

Review Article

Lipids and skin barrier function – a clinical perspective

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The stratum corneum (SC) protects us from dehydration and external dangers. Much is known about the morphology of the SC and penetration of drugs through it, but the data are mainly derived from *in vitro* and animal experiments. In contrast, only a few studies have the human SC lipids as their focus and in particular, the role of barrier function in the pathogenesis of skin disease and its subsequent treatment protocols. The 3 major lipids in the SC of importance are ceramides, free fatty acids, and cholesterol. Human studies comparing levels of the major SC lipids in patients with atopic dermatitis and healthy controls have suggested a possible role for ceramide 1 and to some extent ceramide 3 in the pathogenesis of the disease. Therapies used in diseases involving barrier disruption have been sparsely investigated from a lipid perspective. It has been suggested that ultraviolet light as a treatment increases the amount of all 3 major SC lipids, while topical glucocorticoids may lead to a decrease. Such effects may influence the clinical outcome of treatment in diseases with impaired barrier function. We have, therefore, conducted a review of the literature on SC lipids from a clinical perspective. It may be concluded that the number of human studies is very limited, and in the perspective of how important diseases of impaired barrier function are in dermatology, further research is needed.

Key words: atopic dermatitis; ceramides; cholesterol; free fatty acids; lipids of stratum corneum.
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The establishment of an effective physical barrier segregating the living organism from its surrounding environment is the key function of human skin. Impairment of the barrier function is not only an established aspect of, for example, atopic dermatitis but also of other diseases as well (1–3).

During recent decades, the stratum corneum (SC) of healthy, and to some extent, also diseased skin has been investigated intensively, with special focus on morphology, barrier function, lipid composition, and the penetration of drugs. Most research has been performed using *in vitro* SC models and in animal models, but human skin has been studied only to a lesser extent. This research has substantially increased our understanding of the SC structure and function on the molecular level, but there is still limited knowledge on the linkage to a clinical perspective in humans.

This review summarizes the current knowledge from the clinical perspective and focuses on SC lipids in relation to skin barrier function. The roles

of the 3 major SC lipids ceramides, free fatty acids, and cholesterol are assessed in an attempt to identify which areas are well clarified and which needs more research.

Background

Lipids of SC

The 3 major SC lipids are ceramides, free fatty acids, and cholesterol. The lipid synthesis is believed to take place in the stratum granulosum (4) where small cytoplasmic inclusions (lamellar bodies) are formed and packed in multilayered stacks (5). At the stratum granulosum–SC interface, lamellar body exocytosis takes place to form the lipid lamella (6).

Structure of SC

The SC is built of keratin imbedded in lipids. A classical model used to describe SC is the *Brick*

and *Mortar model* (7), with the bricks being annucleated, keratin-rich corneocytes and the mortar the extracellular, lipid-enriched matrix organized into lipid bilayers. This 2-compartment model is still the 1 most often referred to, although the understanding of connection of the 'bricks' by desmosomes and penetration through the 'mortar' has been modified (8) by more recent knowledge of the molecular organization, obtained from structural investigation with methods such as X-ray diffraction and Raman spectroscopy, and propose that different phases and organization of the lipids play a functional role to the SC. The phases are liquid packing/liquid crystalline phase, hexagonal packing/gel phase, and, as the most solid phase, the orthorhombic packing/crystalline phase (9).

In the *Domain Mosaic model*, the mortar is described as a non-homogeneous mass, with polar lipids in a crystalline phase surrounded by polar lipids in the gel phase (10). As a direct outcome of this model, the *Single Gel Phase model*, describing the lipids as 1 single coherent lamellar gel phase, was more recently proposed by Norlén (11). The *Sandwich model* proposed by Bouwstra et al. (12) describes the lipids in highly ordered bilayers, with a molecular arrangement in a 13-nm periodicity and with an alternating crystalline-phase layer and fluid-phase layer.

These models all describe important aspects of the rather complicated and highly developed roles of the SC, but new models are still being proposed (13). No model has hitherto integrated all the various aspects of the skin barrier function.

1 limitation of these models is that the extrapolation of animal studies to human skin may be difficult. Observations by X-ray diffraction, comparing pig skin and human skin, show that whereas pig SC lipids mostly form a hexagonal phase, human SC lipids are predominantly organized in an orthorhombic phase (14, 15), and there are indications that the differences in the ceramides between humans and pigs have influence on the phases that the SC lipids form. Human ceramide 1 appears to have an important role in this (16), which is an interesting observation in relation to the finding in atopic dermatitis (see below).

In recent years, the role of the protein filaggrin for the integrity of SC has been investigated. Filaggrin aggregates the keratin filaments and causes the flattened shape of the corneocytes (17). Seguchi et al. found in 1995 a decreased expression of filaggrin in patients with atopic dermatitis (18). However, only recently, it was found that loss-of-function mutations in the gene encoding for filaggrin cause ichthyosis vulgaris (19), and shortly after, the same research group found that

the same defect is a predisposing factor for atopic dermatitis (20). Filaggrin mutations are reported in 15–25% of all patients with atopic dermatitis. This inspired Lerbaek et al. to investigate a possible association between hand dermatitis or contact allergy and filaggrin mutations. The study was performed on a twin cohort with hand dermatitis, and no association was found (21). Although the findings of filaggrin mutations are extremely interesting and important, it should not prevent the search for other defects in SC related to barrier defect.

Method

We have made a review of the literature on skin lipids and skin barrier function in humans. Searching relevant databases (PubMed, EMBASE, Google, Biosis, and Cochrane), we used the following search criteria: skin barrier, skin lipids, and SC. We identified 580 papers. Most papers were only slightly relevant from a clinical point; focusing on human studies and clinical aspects narrowed the number to 58.

Lipids in healthy skin

Ceramides. Ceramides consist of a long-chained base with an amide-linked fatty acid. The most common long-chained base is sphingosine (sphing-4-enine). In the human SC, at least 9 different classes (ceramides 1–9) of ceramides have been described (22). Neosynthesis of the ceramides takes place in the keratinocytes in the stratum granulosum and is delivered to the SC in lamellar bodies as described previously. The rate-limiting enzyme for the synthesis of ceramide is serine palmitoyl-CoA transferase (SPT). SPT is the initial step in the synthesis of all sphingolipids condensing 1 serine and 1 palmitoyl-CoA to ketosphinganine (Fig. 1). Its importance is indicated by the fact that barrier damage increases the activity of this enzyme and topical application of SPT inhibitors leads to delayed barrier repair (23). SPT is also upregulated by ultraviolet (UV)B light (24, 25). Ceramides are also metabolized from glycosphingolipids (e.g. cerebroside) and sphingomyelin, as shown in Fig. 1. Hydrolysis of cerebroside through β -glucocerebrosidase is considered to be the major pathway for ceramide synthesis in the SC (26), although it has also been shown that hydrolysis of sphingomyelin by the acid sphingomyelinase also is essential for the formation of a fully functional SC (27, 28). A possible ceramide gradient in the SC has been examined by several groups. In 1 study including 6 human volunteers, different layers of the SC were analysed

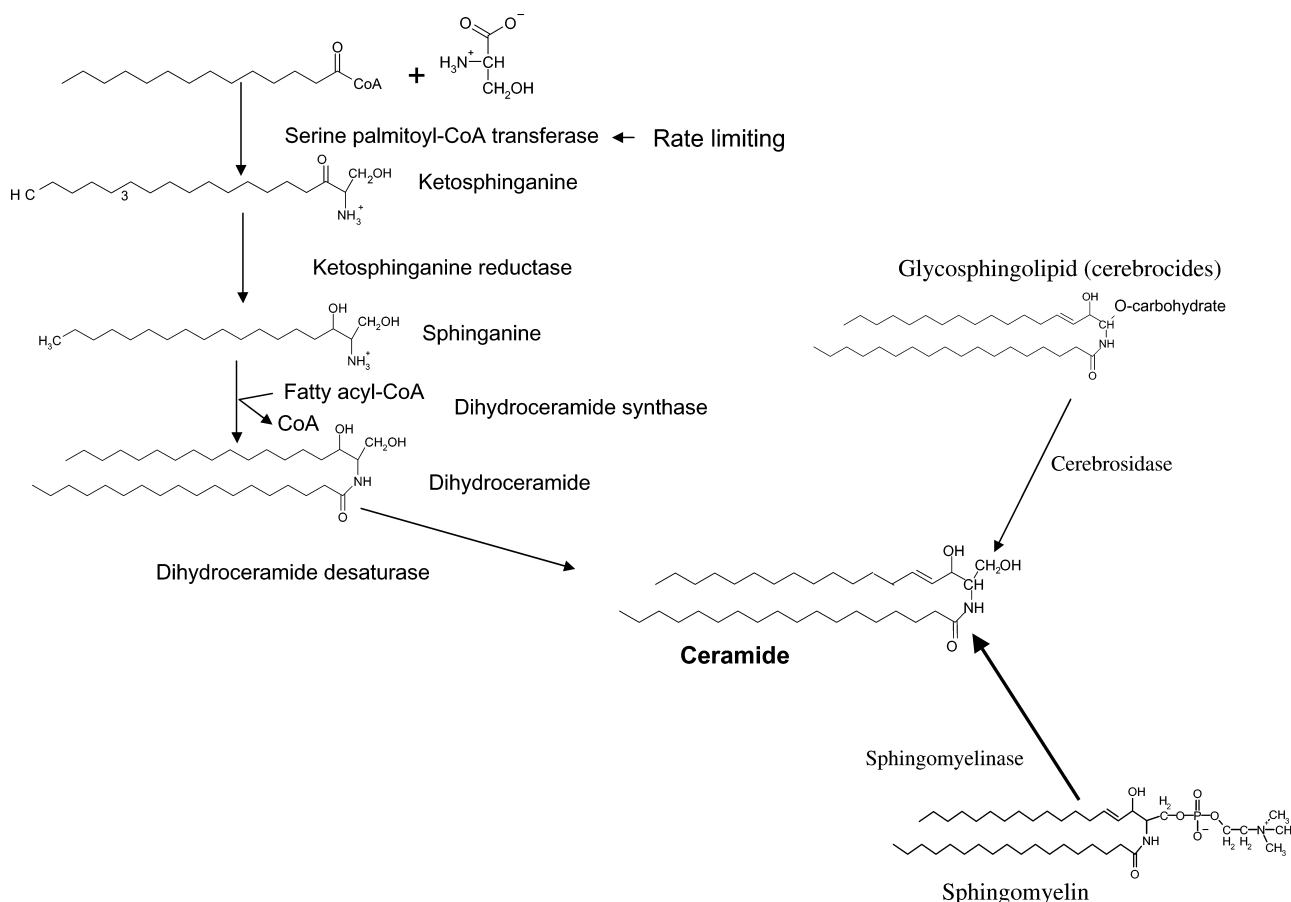


Fig. 1. Ceramide synthesis pathways.

by tape stripping (29), and a decrease in total ceramides and an increase in phospholipids were found in the deeper layers of SC compared with the more superficial ones. Another study, including 18 healthy volunteers, found no gradient in SC in either the total amount or the amount of any subgroups of ceramides (30). Weerheim and Ponc (31) using tape stripping in healthy volunteers also found no ceramide gradient. The cholesterol/ceramide ratio has been shown not to differ in the inner SC compared with outer SC (32).

As mentioned above, ceramides, especially human ceramide 1, seem to have an important role in the organization of SC lipids in an orthorhombic phase (16, 33).

Cholesterol. Another lipid in the SC of importance for the barrier function is cholesterol. Cholesterol synthesis is a highly complex process, starting with the formation of the isoprenoid unit isopentenyl pyrophosphate from acetyl-CoA. In the next steps, 6 isoprenoid units are condensed in the synthesis of squalene, and cholesterol is finally formed through cyclization and decarboxylation of squalene. The rate-limiting step in cholesterol synthesis is the formation of mevalonate

from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalysed by the HMG-CoA reductase. Interestingly, several enzymes are upregulated after acute barrier disruption, particularly HMG-CoA reductase, HMG-CoA synthase, farnesol diphosphate synthase, and squalene synthase (34, 35). Knowledge about cholesterol is almost entirely based on animal studies, which means that the findings are not necessarily the same in humans. The importance of cholesterol synthesis for recovery of barrier function is illustrated by an experiment in mice, where topical application of HMG-CoA reductase inhibitors caused a delayed barrier recovery following barrier disruption with acetone (36). The same group also looked at the outcome of using SPT (ceramide-regulating enzyme) with and without application of HMG-CoA reductase inhibitor, with diverging results (37). However, as already mentioned, the results are difficult to interpret in a human perspective.

Another *in vitro* study used topical application of statins, which are HMG-CoA reductase inhibitors, to inhibit cholesterol synthesis and found that in their cultures of human keratinocytes,

a 90% inhibition of cholesterol synthesis took place, but the effect on barrier disruption was limited (38). They showed that following barrier disruption, an upregulation of scavenger receptor class B type 1, a cell surface receptor for high-density lipoprotein in the basal layer of epidermis that mediates uptake of cholesterol from the circulation, is upregulated (38). These data might be interpreted as a result of a high level of extra epidermal cholesterol uptake. Another interesting study including patients in systemic statin treatment and healthy volunteers has been undertaken to show whether patients receiving statins had a deteriorated skin barrier function. After irritation with sodium lauryl sulfate (SLS), the erythema was significantly less pronounced in the statin-treated group, but no effect was found on the water barrier as evaluated by transepidermal water loss (TEWL) (39). The overall role of cholesterol in the SC is as a stabilizer on the structure of the SC (11), at least in respect to temperature changes (40). However, more research is needed to establish the exact role of cholesterol in human SC.

Free fatty acids. Free fatty acids are, together with cholesterol sulfate, the only lipids in the SC with a charged/ionizable headgroup (41) and therefore probably necessary for the formation of the SC lipid bilayer formation. The free fatty acids are responsible for the acidic pH of approximately 4–5.5 at the SC surface (8) and can influence phase behaviour (42).

The rate-limiting step in synthesis of fatty acids is carboxylation of acetyl-CoA to malonyl-CoA, catalysed by acetyl-CoA carboxylase. Malonyl-CoA is the substrate for synthesis of fatty acids by the fatty acid synthase (34, 35). It has been shown that after barrier disruption, the messenger RNA levels for these enzymes increase suggesting increased synthesis (34).

What happens with the lipids in atopic dermatitis/eczema?

Atopic dermatitis. Only a few studies have reported data from diseased human skin (Table 1).

Nardo et al. (43) studied patients with atopic dermatitis, subdivided into patients with no active signs of atopic dermatitis and patients with active lesions, and compared SC lipids with those of healthy volunteers. SC was collected from cyanoacrylate strips, and lipid analysis showed significant reduction in ceramides 1 and 3 and significant increase in cholesterol in atopic dermatitis patients with active lesions compared with healthy volunteers. The group with atopic dermatitis without current eczema had intermediate values. Furthermore, they reported a significant negative correlation between TEWL and the quantity of ceramide 3 (43). Imokawa et al. (44) has performed a similar study examining lesional and non-lesional skin in patients with atopic dermatitis. Both areas had significantly lower levels of ceramides, especially ceramide 1, compared with healthy controls (44). Yamamoto et al. conducted a small study and found no statistical difference between atopic dermatitis patients and controls with respect to the total lipid amount, but a decreased level of ceramide 1 in atopic dermatitis patients (45). Similar results were found in a study by Bleck et al. (46). Interestingly no significant differences regarding relative lipid composition were found in a study by Farwanah et al. (47).

Attention has also been paid to the role of altered sphingomyelinase activities. Jensen et al. (28) looked at both lesional skin and non-lesional skin from patients with atopic dermatitis compared with healthy individuals and found decreased amount of acid as well as neutral sphingomyelinase in lesional and non-lesional atopic dermatitis skin. Based on these results, it was suggested that this disturbed hydrolysis of sphingomyelin could explain the reduced ceramide levels found in atopic dermatitis.

Another group found that in atopic dermatitis, most of the sphingomyelin hydrolysis was not performed by sphingomyelinases (Fig. 1) but by sphingomyelin acylase that does not release ceramide but free fatty acid and sphingosyl phosphorylcholine (48).

Table 1. Human studies on SC lipids in patients with AD

Patients	Collection of SC	Lipid analysis	Results	Reference
47 AD patients with lesional skin from 28 and non-lesional skin from 19	Cyanoacrylate strip	HPTLC	Reduced total ceramides Reduced ceramides 1 and 3 Increased cholesterol	43
35 AD patients with both lesional and non-lesional skin	Cyanoacrylate on a glass slide	TLC	Reduced total ceramides Reduced ceramide 1	44
6 AD patients with non-lesional skin	By rinsing with ethanol	HPTLC	Reduced ceramide 1	45
10 AD patients with non-lesional skin	Cyanoacrylate strip	HPTLC	Reduced ceramides 1 and 3	46

AD, atopic dermatitis; HPTLC, high-performance thin-layer chromatography; TLC, thin-layer chromatography.

Table 2. Differences in barrier parameters for humans and animals

Barrier parameter	Differences in humans and animals
Stratum corneum thickness	Has been reported in humans to be 6–19 μm and 11.7 μm mean respectively (60, 61) were pig ears is reported to be 17–21 μm and a mean of 8.5 μm respectively (61, 62)
Corneocytes	Humans has 18–20 layers were mice has 6–7 layers (63)
Epidermis total lipids	Humans has 10% lipids of epidermis dry weight compared to pigs 8% and rat 20% of epidermis dry weight (64)
Barrier repair time	Is reported to be 60–80 hours in humans and 24 hours in mice as measured by TEWL (63)
Ceramides	Human ceramide 1 and 4 are quite unique in its structure because it has a linoleic acid linked to a omega-hydroxy fatty acid, which pig ceramide 4 and other animals has not (65)
TEWL	Measured from the human volar forearm TEWL values ranging from 2,8 to 8,1 $\text{g/m}^2/\text{h}$ has been reported (66), which is near the values of a pig study with values of 6,29 $\text{g/m}^2/\text{h}$ (67). A study with hairless rats showed a mean TEWL at 7,2 $\text{g/m}^2/\text{h}$ (68)

Another explanation for the impaired barrier in atopic dermatitis is given by a study using electron micrographs of different layers of punch biopsies from human atopic non-eczematous dry skin compared with those of healthy controls. They did not find any epidermal lipid structure abnormalities, but in contrast to normal skin, they found lamellar bodies in much more superficial layers of the SC (49). This could possibly indicate that it is not the synthesis of lipids that is affected, but the lipids are retained and not readily available to the SC.

Dermatitis. Attention has been given to the function of ceramides in SC in relation to experimentally induced dermatitis. A study of healthy females found that volunteers with a low level of ceramide 1, ceramide 6I, and ceramide 6II have a higher score of either erythema or TEWL after experimental induced skin irritation, indicating a lower threshold to irritant contact dermatitis being related to decreased levels of these ceramides (50). The influence of tape stripping on the quantitative amount of lipids in the SC was studied in healthy volunteers (51), and an increase in ceramides 1 and 2 and a decrease in the other ceramides were found in the clinically scaly skin following tape stripping. Similar results were found in the same group after exposure to SLS (51).

As mentioned earlier, the predominant phase of the lipids in human SC is orthorhombic, and there are indications that especially ceramide 1 is responsible for this. The electron diffraction method has also been used on atopic dermatitis patients to explore the lamellar organization of the SC. It has been shown that compared with normal skin, the presence of hexagonal phase is increased with respect to orthorhombic packing (52).

Effects of UVB and steroids

Knowledge of the role of commonly used therapies on the composition of the SC is of considerable importance in diseases in which barrier

disruption plays a role. 1 such external therapeutic factor influencing SC lipids is UV-light. In a study including 20 healthy volunteers to UV treatment 3 times weekly for 3 weeks, they collected SC lipids by organic solvents and found that the UV light led to an increase in SC ceramides (53). Another study made by the same group found that after exposing 30 healthy individuals to UVA and UVB, respectively, both groups were less vulnerable to SLS than untreated controls, with no difference between the 2 light sources (54). Their lipid profile was also examined, and it was found that UVB and to some extent UVA increase the amount of all classes of lipids including all subclasses of ceramides except ceramide 1 (54). Different topical regimes with SC lipids have shown different results. The use of lactic acid (the L-lactic acid isomer) has, in 1 study, been shown to increase the level of ceramides in human SC and to decrease the susceptibility to TEWL as evaluated by TEWL measurement (55). In another study, the role of short-term glucocorticoid treatment was investigated with respect to barrier disruption, SC cohesion, and influence on lamellar bodies, and whether the topical application of an equimolar amount of free fatty acids, ceramides and cholesterol after the glucocorticoid treatment prevented any of the damages (56). It was concluded that the integrity of the SC was significantly reduced as evaluated by TEWL after tape stripping and that glucocorticoid treatment results in a decrease in lamellar body production and secretion. In animal models, equimolar amount of free fatty acids, ceramides, and cholesterol was applied on a glucocorticoid-treated and tape-stripped area, and this was found to accelerate barrier recovery compared with a vehicle-treated area (56).

Discussion

The vast majority of the very limited transport of substances across the SC takes place through the

lipid bilayer, which makes it essential for the barrier function. Each of the 3 major SC lipids has an important role for the barrier function. Especially ceramides, but also cholesterol, seem to have influence on (and are influenced by) the integrity of the SC. Free fatty acids are believed to play a major role in the bilayer formation and pH. In this review, we have looked at both normal and diseased skin.

The normal skin barrier is exposed daily to numerous external factors such as changes in air humidity, sun exposure, detergents, moisturizers, etc. However, the influence of these factors on barrier lipids (synthesis, structure, and ratio between different lipids) has not been thoroughly elucidated. A recent paper states, with respect to aged skin, that the pH of the SC increases, and this leads to an impairment of the lipid processing (57); this is in good agreement with 2 papers that found decreased levels of lipids in aged human skin (44, 58). 2 studies have looked at seasonal variations and found a decrease in absolute amount of lipids in winter when comparing the same group of people in both seasons (58, 59). Apart from this, few studies have reported data on influence of age, hormonal status, or seasonal variation in skin lipids.

It has been shown that in diseases related to impaired skin barrier such as atopic dermatitis, the level of some or all ceramides are decreased, while the level of cholesterol is increased (43–45). The exact consequence of this is uncertain, and more research is needed.

UV light is a therapy commonly used for a number of dermatological diseases. The influence of the UV treatment, as well as the daily exposure to the sun, on the SC lipids is an area with only few human studies. However, it seems that UV light leads to an increase in the lipids in general (53, 54).

Topical glucocorticoids are frequently used in dermatology. A paper indicated that topical glucocorticoids lead to a decrease in the amount of lamellar bodies and therefore a decrease in SC lipids (56), which is of concern when treating diseases with impaired barrier function like atopic dermatitis.

Conclusions

Detailed knowledge on SC lipids and morphology has been obtained from *in vitro* and animal studies, and much progress has been made with respect to understanding the barrier function of the SC. However, when it comes to human studies, diseased skin, and effects of treatment, data are limited. There are good indications that ceram-

ides are reduced in atopic dermatitis patients, that UV exposure leads to an increased synthesis of SC lipids, and that topical glucocorticoids decreases the total amount of SC lipids. Future research in humans should pay attention to physiological changes in SC lipids because of sex, age, seasonal variation, etc. and should include regulations of enzymes and level of changes in gene expression.

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