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"a new tool for the evaluation of skin barrier overall wellbeing"

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1. Introduction

The skin is the outermost surface of the body playing a primary function in providing a physical, chemical, and immunological barrier that protects the body from the external environment [1].In addition to this, it is responsible for the maintenance of the omeosthasis and preventing the excessive transepidermal water loss (TEWL) [2] [3]. Given all the fundamental functions it performs, the integrity of the skin is essential for maintaining the general health. The integirity of the barrier is particularly related to the complex composition and organization of the stratum corneum (SC), 10− 20 μm thick [4] [5], which is the outermost layer of the epidermis. The stratum corneum is made up of two major components: corneocytes and intercellular lipids, resembling a structure called "brick and mortar", forming a hydrophobic matrix. [6] Within the corneocytes, structural proteins are highly cross-linked and concentrated both intracellularly and at intercellular junctions, providing mechanical strength and contributing to the integrity of the skin barrier.[7] Among these classes of elements, lipids play a fundamental role in maintaining barrier function [8] with an organization in lamellar structures due to the hydrophobic and hydrophilic regions. Several lipid classes are present in the intercellular matrix of the stratum corneum: cholesterol, free fatty acids (FFA) and ceramides. Of this lipid matrix, ceramides represent approximately 50% by weight [9]. Ceramides include the most diverse types: they are amphiphilic molecules, exhibit varying molecular structures that lead to their classification into multiple groups based on the combination of a sphingoid base and different fatty acids. In particular, the majority of ceramides belong to the non-hydroxy family, constituting 55% of the total free ceramides [8] [10]. SC ceramides are highly complex but generally the most common are cer[NP] and cer[NS] [11] [12]. The quantity and class distribution of ceramides have been linked to barrier function quality so variations in ceramide levels influence skin condition [8] . External (pollution, chemicals, climate, season) and internal factors (age, sebum secretion, pH modulation) can affect the lipid composition of SC leading to dryness and barrier impairment. [11], [13], [14]. In addition, several dermatological conditions, sucha as atopic dermatitis, psoriaris, xerosis for instance, are associated to the alteration of the lipid matrix of the stratum corneum, resulting in an altered barrier function and also increased tewl and allergen penetration [3] [14] [15]. Therefore, understanding the relationship between SC lipid and skin status is important to determine the clinical and cosmetic potential of lipid restoration in different types

of skin. In this context, ceramides stand as the main structural components of lamellar bodies and the multilamellar barrier. [10] Given the importance of this highly organized lipid architecture for barrier function, it is crucial to investigate both total ceramide content and the ratios among specific ceramide subclasses to detect alterations associated with skin dysfunction.[5] This study aimed to focus on the central role of ceramides for the development of an index capable of predict or identify conditions and alteration of the skin barrier. To achieve this goal, an in vivo characterization study to investigate the ceramide content in the stratum corneum, normalized to protein content, and its correlation with biophysical parameters related to skin barrier properties in a heterogeneous panel of female subjects were carried out. In particular, the integration of cer-NP with functional parameters of skin health want to be the basis for a comprehensive index of skin barrier status. By characterizing the ceramide-to-protein ratio in the stratum corneum and correlating these values with TEWL, and hydration levels, this research want to propose a novel framework for evaluating the condition of the epidermal barrier. This consolidative approach may lay the groundwork for the development of diagnostic systems and advanced, personalized skincare tools, with the potential to predict and manage skin barrier-related conditions, with a further step in microbiota correlation.

2. Materials and Methods

An in vivo characterization study on a heterogeneous female panel was performed to evaluate skin type-specific ceramide profiles. The single center study particularly investigated the relationship between stratum corneum ceramide levels, specifically ceramide NP, and protein content of the stratum corneum and key biophysical properties of the skin, such as skin hydration, trasp-epidermal water loss and skin sebum. This integrated approach was designed to support the development of an index capable of reflecting the status of the skin barrier and its healthy features, by linking biophysical parameters to ceramide profile and eventually to skin microbiota. The index should create a framework to identify the skin condition so to optimize clinical or cosmetic treatment of the skin.

Volunteers

The in vivo study involved 90 European Caucasian women aged between 27 and 65 years. Participants were recruited in northern Italy -Lombardy- and the enrollment took place in late autumn/winter season 2024. Inclusion criteria regarded the good state of general health, and the absence of diseases, lesions in the involved area and no breastfeeding or pregnancy state. All the subjects signed an informed consent form according to the Italian law (GDPR 2016/679) and the analyses were performed in compliance with the principles of Helsinki declaration [16]. Skin assessments were conducted on distinct anatomical sites: based on a randomization mode, some participants were assigned to facial analysis in the zygomatic bone while others underwent body analysis in the lower leg.

Instrumental assessment

Measurements were conducted in an air-conditioned room with controlled temperature and humidity (T 22°C, r.h. 50 ± 5%). Subjects acclimatized for 15 minutes before data acquisition and they were instructed not to apply any topical products in the test area during the 24 hours preceding the analysis. All the procedures involved the contact between skin and probes without causing discomfort, pain or damage.
The study involved the determination of the ceramide content and the evaluation of correlated skin parameters such as SC water content, trans-epidermal water loss (TEWL) and sebum.

Protein and Ceramide content

The analytical approach considered the ratio of ceramide (cer-NP) content to the protein content of the stratum corneum. Ceramide data were obtained through tape stripping, extraction methods, and HPLC analysis. Specifically, the assessment was conducted by evaluating the content of ceramide isolated from the skin using tape stripping as the sample collection method. It involved applying an adhesive tape of 14 mm of diameter (D101- D-Squame Stripping Discs, Cuderm, Dallas, TX, USA) on the skin surface with a constant pression of 225 g/cm^2 to the disc surface, and then removed to collect layers of the stratum corneum. Strips were analyzed to determine at the beginning the protein content taken from stratum corneum using the infra-red densitometry (IRD) technique at 850 nm. Results were expressed as protein content µg/cm^2 obtained using the following equation: µg/cm^2= 1.366*Abs%-1.557 [17]. Each strip was then subjected to a ceramide extraction process with a solvent mixture, consisting of Methanol and Ethyl Acetate in an 80:20 (v/v) ratio, to obtain the extraction by ultrasonic bath for 60 minutes, then removing the solvent by evaporation through a controlled nitrogen flow. The dried sample is resuspended in a solvent mixture of Chloroform and Methanol and incubated at 4°C overnight then filtered inside the vials for HPLC analysis by HPLC-CAD. Peak analysis was performed using Thermo Scientific™ Chromeleon™ Chromatography Data System Software 7.3 (60919), Waltham, MA, USA. Analysis to determine the amount of Ceramide NP (Cer-NP) were conducted after a proper calibration curva using a standard solution of Cer-NP.

Stratum corneum (SC) water content

The stratum corneum (SC) water content was measured with a Corneometer CM 825 (Cutometer MPA580, Courage&Khazaka, Cologne, Germany) on an arbitrary scale (0-100 U.A) using a device equipped with a 49 mm^2 surface probe measuring within a 10-20 m depth range in the stratum corneum.

Trans epidermal water loss

The Transepidermal water loss (TEWL) was measured with a skin evaporimeter made of a small cylindrical open chamber (1 cm in diameter, 2 cm in height) with a couple of hygrometric sensors connected to a microprocessor plugged into a computer workstation (TM 300 W, Cutometer MPA580, Courage&Khazaka, Cologne, Germany) expressed as g/m^{2}h.

Sebum

The sebum was measured with a Sebumeter (Cutometer dual MPA580, Courage & Khazaka, Cologne, Germany) based on grease-spot photometry. A special tape becomes transparent in contact with the sebum on the skin surface. A photocell measures the transparency. The light transmission represents the sebum content on the surface of the measuring area and is displayed in µg sebum/cm^2.

Statistical analysis

The data collected were processed using both descriptive statistical analysis and statistical testing with specific comparisons for parametric and non-parametric data. A significance level of 5% was chosen, so variations were considered statistically significant for $p < 0.05$.

3. Results

Instrumental data were collected on a panel of 90 Caucasian women. They were divided into two major groups based on the anatomical site involved in the evaluation: face (n° 50 subjects) or body (n° 40 subjects).

Skin type classification

Participants for face analysis were classified into three categories (dry, normal or oily skin) according to the relationship between SC water content and sebum levels, giving to these specifics: dry skin (hydration values below 30 ± 2 arbitrary units (A.U.) and sebum levels lower than 80 µg/cm²); oily skin (sebum content exceeded 80 µg/cm², regardless of hydration levels); normal skin (all other combinations that did not meet the criteria for dry or oily skin).[18]

Participants for body analysis were classified according to the SC water content results due to the anatomical and physiological differences in sebaceous gland distribution. The lower leg is characterized by low presence of sebaceous glands, which results in minimal sebum production. Then, sebum measurements were not included.[19]

Results are presented considering both the anatomical location and each individual's skin type. This approach allowed for the exploration of how ceramide levels vary with skin type and region.

Face analysis

50 subjects were included in face analysis. According to the results, skin types were distributed as follows: 40% dry, 40% normal, 20% oily (Table 1).

Table 1. Face data: biophysical parameters according to different skin types

Skin type	N° subjects	SC water content	TEWL	Sebum
Dry	20	24.98 ± 4.41	11.73 ± 2.92	29.80 ± 14.12
Normal	20	36.40 ± 4.80	9.99 ± 2.09	51.05 ± 16.60
Oily	10	47.88 ± 6.09	11.87± 3.41	123.10 ± 29.89

Body analysis

40 participants were included in body analysis. According to the results, skin types were distributed as follows: 100% dry (Table 2).

Table 2. Body data: biophysical parameters according to different skin types

Skin type	N° subjects	SC water content	TEWL
Dry	40	25.61 ± 3.63	8.35 ± 1.66

Ceramide/protein ratio

Differences in ceramide/protein ratios were evaluated across skin types and anatomical region, as shown in Table 3.

Table 3. Ceramide and Protein Content by Area and Skin Type

Skin type/area	N° subjects	Ceramide content	Protein content	Ceramide/protein ratio
Face - Dry	20	4.59 ± 0.87	29.01 ± 6.42	16.82 ± 5.67
Face - Normal	20	5.79 ± 0.83	24.02 ± 5.54	25.26 ± 6.13
Face - Oily	10	5.10 ± 1.30	23.83 ± 6.34	22.30 ± 6.33
Body - Dry	40	4.30 ± 1.53	30.26 ± 6.76	14.74 ± 5.45

The approach then combined biophysical parameters to ceramide/ protein content ratio, especially regarding skin hydration and and transepidermal water loss (TEWL).

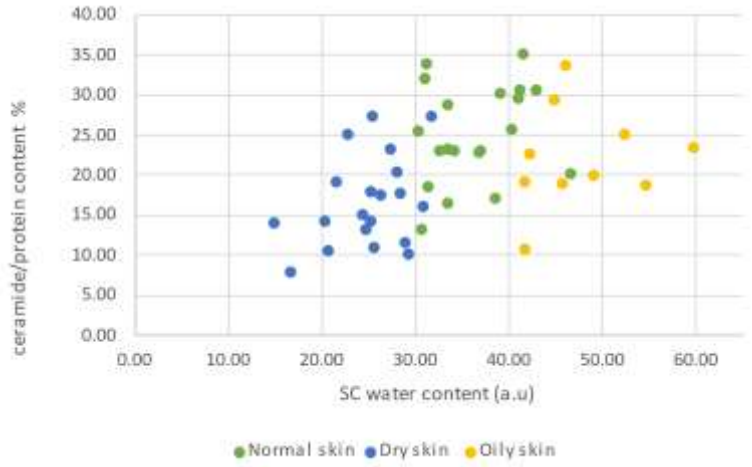

Figure 1. Scatter plots between SC water content and the ceramide/protein content ratio across different skin types: normale (green) dry (blue) and oily (yellow).

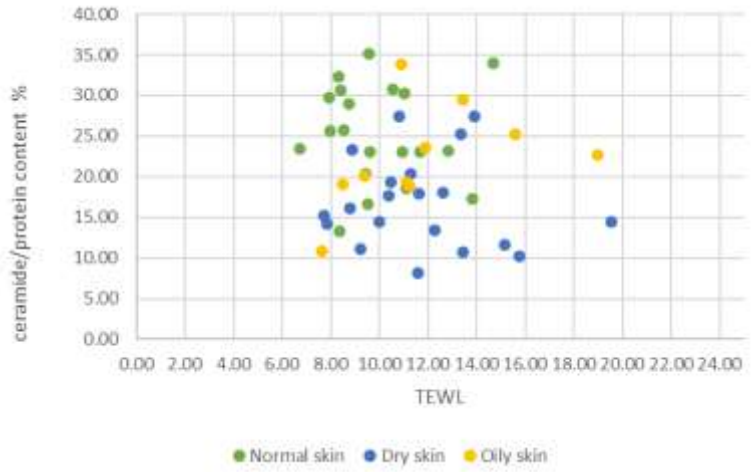

Figure 2. Scatter plots between TEWL and the ceramide/protein content ratio across different skin types: normale (green) dry (blue) and oily (yellow).

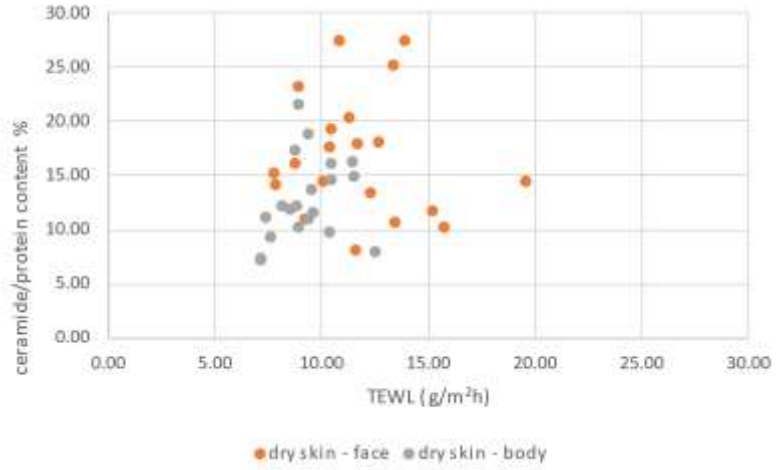

Figure 3. Scatter plots between SC water content and the ceramide/protein content ratio across different body region for dry skin: face (orange) and body (grey).

4. Discussion

In the present study, we compared the ceramide/protein ratio between the participants with different skin type across different body regions. The main objective of this study was to link biophysical parameters to the ceramide content (cer-NP). To that end, an in vivo investigation was conducted on 90 European Caucasian women divided into two anatomical-region groups. Different type of skin were compared: dry, normal, oily.

The biophysical data enabled the preliminary classification. On the face, a heterogeneous distribution was observed, with subjects falling into distinct categories, while on the body, the skin was characterized by a more homogeneous profile, predominantly presenting a single feature. In fact, all subjects exhibited characteristics consistent with dry skin in this region, as expected based on site-specific physiology and seasonality of the data acquisition. Based on these classifications, it became relevant to explore the correlation between the ceramide/protein ratio and the biophysical parameters. Figure 1) reveals a clear clustering pattern based on skin type, suggesting a relationship between stratum corneum hydration levels and the relative abundance of ceramides normalized to protein content. Dry skin has a lower ceramide/protein ratio, mostly <20%, indicating a trend towards a barrier function with an impaired lipid-protein balance. Normal skin represents the central one with a ceramide/protein ratio between 20–35%. Normal skin reflects a balanced barrier profile. Oily skin, showing the highest hydration levels (>40 a.u.), has a broader distribution of ceramide/protein ratios. This may reflect different conditions, or sebum interference. Generally, the data suggest that while SC water content increases progressively from dry to normal to oily skin, the ceramide/protein ratio also follows this trend, even if there greater variability in the oily skin group. Ceramide to protein content normalization can act as a marker to distinguish between skin types and maybe reflect the functional state of the skin barrier. This framework supports the rationale for integrating ceramide/protein data with biophysical measurements in the development of a comprehensive skin barrier health index. In addition, the distribution pattern of this ceramide/protein ratio in dry facial skin is similar to that observed in dry skin on the body, suggesting a common profile even in different regions. On the contrary, Figure 2 reveals no clear clustering pattern based on skin type for TEWL. Dry skin has a lower ceramide/protein ratio and a broader range of TEWL values, as well as oily skin. Normal skin shows a more uniform distribution. Overall, the data suggest that TEWL does not follow a distinct pattern.

5. Conclusion

The integrity of this barrier is crucial for overall health and is closely linked to the structure and composition of the stratum corneum. The SC's barrier function relies on a complex lipid matrix, primarily composed of ceramides, which represents around 50% of the intercellular lipids.
Given the essential role of ceramides in maintaining skin barrier integrity, understanding their relationship with skin health is crucial. This study aims to explore the role of ceramides in skin barrier function, focusing on the ceramide-to-protein ratio in the SC and its correlation with skin health parameters like hydration and TEWL. The goal is to develop a comprehensive index for assessing skin barrier status. The results demonstrated that ceramide content, when normalized to protein content, is comparable between the face and body within the same skin type, according to the standard classification of dry, normal, or oily skin. This highlights that ceramide levels appear to be independent of the analyzed area but are closely associated with skin classification. These findings define the relationship between ceramide content and skin type. This causal link represents a critical step toward the development of an index capable of defining skin status—an overall skin barrier health score—that integrates quantitative ceramide data with biophysical data. Additional analysis on microbiota will contribute to refining the scale and constructing an overall skin barrier health score, paving the way for innovative tools to evaluate skin barrier health and guide the development of tailored skincare solutions.

6. References

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