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► **To cite this version:**

Frédéric Bonté. Skin lipids : Their origin and function. Recent research development in lipid research, pp.43-62, 1999. hal-03316280

**HAL Id: hal-03316280**

**<https://hal.science/hal-03316280v1>**

Submitted on 5 Sep 2021

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**Skin lipids : their origin and function**

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**Abstract:**

Epidermis is a very dynamic structure in which lipids play two distinct roles. Specific lipids, mainly ceramides, cholesterol and free fatty acids are delivered in the last steps of the keratinocyte differentiation. By forming a highly organized structure, they are considered as responsible of the skin barrier properties. New data show that sebum lipids are also involved in skin barrier. Intracellular lipids, contribute to the natural control of epidermal homeostasis and in the repair metabolism.

Running title: Epidermal lipids

## INTRODUCTION

Skin is essential for human survival; it protects the body against UV radiations, loss of endogenous material (water, etc.) and any uncontrolled percutaneous absorption of chemicals or toxic materials from the environment. At the top of the skin, sebum secreted by sebaceous glands forms an amorphous hydrophobic film that protects the skin. The barrier properties of the skin are mainly due to the horny layer or stratum corneum, thin outer organized layer composed of hydrophilic and hydrophobic domains. The composition and structure of the stratum corneum are the main factors that ensure the barrier function of the skin. But the stratum corneum is no longer viewed as a dead layer of bricks (corneocytes) and mortar (lipids), but, more a living, highly regulated and dynamic structure.

The epidermis is a very dynamic structure in which there is a permanent balance between cornification and desquamation. The stratum corneum undergoes continual biochemical changes from the underlying epidermis, cell renewal and differentiation, in order to maintain its functions. Differentiated keratinocytes (Fig.1), migrate through the suprabasal layers and are transformed into resistant keratinized

corneocytes. They then become embedded in an organized lipid matrix leading to a tortuous pathway (1, 2). At the same time, there is desquamation of about one corneocyte layer a day. Desquamation requires proteolytic activity. Inhibition of the action of serine protease enzymes (trypsin and chymotrypsin like) on corneodesmosomes leads to peeling or dry skin. Any disturbance in skin lipids will also disrupt the epidermal barrier to water loss and cell-to-cell adhesion. Skin surface lipids are also involved in protecting the body by inhibiting the adhesion of pathogens such as *Candida albicans* to the stratum corneum (3).

The skin is also a reactive structure. If the barrier function is perturbed (soap, detergents) or attacked, lipid mediators, ions and cytokines will be released and mediate recovery. The stratum layers interact continuously with the underlying epidermis, especially through lipids which are thought to be true second messengers.

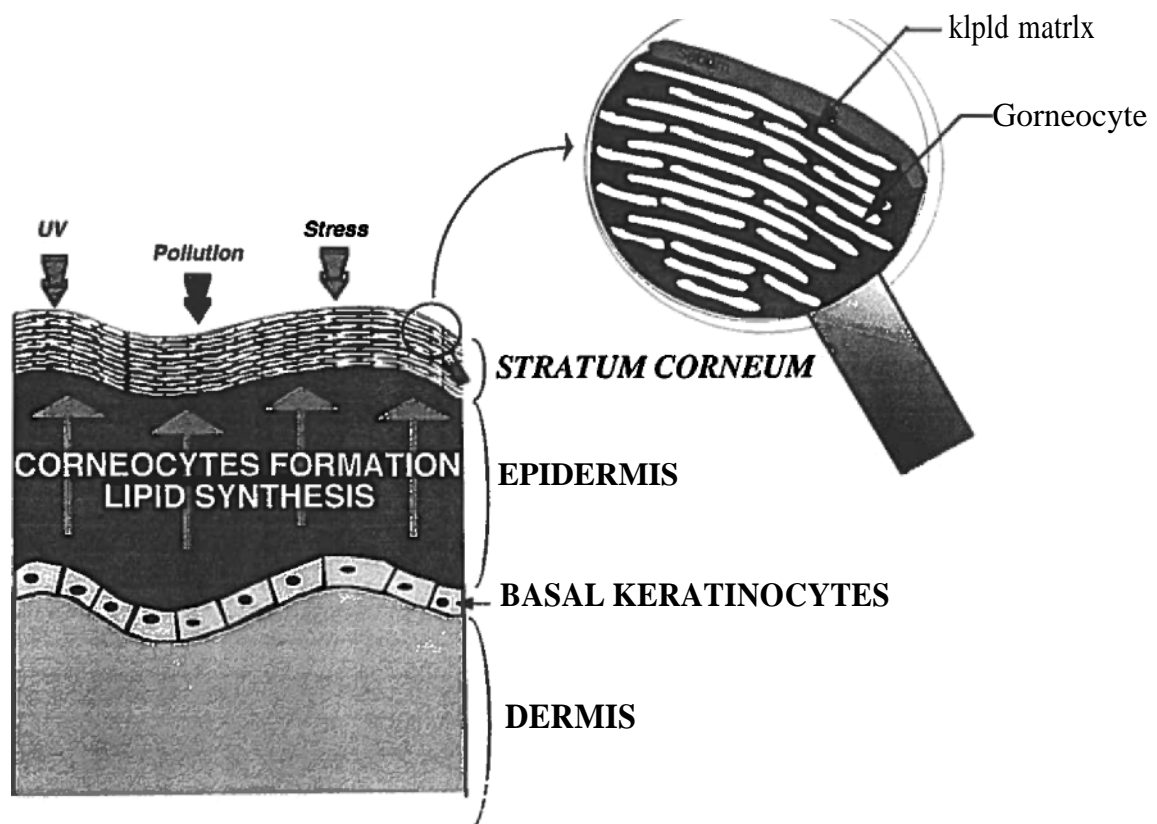


Figure 1 : Diagram of the epidermis and its functions

This paper focuses on the importance of various skin lipids from the stratum corneum, sebocytes, nails, their synthesis and their biological properties. We will emphasize their properties in relation to their structure, and their role in the control of epidermal homeostasis.

### STRATUM CORNEUM L&IDS

#### Comnosition

The stratum corneum intercellular lipids are made up of 20-25% free fatty acids, 20-25% cholesterol and 50% ceramides by weight which corresponds to equimolar

concentrations (1/1/1) of the three. More detailed information on the composition of human stratum corneum lipids can be found in the literature (4-7). The human stratum corneum usually contains fatty acids, cholesterol, ceramides, and also 10% cholesterol esters, 1-2% cholesterol sulfate and little or no phospholipid. The lipids composition can vary with the season, age, sex, anatomical region, UV, atopic dry skin and atopic dermatitis which can influence its function as an efficient barrier (8-14).

### Synthesis

Desquamation is a physiological process linked via unknown signals to such underlying changes as keratinocyte division, differentiation and the corresponding synthesis of new lipids. The supra basal keratinocytes synthesize lipid **precursors** and glycolipids are transformed into ceramides and phospholipids into free fatty acids. Lipid synthesis takes place in the endoplasmic reticulum (ER) and is processed in the Golgi apparatus to produce molecules suitable for the lamellar bodies supply in the trans Golgi network. The lamellar bodies accumulate proteins, enzymes and lipids. The epidermal lamellar bodies are delivered selectively to the intercorneocyte spaces. It is generally recognized that lipid synthesis by keratinocytes is not linked to nutrition, except for free fatty acids. Linoleic and linolenic acids must be provided by the blood and are essential for barrier **formation**.

### Free fatty acids

The main free fatty acids in the stratum corneum are C16-C18 and C14-C24 molecules, but there are also more specific saturated long-chain as (C22-24) fatty acids (2). Living organisms are able to produce palmitic acid (C16) from acetyl CoA. The carbon chain is then extended by 2C (from malonyl CoA) to form stearic acid (C18). Desaturase activity then produces palmitoleic acid (C16:1) and oleic acid (C18:1). Only plants can produce linoleic (C18:2) and linolenic acids (C18:3), they are therefore essential fatty acids. Keratinocytes do not possess an elongation enzyme able to produce arachidonic acid (C20:4) from linoleic acid. But they do possess a fatty acid-binding protein that is probably involved in recycling free fatty acids from the stratum corneum (15-17).

Polyunsaturated fatty acids are also precursors of the lipids involved in inflammation (18-19). It is not clear if the free fatty acids found in the stratum corneum are all specifically synthesized by the keratinocytes. There is a possibility that some fatty acids are also derived from the extracellular degradation of precursor lipids. The recovery of the stratum corneum barrier after perturbation requires extracellular phospholipase A<sub>2</sub> activity (20, 21).

### Cholesterol

Cholesterol accounts for one third of the stratum corneum lipids and is synthesized in the epidermis. In fact, the skin is the major storage site of cholesterol in the body. Multistep enzymatic reactions are involved in the synthesis of cholesterol from acetyl CoA. The rate limiting step is the hydroxy methyl glutaryl CoA reductase (HMG-CoA) involved in the condensation of farnesyl pyrophosphate. Cholesterol is very important for skin function and its synthesis increases following barrier perturbation (22,23). A minor fraction is sulfated by a cholesterol sulfotransferase to form cholesterol sulfate, a more polar, that is probably important in the structure of intercorneocyte lipid bilayers (24).

### Sphingolipids and ceramides

Sphingolipids are major class of lipids in the epidermis and are the precursors of ceramides. They contain more long-chain saturated fatty acids than do esterified phospholipids and are considered to be the main precursors of these fatty acids in the stratum corneum (25, 26). Sphingomyelin and glucosylceramides are the major sphingolipids found in epidermal lamellar bodies.

### De novo synthesis

Sphingolipids are derived from long-chain sphingoids. Sphingolipid synthesis occurs in the ER and Golgi apparatus. A sphingoid base is formed at the cytosolic **surface of** the ER by the condensation of L-serine with palmitoyl-CoA to form 3-dehydro-sphinganine. This is then converted by ceramide synthase to dihydroceramides, and desaturated to give ceramides. The ceramide molecule is then modified by the addition of phosphorylcholine to form sphingomyelin, on the luminal **surface of** the Golgi apparatus. Glycosylation takes place first at the cytosolic surface of the Golgi, then at the luminal surface. All products are then exported to the plasma membrane by exocytosis. The two main sphingoid **precursors** of ceramide synthesis in the epidermis are sphingosine (4-sphingenine) and phytosphingosine (4-hydroxy-sphinganine) (Fig.2).

### Sphingomyelin cycle

The breakdown of sphingomyelin is another source of ceramides (27, 28). Sphingomyelinase (acid or neutral) cut sphingomyelin to give phosphorylcholine and ceramides. Then ceramidases (deacylases) remove the amide-linked fatty acid moiety to give sphingosine and fatty acids. There are Mg<sup>2+</sup> dependant and independant isoenzymes of sphingomyelinase and any lack of physiological degradation of ceramides leads to severe disease. As all plasma membranes are asymmetric, any breakdown of sphingomyelin results in a physical alteration in the membrane which can trigger other signals.

This generation of intracellular ceramides influences such major cellular functions as proliferation, differentiation and apoptosis.

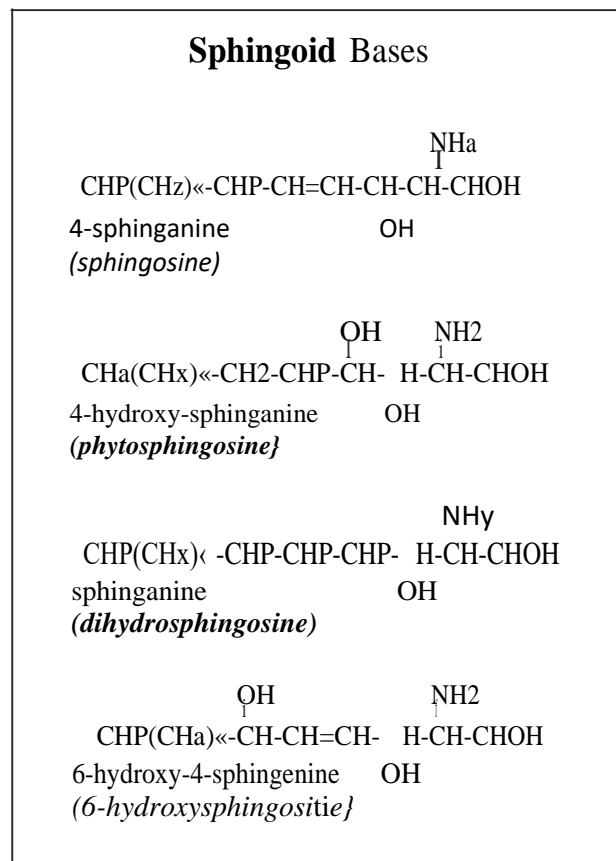


Figure 2 Sphingoid bases of human ceramides

### Formation and secretion of lamellar bodies

Lamellar bodies are the lipid-rich organelles present in differentiated keratinocytes. Lamellar bodies (LB) are secreted by the apical cytosol of the outermost granular cells at the stratum granulosum - stratum corneum interface by a controlled series of events (29, 31) (Fig.3). They fuse with the plasma membrane, delivering lipids and hydrolases to the interstices of the stratum corneum. Some glycosylated lipid forms can be removed at this step. The enzymes are phospholipases, sphingomyelinases, steroid sulfatase, glucocerebrosidases and acidic-neutral lipases.

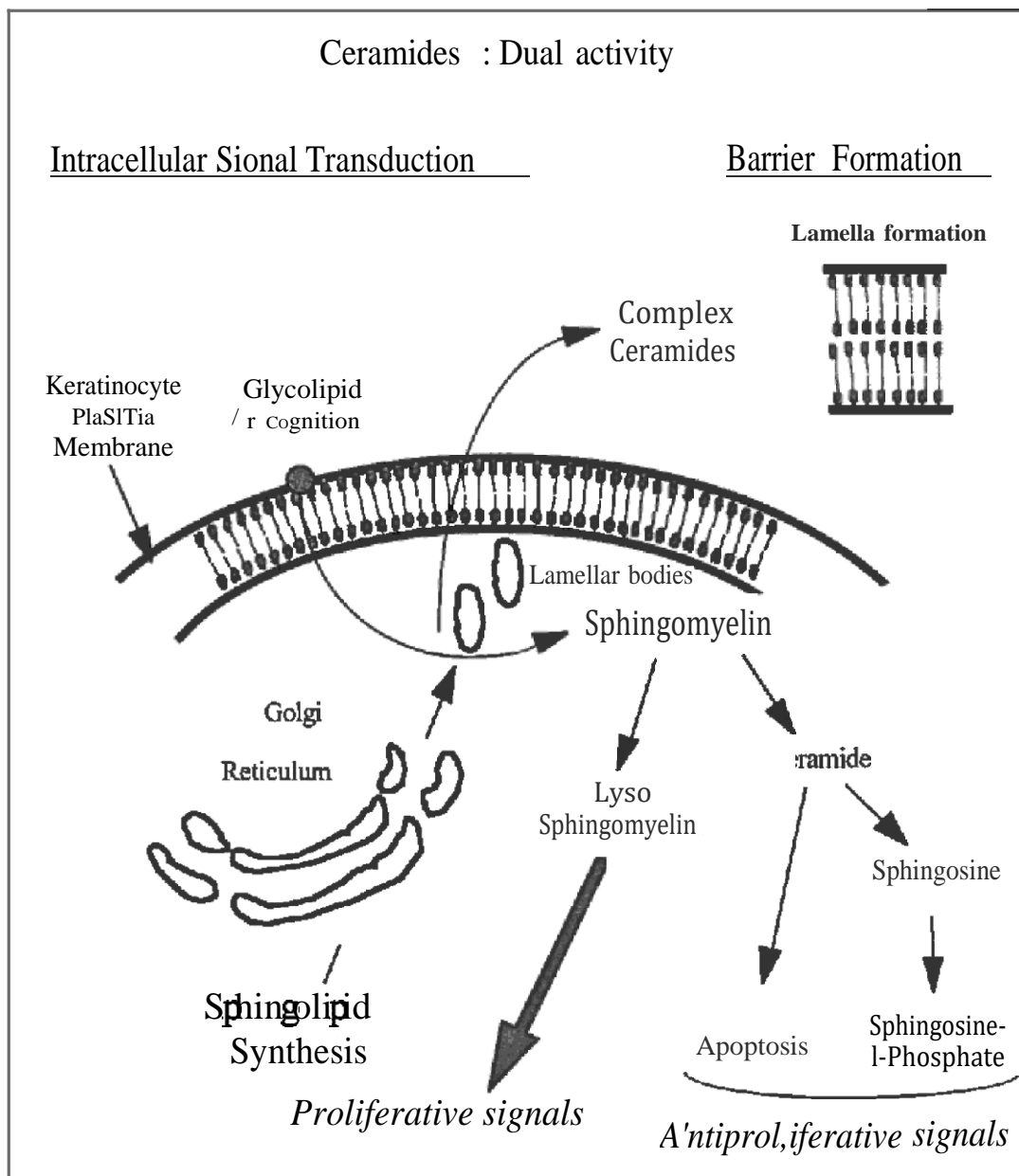


Figure 3 : From lipid synthesis to lamella formation

These enzymes are very often sensitive to pH (note that pH 5-6 is required for glycosylceramidase activity) and could be regulated by any disruption of the barrier. It is not clear if there is a pH gradient, but microdomains of pH are probably present at this skin level (32). All the metabolic intermediates in the upper layer lipid degradative pathways can probably act as

signals and these organelles are very rapidly secreted in response to barrier environmental stress or disturbance. Lipids from the lamellar bodies are chemically different from those found between corneocytes, indicating post-secretion maturation. Enzymatic steps that occur in the lower 3-4 layers of the stratum compactum are involved in this maturation

leading to chemical modifications and lamellae formation. Secreted phospholipase At, which is very active at neutral pH, is first cleavage step leading to free fatty acids; this is then followed by  $\beta$ -glucocerebrosidase which requires a more acid pH to transform glucosylceramides into ceramides.

The physiological function of the lamellae is to incorporate water molecules into the stratum corneum, to produce an adaptative, organized and regulated barrier against our "dry" environment (33). A widely accepted water profile in the skin is 80% in basal layer, 65% in stratum granulosum, followed by a drop to 30% at the stratum granulosum-stratum corneum interface and 10-15% at the desquaming surface. This water gradient in skin is probably related to the nature of the biological processes involved.

#### Lipid composition, three dimensional organisation and physiological impact

The corneocyte envelope formed from involucrine by activation of transglutaminase is known to have a lipid microlayer covalently linked to its surface (34-35) During the formation of the lipid lamellae, the bound lipid, mainly ceramides, orientate the intercellular lamellae. This lipid-bound envelope is essential for lamella formation, its orientation and its barrier properties (Fig.4). Omega hydroxy-ceramides are the main lipids that are covalently linked to the corneocyte envelope. Their formation is cytochrome P450 dependent and they appear to be of prime importance for epidermal permeability. Lamellae are composed of ceramides, cholesterol and free fatty acids organized in a multilamellar arrangement between layers of corneocytes.

This organization of the lipids in the stratum corneum has been examined in greater detail

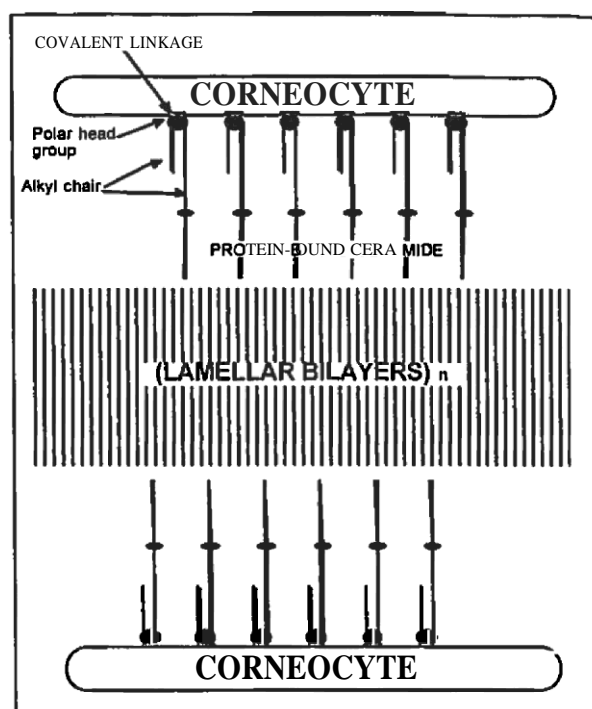


Figure 4 : Corneocyte envelope and lamella organization

following recent work on the molecular structures of human ceramides. Knowledge of molecular details is necessary to understand the exact structures and their implications for the penetration of drugs and/or protection against water loss.

A new structure-based **nomenclature** has been proposed for the human ceramide family. Ceramides differ from each other by their head group and the length of the fatty acid chain (16 to 30 carbon atoms) (Fig.5). These include 7 free ceramides cer 1 0 cer 7 and two protein bound ceramides, i.e a very long chain «i-hydroxyacid amide linked to sphingosine and a new in-hydroxyacid derivative of a new base, 6 hydroxy-4-sphingenine (36).

Ceramide 1 : contains linoleic acid linked to a long chain ni-hydroxy acid this is very important for the lateral packing of lipids and for long range ordering which is the



basis of the barrier organization. It is bound to the corneocyte envelope in pig skin.

Ceramide 2 : consists mainly of 22, 24, 26 carbon straight chain saturated fatty acids amide-linked to sphingosine base.

Ceramide 3 : contains a similar pattern of long chain fatty acids amide-linked to phytosphingosine base.

Ceramide 4 : is an acyl ceramide bound to an acylated  $\omega$ -hydroxy acid-linked to a new trihydroxy sphingosine base.

Ceramides 5 and 6 : contain alpha hydroxyacids amide-linked to sphingosine or to a phytosphingosine base.

Ceramide 7 : is a hydroxyacyl-6-hydroxy-4-sphingemine.

There are 2 ceramides bound to the human corneocyte envelope, one has  $\omega$ -hydroxyacids amide-linked to sphingosine (A) and the other one has  $\omega$ -hydroxyacids amide-linked to 6-hydroxy-4-sphingemine (B).

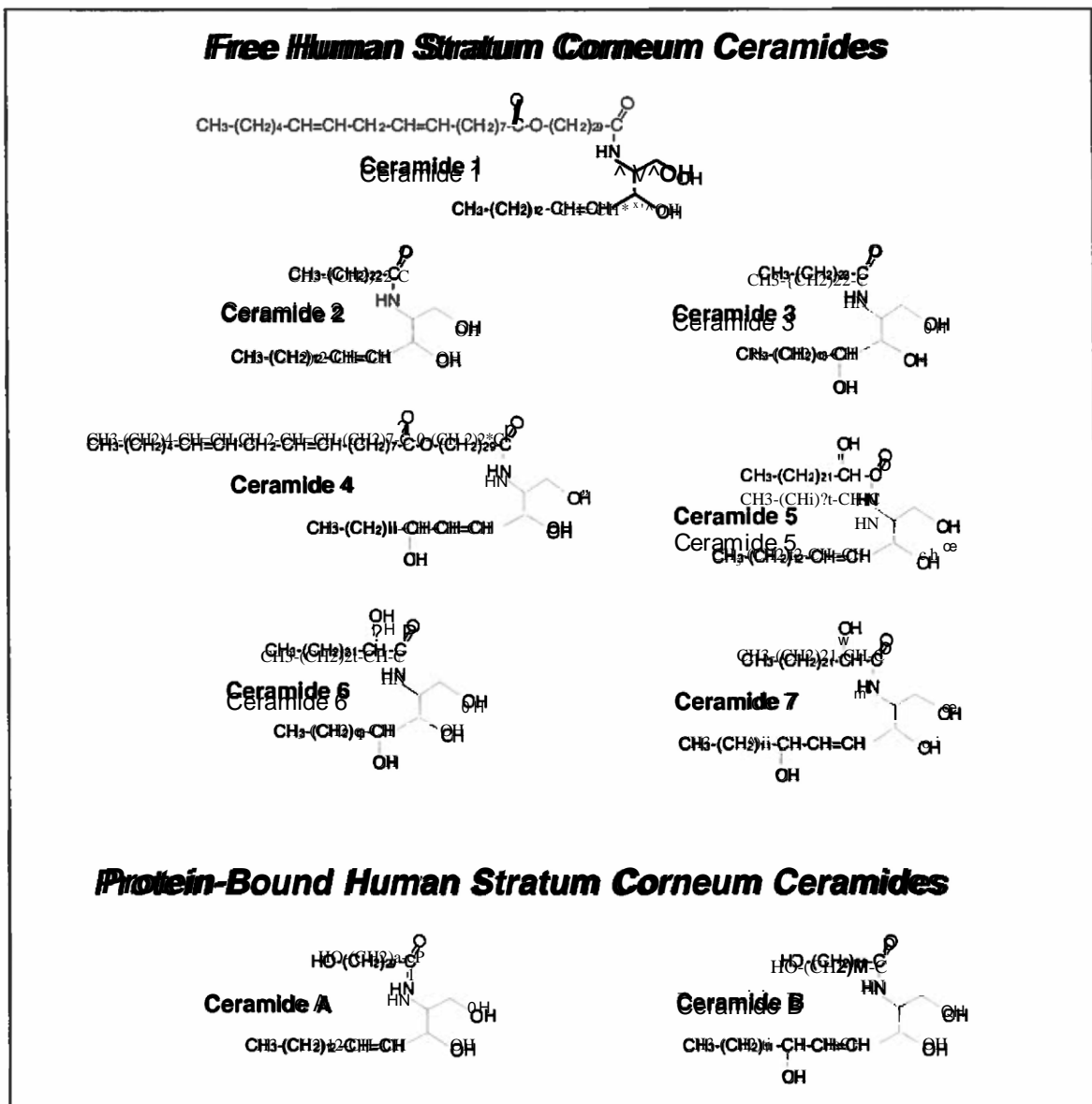


Figure 5 : Human ceramide structures

Free bases are also found in the stratum corneum (37-39). Their biological role is not yet clear, although they are known to inhibit protein kinase C. Sphingosine and/or its derivatives stimulate EGF (epidermal growth factor), interact with other stratum corneum lipids and may be possible regulatory signals to the viable epidermis (40-42).

Despite natural physiological fluctuations, due to nutrition, environmental stress (UV, pollutants like ozone), aging, local perturbation, the efficiency of the global barrier is in large measure due to lipids (43). Any change in the lipid will disturb the 3-D organization of the skin and thus modify its properties. The stratum corneum is not physically uniform through its entire thickness. It has been recently demonstrated there may be a gradient in the composition of the upper stratum corneum in vivo (44). The amount of extractable lipid decreased with depth of the stratum corneum. Less ordered lipids were found near the surface, in agreement with the fact that the upper layers are destined for desquamation. It seems clear that the lipid layers or lipid domains with a specific composition and organization in the middle and lower part of the stratum corneum govern the main skin barrier properties.

A change in pH affects the lamellar ordering of a mixture of cholesterol ceramides and free fatty acids, which are major components of the stratum corneum lipid, but not the lateral packing. The lipids in the long periodicity phase always crystallize in the same orientation at pH 7.4 but not at pH 5. The inclusion of a small amount of cholesterol sulfate markedly **increases** the solubility of cholesterol in cholesterol / ceramides / free fatty acids mixture, indicating a more fluid global structure (45). Recent studies have also indicated that cholesterol sulfate inhibits the proteases

involved in the natural desquamation of stratum corneum. This can be correlated with X-linked ichthyosis, in which hyperkeratosis is mediated by inhibition of the desmosome serine proteases and cholesterol sulfate accumulation (46-47).

The 3-D lipid organisation of the stratum corneum has been the object of many studies over the past few years. Previously, we need to introduce that lipid systems are able to exhibit varied phases depending on their aqueous environment. Simplified view led us to consider solid crystalline, gel and liquid crystalline phases. In the gel crystalline phase, the lipids were assumed to be rigid with a specific orientation with respect to each other. In the liquid crystalline phase, alkyl chains were assumed to have lateral mobility and no ordered arrangements. The phase transitions were also assumed to depend on the temperature, the local water content and composition of the lipid mixture (48-51). X-rays diffraction (small angle and wide angle) studies on sheets of stratum corneum or on lipid mixtures demonstrated the existence of lamellar phases with periodicities of 6.4 and 13.4 nm oriented parallel to the surface of the skin. A long periodicity is expected to be correlated with a strong barrier effect. In the human stratum corneum, orthorhombic and hexagonal lateral packing can also be present. It has been suggested that phase separated cholesterol crystals are intercalated in the lamellae. In fact, the bilayers of the stratum corneum are in a relatively anhydrous (15% water) environment and tightly packed. The presence of water pools close to rough lipid structures found in intercellular regions may help to explain the penetration of small relatively hydrophilic compounds (52). Equimolar mixtures of cholesterol and ceramides form a lamellar arrangement

without any segregation of cholesterol (53). It can be speculated that the exact molecular structure of ceramide (cer 1 to 7) will influence the structures formed by ceramide/cholesterol mixtures depending on their molecular ratio. It has been postulated that small amounts of polar lipids are also involved in the insertion of cholesterol by interacting with the ceramide matrix to modify the period of the lamellar structure (54). Exogenous ceramides, cholesterol and polar lipids are usually found in cosmetic skin care formulations such as skin creams, containing or not liposomes, and therefore can interact with a more or less deficient natural barrier.

Transport across sebaceous lipids, which cover large areas of the skin and are partially incorporated in the upper layer of the stratum corneum lipids, is also recognized as a possible rate-limiting step in percutaneous absorption. Interestingly, recent studies have demonstrated that the components of sebum (triglycerides, wax esters, squalene, and some free fatty acids) can be incorporated into the stratum **corneum** lipids *stricto sensu*, possibly by lipid chain interpenetration (54). This raises the question of the physicochemical interactions of topically applied exogenous skin care products (containing components that are often similar in chemical nature to sebum lipids) with the lipids of the upper layers of the stratum corneum in the control of the skin penetration. In fact, the stratum corneum lipid lamellae may possess local domains of lamellar, hexagonal, crystalline or even cubic phases that can interact, depending on the corneocyte configuration and/or local lipid composition. Other variations in **local** lipid composition or organization may also be associated with barrier perturbation, such as with alcohol, occlusion, penetration enhancers, over-hydration and may be restored with

exogenous lipid and/or modulators of lipid synthesis such as those in skin care products (55).

### **IMPORTANCE OF SEBUM LIPIDS**

While the stratum corneum lipids have been extensively studied, little is known about the importance of the sebum lipids in the control of skin penetration. The stratum corneum is recognized as the major skin barrier for a majority of compounds. This has been deduced from the fact that removal of the stratum corneum by tape stripping leads to a significant increase in trans-epidermal water loss (TEWL). Solvent extraction of the intercellular lipids also removes the skin barrier. Solvents also extract the sebum lipids (54). Sebum lipids form a film of 10-500 pg/cm<sup>2</sup> over almost the whole body surface and are involved in the penetration of xenobiotics when mixed with topically applied formulations. They are also involved in comedone and acne. Sebum is mainly triglycerides (40-60 %), wax esters (19-26%), squalene (11-15 %) and some free fatty acids secreted by the sebocytes. Sebaceous glands are associated with hair follicles, the afferent duct joining the hair **canal** at 0.6 mm below the skin surface.

Sebaceous glands contain lobes of sebocytes connected to the afferent duct. In these holocrine glands, the cells break down to release the secreted lipids. Sebaceous glands are found in almost all parts of the body and their density, size and activity vary with their location and a person's age. There are none on the soles of the feet, on palms of the hands but there are 50-100 sebaceous glands/cm<sup>2</sup> on the inner forearm and 400-900/cm<sup>2</sup> on the face, the back and the scalp (56).

The development and differentiation of sebaceous glands is regulated by androgens.

They cause cells to grow, proliferate and synthesize lipid. Dihydrotestosterone, a metabolite of testosterone synthesized by 5 $\alpha$  reductase (type I) enzymatic activity, has a high affinity to the androgen receptor and is the androgen regulator of sebum secretion. Recent investigations have also shown that the lipid patterns in freshly isolated glands and the in vitro secretion of human sebaceous glands are different (57-59). Inhibition of 5 $\alpha$ -reductase have been suggested as an useliil treatment for oily skin (60). Skin dryness following the topical application of retinoic acid in vivo has been linked to changes in the sebum lipids, with a decrease in wax esters and squalene, but has no effect on stratum corneum lipids, especially on the synthesis of ceramides (61).

It has also been shown that human sebum can be incorporated into the lipids of the upper layers of the stratum corneum without disturbing their phase behaviour (54). This indicates that sebum lipids contribute to the skin barrier, the overall skin integrity and the varying sensitivity of areas of the body. This can be important for the action of cosmetics and tolerance of them. These data also suggest that penetration varies depending on the lipid content of the site, and its organization, and that ex vivo or in vivo measurements only give an overall view of the phenomenon.

### NAIL LIPIDS

The nails form conved horny plates covering the dorsal sides of the fingers and toes. The nail plate grows out from an imagination of the epidermis and adheres to the nail bed and the proximal nail fold surface (62,63). Nails protect the nail bed and provide counter pressure for the pulp, they are essential for finger tactile sensation. The 0.5 - 0.7 mm thick nail plate is formed

of about 100-150 layers of stratified keratinized corneocytes (600 pm<sup>2</sup> in infants to 1000 pm<sup>2</sup> in adult).

There is little published information on nail lipids. We estimated the composition of the nail lipids of a panel of young Caucasian women (mean age 29 years, n=8), who wore no nail enamel for at least six months (table 1). Lipids are carefully extracted 3 times with cyclohexane/ethanol 2/8 then 3 times with cyclohexane/ethanol 5/5 and analyzed by HPTLC/AMD (5).

Knowledge of nail lipids is important for dermatological purposes **and to cosmetics** manufacturers as nail make up is formulated in non-polar solvents (64).

Table 1 :the lipid composition of finger nails (mean value).

	$\mu\text{g} / \text{cm}^2$	% total lipids
Phospholipids	1.4	2.1
Glucoceramides	ND	ND
Cholesterol 3 sulfat	0.6	0.8
Ceramides	14.7	22.7
Cholesterol	11.0	1d.9
Free Fatty Acids	33.5	51.7
Sterols Esters	4	d.2

### SKIN LIPIDS AND CELL SIGNALING

The homeostasis of the epidermis is the results of a closely and highly regulated balance between cell proliferation and cell death (Fig.d).

#### Phospholipids : a source of multiple messages

Phospholipids, especially phosphatidylcholine is a source of lipid messenger molecules (65). Phospholipids are major component of the keratinocyte plasma

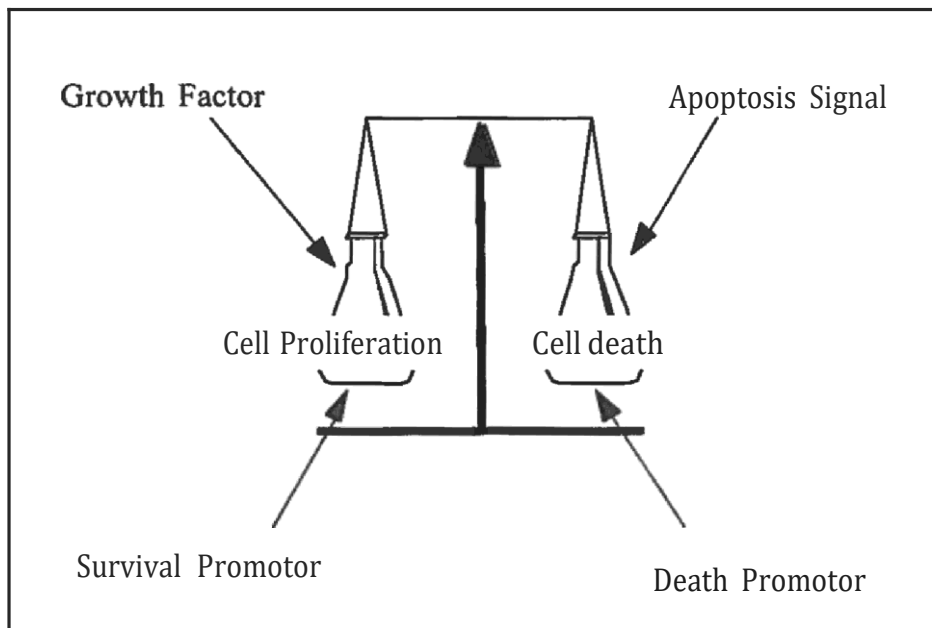


Figure 6 : Cell homeostasis

membrane. The physiological activities of hormones, neurotransmitters and growth factors require a transmission of the signal from the exterior of the plasma membrane to the interior of the **cell**. It involves the formation of a second messenger, which requires distinct pathways, G-protein-linked activation, or the activation of a tyrosine kinase (66). Phosphatidylcholine is a major component of the plasma membranes and can be hydrolyzed by phospholipases C, D and A2. Phospholipids are hydrolyzed by phospholipase C to give inositol 1,4,5-phosphate, which stimulates an increase in free intracellular calcium, and sn 1,2 diacylglycerol, a stimulator of protein kinase C. Phospholipase D generates phosphatidic acid whose action is unclear. It may act as a mitogen following conversion to lyso-phosphatidic acid and interaction with a G protein-linked receptor. Phosphatidic acid inhibits membranes adenylate cyclase activity and stimulates protein kinase activity. Phospholipase A2, which exist as cytosolic and membrane bound forms, generates lyso-phosphatidylcholine and

arachidonic acid which may be a source of eicosanoids.

The enzyme may be phosphorylated in the membrane and then translocated to the cytosol. This can be linked to key events in apoptosis, which include the activation of PKC, increased cytosolic  $Ca^{2+}$ , activation of protein tyrosine kinase (especially in stress) and the accumulation of cAMP and ceramides.

Lastly, protein kinase C elevation or high intracellular concentrations of cAMP can have positive or negative actions on apoptosis, depending on the situation and cell type. The arachidonic acid released by P 2 may also be involved in modulating the sphingomyelin cycle.

Phospholipids and their fatty acid metabolites, such as those derived from arachidonic acid and from the lipoxygenase pathway, are also reactive oxygen species. These reactive oxygen species (ROS) are involved in the induction of genes associated with inflammatory and immune responses. Exogenous fatty acid metabolites

can also promote programmed cell death. This is another potential source of signals for programmed cell death that complements the recognized ROS produced by the mitochondrial respiratory chain.

### Ceramides

Extracellular ceramides with long-chain fatty acids are key components of the stratum corneum barrier. Recent studies have shown that sphingolipids, especially ceramides, with smaller fatty acids chains and/or sphingosine also act as intracellular messengers in various mechanisms such as cell growth, differentiation and apoptosis (67,68). Intracellular ceramides are also produced by keratinocytes in the skin and are involved in the regulation of epidermis.

The balance of cell growth is regulated by sphingolipid metabolites : ceramides can be thought as pleiotropic molecules because they inhibit proliferation, initiate apoptosis and induce differentiation (28, 69) (Fig. 7, 8). Ceramide may be involved in the senescence of fibroblasts cell line and human epidermal tissue by altering sphingomyelinase activity (70,71).

UV irradiation, IU and heat shock can all stimulate sphingomyelinase leading to the production of ceramide and stimulation of various biological pathways (72). Fumonesin, a mycotoxin from *fiisarium* and **D-MAPP** (N myristoylamino-1-phenyl-1-propanol) are both pharmacological modulators of ceramide metabolism.

Sphingosine, sphinganine and impaired mitochondrial uptake of palmityl CoA cause apoptosis (programmed cell death) in keratinocytes. Free sphingosine is found in the human epidermis, and may diffuse from the stratum corneum and hence be a signal to the underlying epidermis.

Ceramide signaling is part of the pathway by which UVA irradiation causes the transcription of certain human genes in

keratinocytes including the **encoding** intercellular adhesion molecule (ICAMI) (73). Oncogenes Bcl2 and Bax regulate programmed cell death by regulating the release of certain caspase (Cysteinyll

Aspartate Specific Proteinase) activators or cytochrome C by mitochondria and are involved in tissue homeostasis (74, 75).

Bcl2, an anti apoptotic signal protein involved in epidermal homeostasis, protects cells from killing induced by ceramide (76-78). The adipocytes, deeper in the skin, can also be regulated via the ceramide pathway. Insulin is known to govern the glucose uptake by the adipocytes, the first step of the mechanisms leading to triglycerides and lipogenesis. TNF  $\alpha$  and permeant ceramide both inhibit insulin signaling in 3T3-L1 (preadipocyte cell line) by stimulating the P55 TNF receptor. TNF  $\alpha$  mediated activation of the membrane-bound neutral sphingomyelinase is likely a major pathway in insulin receptor inhibition and subsequent phosphorylation—dephosphorylation cascade of events (79, 80). TNF $\alpha$ , sphingomyelinase-activated metabolites (sphingoid **bases, ceramides**) should probably act similarly on the insulin signaling pathway and thus decrease the differentiation of cells.

# How sphingolipid metabolites regulate cell life and death

*“de novo synthesis”*

L-serine + palmitoyl-CoA

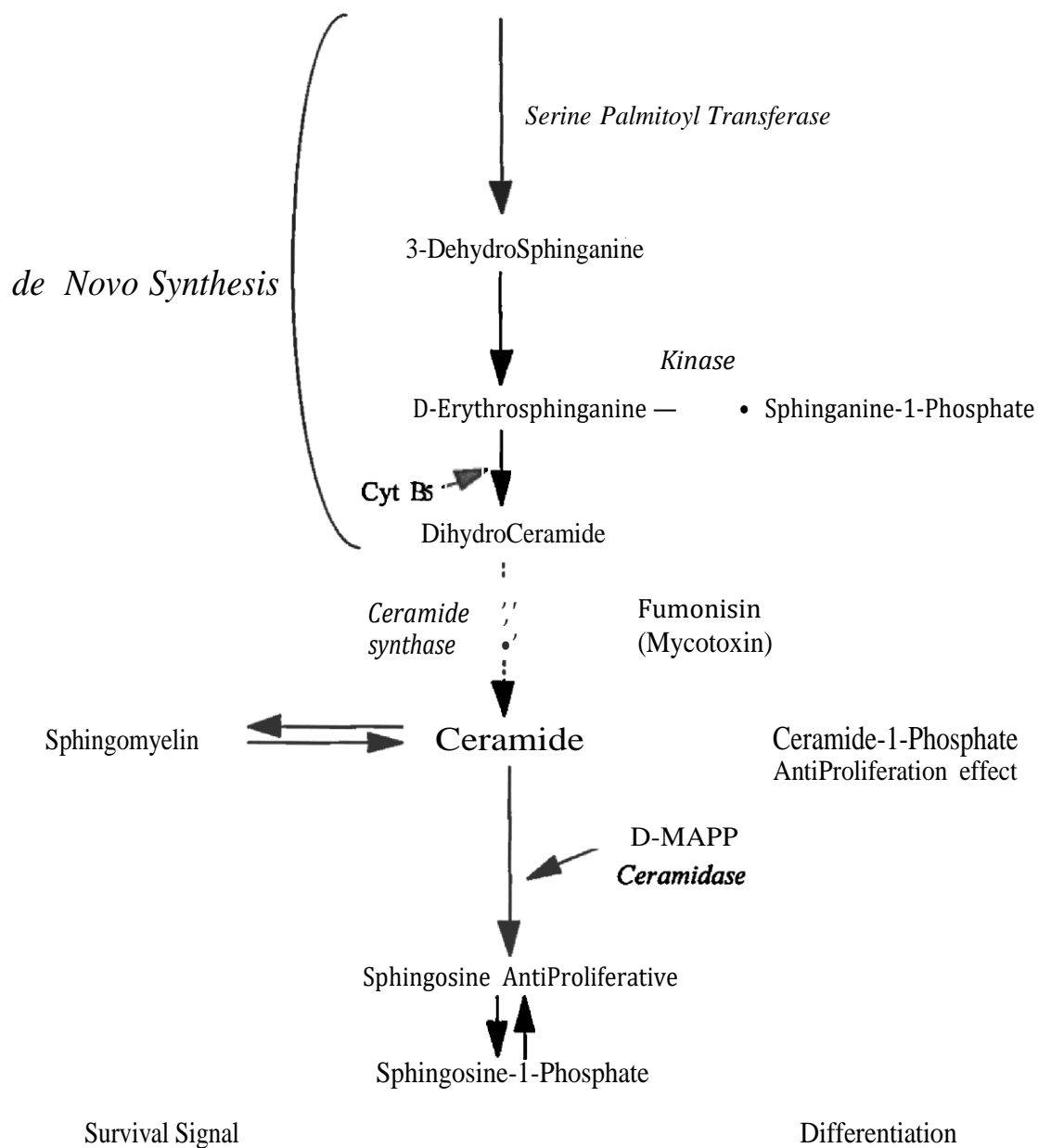


Figure 7 : *de Novo* ceramide synthesis and biological effects

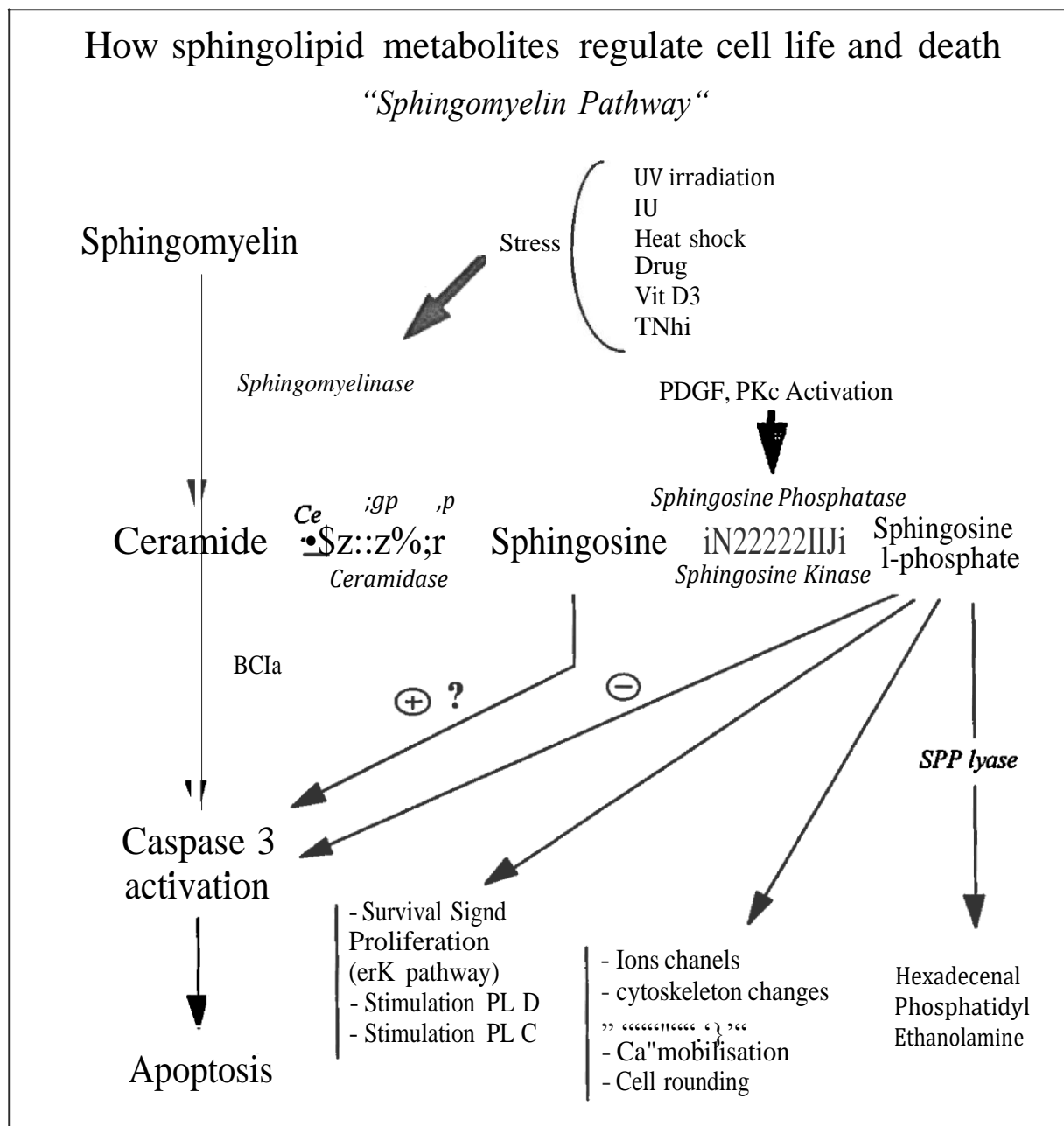


Figure 8 : Sphingomyelin pathway and biological consequences

Disruption of the skin barrier, such as the mechanical disturbance produced by tape stripping, results in a rapid release of the content of lamellar bodies followed by new lipid synthesis. The barrier is repaired rapidly within 1-3 hours, and completely in 24 hours, as measured by TEWL. Increased fatty acids synthesis or cholesterol are important steps (80, 81). Acidic sphingo-

myelinase begins to function a few hours after disruption. It is not clear if stress directly or indirectly via cytokines, etc. stimulates sphingomyelinase. The TNF receptor P55 and ceramides generated by sphingomyelinase breakdown are important for signal transduction in barrier repair (82). Studies by the same authors on mice have shown that decreased acid



sphingomyelinase activity in the inner epidermis and neutral sphingomyelinase in the total epidermis could be linked to reduced barrier capacity with aging (83). This seems to be confirmed in human (73). In fact it seems that while the **TEWL** of aged skin looks very similar to that of young skin, repair capacity in aged skin is delayed and/or reduced.

Organic solvents affect the human skin water barrier. The disruption of barrier by acetone or tape stripping stimulates lipid synthesis and increased production of mRNA of the enzymes involved in epidermal cholesterol, fatty acid and ceramide synthesis. Increased TNF $\alpha$  and IL are involved in the sphingolipid pathways (84 - 86). L-lactic acid, an alpha hydroxy acid, also increases the ceramides in the stratum corneum and skin barrier function (87). Topical application of some free fatty acids in enhancing the permeation of chemicals across the skin, participates to the barrier disruption and may be involved in subsequent signaling and cell death inductions (88, 89).

## **CONCLUSION**

This paper give an overview of the involvement of skin lipids in various phenomena. Sebum lipids appear to be important via their cooperation with stratum lipids, for the penetration phenomenon into the skin. The keratinocytes play a central role in the synthesis of the stratum corneum lipids that are in charge of the skin barrier function. It is also the central target of interaction with the environment, leading directly or indirectly via lipid messengers to epidermal cell homeostasis. Long chain ceramides in the upper layers of the stratum corneum appear to be essential for the skin barrier and drug penetration.

Intracellular ceramides now appear to be, directly or indirectly (via cytokine, TNF, IL $_1$ , etc.) acting as a signal transduction pathway in response to barrier disturbances. Free ceramide bases, and probably some catabolic elements of the upper lipids, can be signals to the underlying epidermis. They can participate in the homeostasis of the epidermis by coordinating such independent biological events as apoptosis and cell differentiation. The biochemical signal pathways by which the stratum corneum and viable epidermis interact are becoming increasingly clear.

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