlying genetic or environmental reasons listed in the previous chapter, sometimes the skin disease aborts the lipid replenishment and the needed rapid repair is not achieved. For this reason, there are already several established methods for the management of the diseased skin barrier such as application of hydrating lotions in combination with anti-inflammatory treatment. In recent years however, certain questions regarding the application of moisturizers on the disrupted skin barrier were raised [78].

As a consequence, several teams have explored whether a topical application of ceramides would help to recover the barrier function in models where the skin is impaired either intentionally with a stress (e.g. sodium dodecyl sulfate [SDS] treatment, tape stripping) or in volunteers suffering from skin conditions with a dysfunctional skin barrier.

For example, treatment with monomodal dispersions containing 0.3% of CER-EOS and CER-EOP formulated with cholesterol allowed the recovery of the LPP arrangement detected by X-ray scattering in a reconstructed epidermal model pre-treated with SDS [79]. In an ex vivo human skin model based on tape stripping to mimic several aspects of the lipid organization in AD skin, the topical application of formulations containing CER EOS or CER NS resulted in a higher fraction of the orthorhombic lateral packing, mimicking more closely the lipid organization in native human skin [80]. Furthermore, in a study performed on human volunteers whose skin epidermal barrier was disrupted by either UVB irradiation or tape-stripping, an improvement of TEWL and of the cohesion of SC was observed after the topical application of product containing C16 ω-OH-phytoceramide [81]. Also, using a pig skin model where the barrier was disrupted by different techniques (SDS, chloroform/methanol treatment or tape stripping), the use of formulations based on CER AP, CER NP, cholesterol and stearic acid could, at least partially, recover the barrier function judging from the permeation behaviour of lipophilic compounds (indomethacin and theophylline) [82]. These results confirm an original observation where a CER NP containing product could also improve the skin barrier function in SDS-treated or tape-stripped volunteers when compared to a petrolatum-containing product [83]. Other studies have shown that topical use of ceramide-containing products could improve the skin barrier in different skin conditions such as senile xerosis [84,85] or psoriatic skin as a companion product of corticoid treatment [86]. However, those later studies were performed with complex products, often without a control product to clearly isolate the effect of the ceramides.

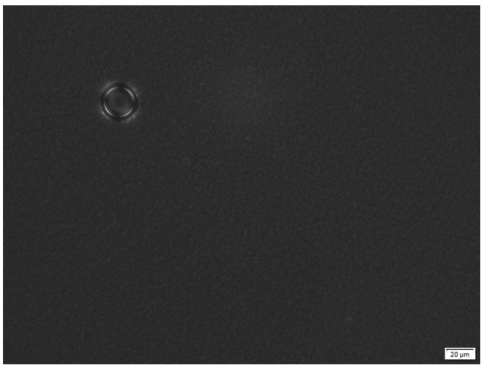
INTRODUCTION OF CERAMIDES IN SKINCARE FORMULATIONS

Challenges in ceramide formulation

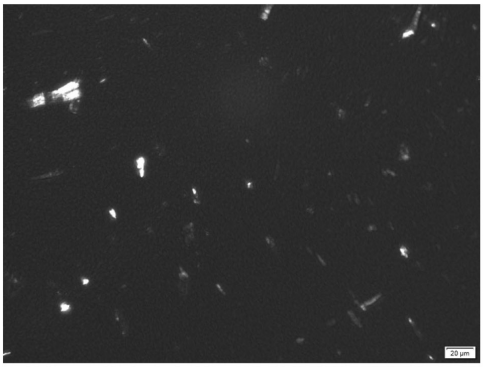
As seen above, delivering ceramides into the skin could be considered as an interesting way to improve impaired skin barrier function to prevent water loss and permeation of xenobiotics and decrease the severity of symptoms of previously cited skin conditions and skin diseases. Development of such products necessitates the solubilization and the dispersion of ceramides in a form that is deliverable and acceptable to the skin. When formulating such compounds, formulators face the challenge of ceramide solubilization. Ceramides need high temperature (above 80°C) to be solubilized [87] in well-chosen oils, emulsifiers and thickeners to avoid recrystallization during the cooling process. This limits the number of formula chassis into which such hot premix can be introduced without destabilizing the emulsion. Different types of emulsions have been described to deliver ceramides in the SC with the use of either conventional, microparticle-, nanoparticle- or liposome-containing emulsions and the use of penetration enhancers [88]. As an example, formulation of phytosphingosine in polar emollient increased its penetration into the skin versus nonpolar ones. It also increased its biological activity measured by the level of mRNA of different epidermal differentiation markers [89].

As another example, an equimolar mix of CER AP and NP with cholesterol and stearic acid showed a better efficacy in the repair of a disrupted pig skin barrier model when the lipids were formulated as liposomes with a lamellar structure than in the form of a droplet suspension [82]. In the same model of disrupted skin barrier, liposomes

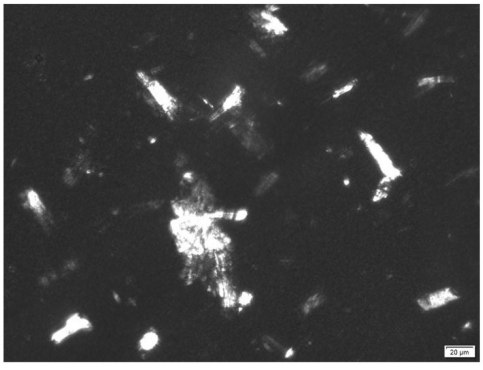
(a) ceramide/cholesterol/FFA lamellar formulation, properly solved ceramides; pH 6.6



(b) ceramide/cholesterol/FFA lamellar formulation, 10% undissolved ceramides, pH 6.6



(c) ceramide/cholesterol/FFA lamellar formulation, 50% undissolved ceramides, pH 6.7



(d) ceramide/cholesterol/FFA lamellar formulation, 100% undissolved ceramides, pH 6.7

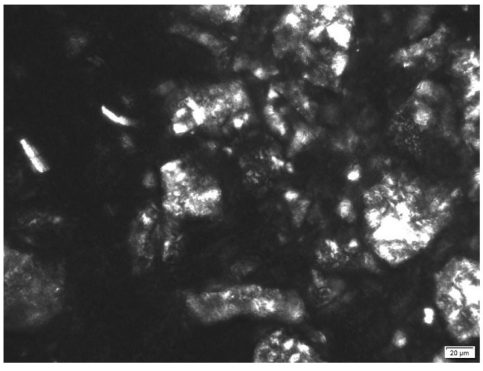


FIGURE 6 Optical microscopy analysis of the different formulas containing lipid mixture and undissolved ceramides at different concentrations. (a) Lamellar formulation with dissolved ceramides and (b) 10%, (c) 50% and (d) 100% of dispersed, undissolved ceramides. Magnification 40-fold, polarization.

prepared with CER NP and CER AP, cholesterol and stearic acid were able to repair the skin barrier, evidenced by the indomethacin permeation and electrical impedance. However, the barrier repair efficacy was higher with large multilamellar liposomes than with small ones with a non-multilamellar structure indicating that ceramides formulated into a multilamellar structure resembling the one present in the skin could be more adapted for barrier strengthening. In the model, the molecular ordering of the lipids measured by FTIR spectroscopy showed that the delipidating treatment with chloroform/methanol (2:1 v/v) induced a loosening in the arrangement of the skin lipids. Treatment with the large lamellar liposomes gave back to the SC lipids with an arrangement close to the non-disrupted barrier, whereas the non-lamellar ones had only a weak effect [90].

In this same article, the authors noticed in some formulations the presence of needle-like crystals due to unproperly dissolved ceramides. Undissolved ceramides and recrystallization can appear when the formula is brought to ambient temperature and during the shelf life of the products.

As the previous studies suggested that the correct solubilization of ceramides in formulation is important to develop efficient products, we have decided to further investigate the impact of the recrystallization of ceramides on the potential of the final formula to deliver its benefits on skin barrier. For this, controlled levels of ceramide crystals have been introduced into an established lamellar emulsion system. When present in crystals, ceramides are not properly incorporated into the lamellar layers in comparison to the original system in which fully dissolved ceramides are stabilized in the lamellar layers (see pictures of the different formulation in Figure 6). Formulations were evaluated in two models: First, a delipidated porcine skin model to assess their ability to repair impaired skin barrier by the measurement of indomethacin permeation through the skin, [90] and second, a human-reconstructed epidermis where the protective/repair effect versus a treatment with SDS was evaluated by measuring cell viability, release of inflammatory marker (IL-1α) and gene expression of filaggrin (FLG) as marker of terminal differentiation and skin barrier formation (See supplement for detailed protocols).

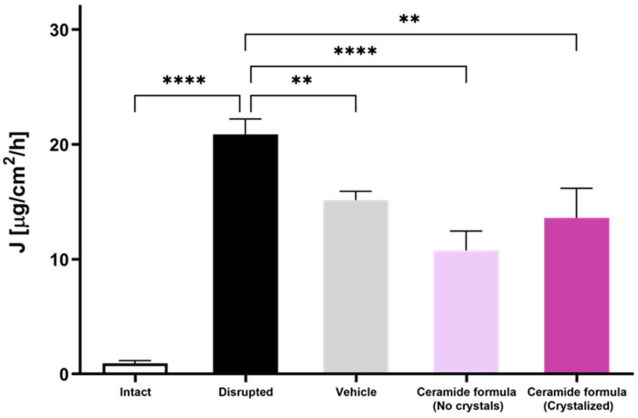


FIGURE 7 Effect of formulas containing ceramides in lamellar or crystalline form on the barrier recovery of disrupted skin. Frozen pig ear skin was placed in a Franz diffusion cell and was delipidated with chloroform/methanol in a 2:1 (v/v) ratio. After pretreatment with the test formulas, permeation of indomethacin into the receptor compartment was measured by HPLC. The skin was either non-delipidated, delipidated (disrupted barrier), delipidated and treated with ceramide-free formula (vehicle), delipidated and treated with formula containing dissolved and undissolved ceramides. Flux values of indomethacin through the native and disrupted skin and the skin treated by samples containing ceramides in lamellar or crystalline form and by the pure vehicle. $n \geq 3$; (*) marks significantly different values ($p < 0.01$** and $p < 0.0001$****) from the control or untreated skins.

Effect on delipidated porcine skin

Porcine skin was treated by chloroform/methanol mixture to disrupt the lipid barrier. Therefore, delipidated skin showed a significant decrease in its barrier function objectivated by the increase in indomethacin permeation expressed by the flux value (1 µg/cm^2/h for intact skin vs. 21 µg/cm^2/h for delipidated skin). The treatment with ceramide-containing formulation improved barrier function as shown by the decrease of indomethacin penetration (11.5 µg/cm^2/h for the ceramide-containing formula vs. 15 µg/cm^2/h for the ceramide-free vehicle). The introduction of ceramide crystals in the formulation partially abolished the positive effect of the ceramide-containing formula on barrier function in this model (13.5 µg/cm^2/h) (Figure 7).

Effect on reconstructed epidermis

Human-reconstituted epidermis treated with SDS showed a significant decrease in cell viability as evidenced by the increase in lactate dehydrogenase (LDH) release (+810%) and a higher release in IL-1α (+1625%). The treatment with a ceramide/cholesterol/FFA lamellar formulation significantly decreased the effect of SDS on each of the parameters (50% reduction in LDH release and more than 50% reduction in IL-1α release) compared to the SDS-stressed vehicle control. Introduction of undissolved crystalline ceramides at all concentrations tested (10%, 50% or 100%) completely abolished the protective effect of ceramide formulation in both parameters.

SDS application leads to a change in filaggrin gene expression, which could be observed to be significantly decreased by SDS treatment compared to the non-stressed vehicle control. Treatment with a ceramide/cholesterol/FFA lamellar formulation led to a significant restoration of filaggrin gene expression, whereas in the presence of undissolved ceramides in the formula, no correcting effect was seen and filaggrin gene expression was even further decreased in samples treated with the formulation including non-properly dissolved ceramides (Figure 8).

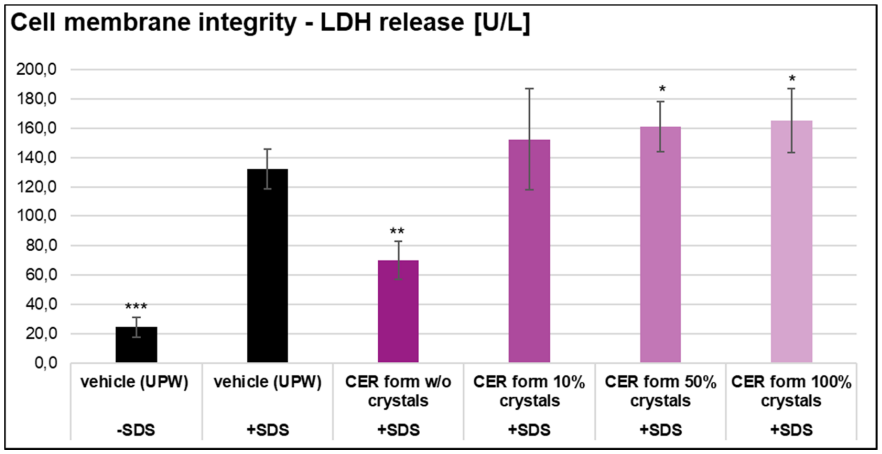
These two experiments suggest that formulas containing lamellar structure of ceramide/cholesterol/free fatty acid mix can deliver beneficial effects on barrier function in models where the lipid barrier has been disrupted. However, adding undissolved ceramides that form crystals decrease the beneficial effect of the formula, thus demonstrating the importance of the care to be brought to ceramide formulation when introducing such compounds in dermo-cosmetic products. The mechanism of the reduced efficacy of ceramides being present in crystals is not yet understood and will be the subject of further investigations.

CONCLUSION

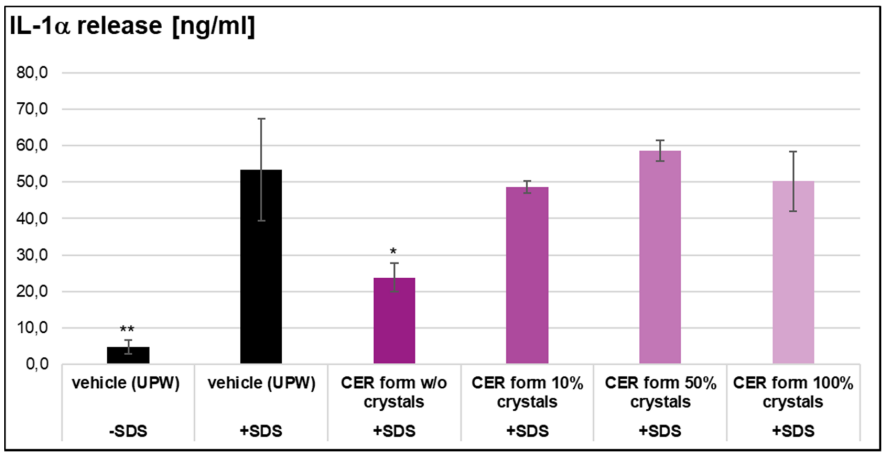
Over the last four decades, a lot of knowledge has been accumulated on the intercellular lipids of the stratum corneum, thanks to the refinement of analytical and biological methods as well as the establishment of new study models. This new knowledge reinforces the primary role that SC lipids play a pivotal role in the vital function of the skin barrier function. It also revealed the complexity of those lipids in terms of biosynthesis, structure and arrangement. In this landscape, ceramides are the cornerstone as these peculiar lipids are the most abundant, specific and diverse components of the stratum corneum lipid barrier and certainly the most complex to study.

One of the actual challenges today is to design studies that could integrate different levels of scientific exploration and link the biology of the cells and the impact on biochemical pathway of ceramide synthesis and metabolism with the effect on lipid arrangement and the impact on the barrier function. This could enable us to have a better picture of the physiological events taking place in lipid matrix in different skin conditions. This integrated understanding of the physiology of the skin barrier could also lead to designing better solutions for improvement

(a) Cell viability



(b) Inflammatory marker (IL1α)



(c) Relative gene expression of filaggrin

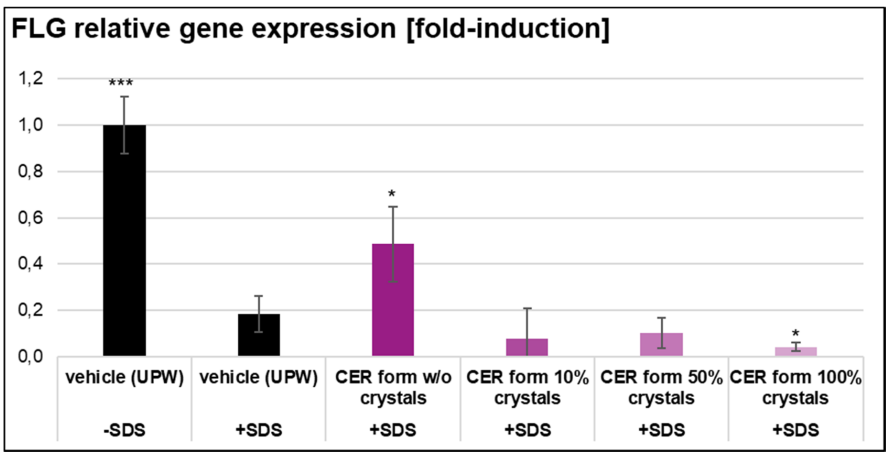


FIGURE 8 Effect of the presence of different levels of non-dissolved ceramides in formulas on the biological response of reconstructed epidermis treated by SDS. (a) Effect on cell viability (LDH release); (b) IL1α release, (c) Relative gene expression of filaggrin. Changes are calculated against the vehicle control + SDS. Statistical significance is calculated using Student's *t*-test with ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

and maintenance of the skin barrier function. On the one hand, these solutions can be based on enzymatically modulating biochemical pathways responsible for the metabolism of sphingolipids; on the other hand, quicker solutions can be designed by topically delivering the lipids that could help to improve the skin barrier function. This starts by setting up large production capacity of natural ceramides to allow their use at an affordable cost in most of the moisturizing products. For this, the establishment of microorganisms able to produce sphingoid bases with the same stereochemistry as the natural molecules, has been the first major step. The second step is now to design products for

topical application where ceramides are properly dissolved to be well tolerated and available to be delivered to the stratum corneum. In our experimental design, we mimicked the conditions where ceramides were imperfectly solubilized and present in a crystalline form. We demonstrated that well-dispersed ceramides could decrease the inflammatory response and restore the impaired skin barrier in different in vitro models. If only 10% of the ceramide mass in the formula are in the crystalline form, the beneficial effect is abrogated. This reinforces the care to be taken by the formulators when working with ceramides, including the choice of other lipids, emulsifiers, and thickeners to keep the ceramide solubilized, preferentially within a lamellar structure. Concerns in formulating ceramides have triggered lipid manufacturers to propose stabilized and ready to use liquid mixtures of lipids containing ceramides preformulated in a lamellar format, on top of pure ceramides in powder form or solid pellets. This format could simplify the production process as no high temperature premix is required. It should also ensure a better solubilization of those lipids in the final products. Nonetheless, the use of those preformulated lipids shall not prevent formulators to check for the final solubilization state of the ceramides and for the efficiency of their products to improve the skin barrier. In addition, it would be interesting to include ceramides with various fatty acid chain lengths into ready to use ceramide mixtures to mimic even further the natural occurrence of ceramides in the skin [91]. Oh et al. showed that the diversity of ceramide fatty acid chains is beneficial for the skin restoration [92].

Furthermore, as any other actives used at low concentration, the chassis of the formula should be optimized to enhance the delivery into the skin by, for example, playing with emollient polarity or using specific formulations that may enhance ceramide penetration in the stratum corneum.

In conclusion, ceramides are certainly the lipid family with the most structural diversity. They are also the most specific lipids of the SC. There, they play a critical role as, together with fatty acids and cholesterol ceramides build the essential lipid barrier. However, changes in their contents, for example, by ageing, seasonal or other external influences result in skin barrier impairment and are often associated with barrier-affected skin conditions and skin disease. Therefore, ceramides, when formulated in proper way, should be considered as ingredients of choice for skincare products to provide maintenance and recovery of skin primary function: an efficient barrier.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Benson HAE. Skin structure, function, and permeation. In Top Transdermal Drug Deliv. 1st ed. Hoboken, New Jersey: Wiley; 2012. p. 1–22.
2. Antunes E, Cavaco-Paulo A. Stratum corneum lipid matrix with unusual packing: a molecular dynamics study. Colloids Surf B Biointerfaces. 2020;190:110928.
3. Iwai I, Han H, Hollander LD, Svensson S, Öfverstedt LG, Anwar J, et al. The human skin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety. J Invest Dermatol. 2012;132:2215–25.
4. Ishida-Yamamoto A, Igawa S, Kishibe M. Molecular basis of the skin barrier structures revealed by electron microscopy. Exp Dermatol. 2018;27:841–6.
5. Voegeli R, Rawling AV. Moisturizing at the molecular level. Int J Cosmet Sci. 2023;45(2):133–54.
6. Talreja P, Kleene NK, Pickens WL, Wang TF, Kasting GB. Visualization of the lipid barrier and measurement of lipid pathlength in human stratum corneum. AAPS PharmSci. 2001;3:E13–E56.
7. Elias PM. Epidermal lipids, membranes, and keratinization. Int J Dermatol. 1981;20:1–19.
8. Feingold KR, Elias PM. Role of lipids in the formation and maintenance of the cutaneous permeability barrier. Biochim Biophys Acta—Mol Cell Biol. 2014;1841:280–94.
9. Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, Roitman E, et al. Human stratum corneum lipids: characterization and regional variations. J Lipid Res. 1983;24:120–30.
10. Bouwstra JA, Nadaban A, Bras W, Mc Cabe C, Bunge A, Gooris GS. The skin barrier: an extraordinary interface with an exceptional lipid organization. Prog Lipid Res. 2023;92:101252.
11. Draelos ZD. New treatments for restoring impaired epidermal barrier permeability: skin barrier repair creams. Clin Dermatol. 2012;30:345–8.
12. Masukawa Y, Narita H, Shimizu E, Kondo N, Sugai Y, Oba T, et al. Characterization of overall ceramide species in human stratum corneum. J Lipid Res. 2008;49:1466–76.
13. Motta S, Monti M, Sesana S, Caputo R, Carelli S, Ghidoni R. Ceramide composition of the psoriatic scale. Biochim Biophys Acta. 1993;1182(2):147–51.
14. Kawana M, Miyamoto M, Ohno Y, Kihara A. Comparative profiling and comprehensive quantification of stratum corneum

ceramides in humans and mice by LC/MS/MS[S]. J Lipid Res. 2020;61:884–95.
15. t'Kindt R, Jorge L, Dumont E, Couturon P, David F, Sandra P, et al. Profiling and characterizing skin ceramides using reversed-phase liquid chromatography–quadrupole time-of-flight mass spectrometry. Anal Chem. 2012;84:403–11.
16. Holleran WM, Takagi Y, Uchida Y. Epidermal sphingolipids: metabolism, function, and roles in skin disorders. FEBS Lett. 2006;580:5456–66.
17. Mizutani Y, Mitsutake S, Tsuji K, Kihara A, Igarashi Y. Ceramide biosynthesis in keratinocyte and its role in skin function. Biochimie. 2009;91:784–90.
18. Kihara A. Synthesis and degradation pathways, functions, and pathology of ceramides and epidermal acylceramides. Prog Lipid Res. 2016;63:50–69.
19. Li Q, Fang H, Dang E, Wang G. The role of ceramides in skin homeostasis and inflammatory skin diseases. J Dermatol Sci. 2020;97(1):2–8.
20. Farwanah H, Wohlrab J, Neubert RHH, Raith K. Profiling of human stratum corneum ceramides by means of normal phase LC/APCI–MS. Anal Bioanal Chem. 2005;383:632–7.
21. Bouwstra JA, Gooris GS, Dubbelaar FER, Weerheim AM, Ijzerman AP, Ponec M. Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res. 1998;39:186–96.
22. Elias PM, Gruber R, Crumrine D, Menon G, Williams ML, Wakefield JS, et al. Formation and functions of the corneocyte lipid envelope (CLE). Biochim Biophys Acta—Mol Cell Biol. 2014;1841:314–8.
23. Uchida Y, Holleran WM. Omega-O-acylceramide, a lipid essential for mammalian survival. J Dermatol Sci. 2008;51:77–87.
24. Fujii M. The pathogenic and therapeutic implications of ceramide abnormalities in atopic dermatitis. Cells. 2021;10(9):2386.
25. Van Smeden J, Boiten WA, Hankemeier T, Rissmann R, Bouwstra JA, Vreeken RJ. Combined LC/MS-platform for analysis of all major stratum corneum lipids, and the profiling of skin substitutes. Biochim Biophys Acta. 2014;1841(1):70–9.
26. Van Smeden J, Janssens M, Kaye EC, Caspers PJ, Lavrijsen AP, Vreeken RJ, et al. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. Exp Dermatol. 2014;23(1):45–52.
27. Merleev AA, Le ST, Alexanian C, Touss A, Xie Y, Marusina A, et al. Biogeographic and disease-specific alterations in epidermal lipid composition and single-cell analysis of acral keratinocytes. JCI Insight. 2022;7(16):e159762.
28. Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol. 2008;17:1063–72.
29. Krieg P, Furstenberger G. The role of lipoxygenases in epidermis. Biochim Biophys Acta. 2014;1841(3):390–400.
30. Tyrrell VJ, Ali F, Boeglin WE, Andrews R, Burston J, Birchall JC, et al. Lipidomic and transcriptional analysis of the linoleoylomega-hydroxyceramide biosynthetic pathway in human psoriatic lesions. J Lipid Res. 2021;62:100094.
31. Behne M, Uchida Y, Seki T, de Montellano PO, Elias PM, Holleran WM. Omegahydroxyceramides are required for corneocyte lipid envelope (CLE) formation and normal epidermal permeability barrier function. J Invest Dermatol. 2000;114(1):185–92.
32. Epp N, Furstenberger G, Muller K, de Juanes S, Leitges M, Hausser I, et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. J Cell Biol. 2007;177(1):173–82.
33. Krieg P, Rosenberger S, de Juanes S, Latzko S, Hou J, Dick A, et al. Aloxe3 knockout mice reveal a function of epidermal lipoxygenase-3 as hepoxilin synthase and its pivotal role in barrier formation. J Invest Dermatol. 2013;133(1):172–80.
34. Bouwstra JA, Gooris GS, van der Spek JA, Bras W. Structural investigations of human stratum corneum by small-angle X-ray scattering. J Invest Dermatol. 1991;97:1005–12.
35. White SH, Mirejovsky D, King GI. Structure of lamellar lipid domains and corneocyte envelopes of murine stratum corneum. An X-ray diffraction study. Biochemistry. 1988;27:3725–32.
36. Madison KC, Swartzendruber DC, Wertz PW, Downing DT. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. J Invest Dermatol. 1987;88(6):714–8.
37. de Jager M, Groenink W, Guivernau RBJ, Andersson E, Angelova N, Ponec M, et al. Novel in vitro percutaneous penetration model: evaluation of barrier properties with p-amino-benzoic acid and two of its derivatives. Pharm Res. 2006;23(5):951–60.
38. Opálka L, Kováčik A, Maixner J, Vávrová K. Omega-O-Acylceramides in skin lipid membranes: effects of concentration, sphingoid base, and model complexity on microstructure and permeability. Langmuir. 2016;32(48):12894–904.
39. Groen G, Poole DS, Gooris GS, Bouwstra JA. Is orthorhombic lateral packing and a proper lamellar organization important for the skin barrier function? Biochem Biophys Acta. 2011;1808(6):1529–37.
40. Uche LE, Gooris GS, Beddoes CM, Bouwstra JA. New insight into phase behavior and permeability of skin lipid models based on sphingosine and phytosphingosine ceramides. Biochim Biophys Acta. 2019;1861(7):1317–28.
41. Uche LE, Gooris GS, Bouwstra JA, Beddoes CM. Barrier capability of skin lipid models: effect of ceramides and free fatty acid composition. Langmuir. 2019;35(47):15376–88.
42. Norlén L, Lundborg M, Wennberg C, Narangifard A, Daneholt B. The Skin's barrier: a Cryo-EM based overview of its architecture and stepwise formation. J Invest Dermatol. 2022;142(2):285–92.
43. Bouwstra JA, Gooris GS, Cheng K, Weerheim A, Bras W, Ponec M. Phase behavior of isolated skin lipids. J Lipid Res. 1996;37(5):999–1011.
44. Bouwstra JA, Gooris GS, Dubbelaar FER, Weerheim AM, Ponec M. pH, cholesterol sulfate, and fatty acids affect the stratum corneum lipid organization. J Invest Dermatol. 1998;3(2):69–73.
45. Abraham W, Wertz PW, Downing DT. Effect of epidermal acylglucosylceramides and acylceramides on the morphology of liposomes prepared from stratum corneum lipids. Biochim Biophys Acta. 1988;939(2):403–8.
46. Bouwstra JA, Gooris GS, Dubbelaar FE, Weerheim AM, Ljzerman AP, Ponec M. Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res. 1998;39(1):186–96.
47. de Jager MW, Gooris GS, Ponec M, Bouwstra JA. Lipid mixtures prepared with well-defined synthetic ceramides closely mimic the unique stratum corneum lipid phase behavior. J Lipid Res. 2005;46(12):2649–56.

48. Pullmannova P, Ermakova E, Kovacik A, Opalka L, Maixner J, Zbytovska J. Long and very long lamellar phases in model stratum corneum lipid membranes. J Lipid Res. 2019;60(5):963–71.
49. Mojumdar EH, Gooris GS, Groen D, Barlow DJ, Lawrence MJ, Demé B, et al. Stratum corneum lipid matrix: location of acyl ceramide and cholesterol in the unit cell of the long periodicity phase. Biochim Biophys Acta. 2016;1858(8):1926–34.
50. Ishikawa J, Shimotoyodome Y, Ito S, Miyauchi Y, Fujimura T, Kitahara T, et al. Variations in the ceramide profile in different seasons and regions of the body contribute to stratum corneum functions. Arch Dermatol Res. 2013;305(2):151–62.
51. Rogers J, Harding C, Mayo A, Banks J, Rawlings A. Stratum corneum lipids: the effect of ageing and the seasons. Arch Dermatol Res. 1996;288(12):765–70.
52. Fujiwara A, Morifuji M, Kitade M, Kawahata K, Fukasawa T, Yamaji T, et al. Age-related and seasonal changes in covalently bound ceramide content in forearm stratum corneum of Japanese subjects: determination of molecular species of ceramides. Arch Dermatol Res. 2018;310(9):729–35.
53. Pappas A, Kendall AC, Brownbridge LC, Batchvarova N, Nicolaou A. Seasonal changes in epidermal ceramides are linked to impaired barrier function in acne patients. Exp Dermatol. 2018;27(8):833–6.
54. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. J Clin Invest. 1995;95(5):2281–90.
55. Mellody KT, Kendall AC, Wray JR, Foster AR, Langton AK, Costello P, et al. Influence of menopause and hormone replacement therapy on epidermal ageing and skin biomechanical function. J Eur Acad Dermatol Venereol. 2022;36(7):e576–e580.
56. Kendall AC, Pilkington SM, Wray JR, Newton VL, Griffiths CEM, Bell M, et al. Menopause induces changes to the stratum corneum ceramide profile, which are prevented by hormone replacement therapy. Sci Rep. 2022;12(1):21715.
57. Takagi Y, Nakagawa H, Kondo H, Takema Y, Imokawa G. Decreased levels of covalently bound ceramide are associated with ultraviolet B-induced perturbation of the skin barrier. J Invest Dermatol. 2004;123(6):1102–9.
58. Suárez-Fariñas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol. 2011;127:954–964.e954.
59. Marenholz I, Nickel R, Rüschendorf R, Schulz FG, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol. 2006;118(4):866–71.
60. Rodriguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown SJ, Cordell HJ, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. J Allergy Clin Immunol. 2009;123(6):1361–70.
61. Shen CP, Zhao MT, Jia ZX, Zhang JL, Jiao L, Ma L. Skin ceramide profile in children with atopic dermatitis. Dermatitis. 2018;29:219–22.
62. Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, et al. Changes in the ceramide profile of atopic dermatitis patients. J Invest Dermatol. 2010;130:2511–4.
63. Bhattacharya N, Sato WJ, Kelly A, Ganguli-Indra G, Indra AK. Epidermal lipids: key mediators of atopic dermatitis pathogenesis. Trends Mol Med. 2019;25:551–62.
64. van Smeden J, Bouwstra JA. Stratum corneum lipids: their role for the skin barrier function in healthy subjects and atopic dermatitis patients. Curr Probl Dermatol. 2016;49:8–26.
65. Boiten W, van Smeden J, Bouwstra JA. The cornified envelope-bound ceramide fraction is altered in patients with atopic dermatitis. J Invest Dermatol. 2020;140(5):1097–100.
66. Ito S, Ishikawa J, Naoe A, Yoshida H, Hachiya A, Fujimura T, et al. Ceramide synthase 4 is highly expressed in involved skin of patients with atopic dermatitis. J Eur Acad Dermatol Venereol. 2017;31(1):135–41.
67. Danso M, Boiten W, van Drongelen V, Gmelig Meijling K, Gooris G, El Ghalbzouri A. Altered expression of epidermal lipid bio-synthesis enzymes in atopic dermatitis skin is accompanied by changes in stratum corneum lipid composition. J Dermatol Sci. 2017;88(1):57–66.
68. Beddoes CM, Rensen DE, Gooris GS, Malfois M, Bouwstra JA. The importance of free fatty chain length on the lipid organization in the long periodicity phase. Int J Mol Sci. 2021;22(7):3679.
69. Uche LE, Gooris GS, Bouwstra JA, Beddoes CM. Increased levels of short-chain ceramides modify the lipid organization and reduce the lipid barrier of skin model membranes. Langmuir. 2021;37(31):9478–89.
70. Kim D, Lee NR, Park S-Y, Jun M, Lee K, Kim S, et al. As in atopic dermatitis, Nonlesional skin in allergic contact dermatitis displays abnormalities in barrier function and ceramide content. J Invest Dermatol. 2017;137:748–50.
71. Ewald DA, Malajian D, Krueger JG, Workman CT, Wang T, Tian S, et al. Meta-analysis derived atopic dermatitis (MADAD) transcriptome defines a robust AD signature highlighting the involvement of atherosclerosis and lipid metabolism pathways. BMC Med Genet. 2015;8:60.
72. Montero-Vilchez T, Soler-Góngora M, Martínez-López A, Fernández-González A, Buendía-Eisman A, Molina-Leyva A, et al. Epidermal barrier changes in patients with psoriasis: the role of phototherapy. Photodermatol Photoimmunol Photomed. 2021;37(4):229–85.
73. Motta S, Monti M, Sesana S. Abnormality of water barrier function in psoriasis. Role of ceramide fractions. Arch Dermatol. 1994;130:452–6.
74. Checa A, Xu N, Sar DG, Haeggström JZ, Ståhle M, Wheelock CE. Circulating levels of sphingosine-1-phosphate are elevated in severe, but not mild psoriasis and are unresponsive to anti-TNF-α treatment. Sci Rep. 2015;5:12017.
75. Moon S-H, Kim J-Y, Song E-H, Shin M-K, Cho Y-H, Kim N-I. Altered levels of sphingosine and Sphinganine in psoriatic epidermis. Ann Dermatol. 2013;25:321–6.
76. Nakajima K, Terao M, Takaishi M. Barrier abnormality due to ceramide deficiency leads to psoriasiform inflammation in a mouse model. J Invest Dermatol. 2013;133(11):2555–65.
77. Tawada C, Kanoh H, Nakamura M, Mizutani Y, Fujisawa T, Banno Y, et al. Interferon-γ decreases ceramides with long-chain fatty acids: possible involvement in atopic dermatitis and psoriasis. J Invest Dermatol. 2014;134(3):712–8.
78. Elias PM, Wakefield JS, Man M-Q. Moisturizers versus current and next-generation barrier repair therapy for the management of atopic dermatitis. Skin Pharmacol Physiol. 2018;32:1–7.

79. Nakaune-Iijimaa A, Sugishimab A, Omurab G, Kitaokaa H, Tashiroa T, Kageyamaa S, et al. Topical treatments with acylceramide dispersions restored stratum corneum lipid lamellar structures in a reconstructed human epidermis model. Chem Phys Lipids. 2018;215:56–62.
80. Berkers T, Visscher D, Gooris GS, Bouwstra JA. Topically applied ceramides interact with the stratum corneum lipid matrix in compromised ex vivo skin. Pharm Res. 2018;35(3):48.
81. Oh MJ, Nam JJ, Lee EO, Kim JW, Park CS. A synthetic C16 omega-hydroxyphytoceramide improves skin barrier functions from diversely perturbed epidermal conditions. Arch Dermatol Res. 2016;308(8):563–74.
82. Čuříková-Kindlová BA, Vovesná A, Nováčková A, Zbytovská J. In vitro modeling of skin barrier disruption and its recovery by ceramide-based formulations. AAPS PharmSciTech. 2021;23(1):21.
83. Kucharekova M, Schalkwijk J, Van De Kerkhof PCM, Van De Valk PGM. Effect of a lipid-rich emollient containing ceramide 3 in experimentally induced skin barrier dysfunction. Contact Derm. 2002;46(6):331–8.
84. Lueangarun S, Tragulplaingam P, Sugkraroek S, Tempark T. The 24-hr, 28-day, and 7-day post-moisturizing efficacy of ceramides 1, 3, 6-II containing moisturizing cream compared with hydrophilic cream on skin dryness and barrier disruption in senile xerosis treatment. Dermatol Ther. 2019;32(6):e13090.
85. Yang Q, Liu M, Li X, Zheng L. The benefit of a ceramide-linoleic acid-containing moisturizer as an adjunctive therapy for a set of xerotic dermatoses. Dermatol Ther. 2019;32(4):e13017.
86. Liu M, Li X, Chen XY, Xue F, Zheng J. Topical application of a linoleic acid-ceramide containing moisturizer exhibits therapeutic and preventive benefits for psoriasis vulgaris: a randomized controlled trial. Dermatol Ther. 2015;28(6):373–82.
87. Zbytovská J, Kiselev MA, Funari SS, Garamus VM, Wartewig S, Palát K, et al. Influence of cholesterol on the structure of stratum corneum lipid model membrane. Col Surf A. 2008;328:90–9.
88. Kahraman E, Kaykın M, Bektay HS, Güngör S. Recent advances on topical application of ceramides to restore barrier function of skin. Cosmetics. 2019;6(52):1–11.
89. Schiemann Y, Wegmann M, Lersch P, Heisler E, Farwick M. Polar emollients in cosmetic formulations enhance the penetration and biological effects of phytosphingosine on skin. Col Surf A. 2008;331:103–7.
90. Vovesna A, Zhigunov A, Balouch M, Zbytovska J. Ceramide liposomes for skin barrier recovery: a novel formulation based on natural skin lipids. Int J Pharm. 2021;596:120264.
91. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res. 2012;53(12):2755–66.
92. Oh MJ, Cho YH, Ch SY, Lee EO, Kim JW, Kim SK, et al. Novel phytoceramides containing fatty acids of diverse chain lengths are better than a single C18-ceramide N-stearoyl phytosphingosine to improve the physiological properties of human stratum corneum. Clin Cosmet Investig Dermatol. 2017;13(10):363–71.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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