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Deciphering the lipid modulation in dry skin to propose dedicated solutions

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1. Introduction

The skin is the body's first line of defense, ensured by the barrier function role of the *stratum corneum* (SC). From a functional standpoint, this outermost layer of the epidermis is composed of stacked corneocytes that are embedded in a brick-and-mortar lipid matrix [1]. This naturally hydrophobic shield prevents water losses from the skin and its efficacy depends on its composition (ceramides, cholesterol and free fatty acids) and structure (conformation and organization) [2] (figure 1).

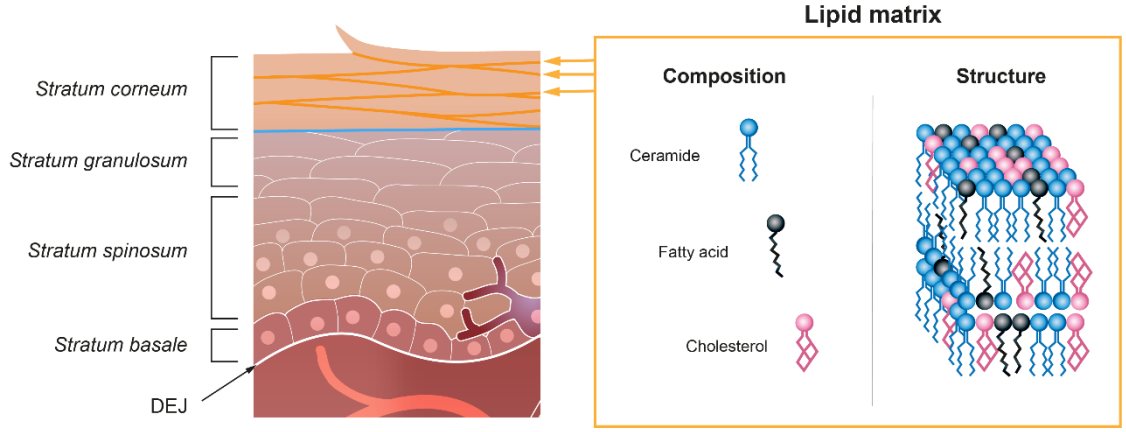

Figure 1. Diagram of the lipid matrix structure.

These two parameters therefore play a fundamental role in maintaining the protection capacities of the SC. When this strategic organization is altered, the skin progressively loses its capacity to defend itself from aggression, making it vulnerable to dehydration and redness, but also to premature and visible signs of skin aging. Dry skin is characterized by a weakened cutaneous barrier function [3]. This skin type results from an alteration of the lipid component of its SC. Even if the relationship between perturbation of lipids and modification of the barrier

function is generally admitted, in most cases, however, the real extent of modifications of the lipid matrix remains poorly understood. Available scientific data at the present time provides only a partial vision of the extent of changes caused in the context of an alteration of the cutaneous barrier.

In this context, this study aimed to investigate lipids from the SC of dry skin directly *in vivo* by combining mass spectrometry-based lipidomics and Raman microspectroscopy. The efficacy of a natural cosmetic active ingredient on the biological needs of dry skin was then investigated.

2. Materials and Methods

A clinical study was conducted on Caucasian women calves. A normal skin group was composed of 19 volunteers (mean age of 56 ± 6 years old with a mean TEWL of 3.9 ± 0.6 g/h/m² and a mean hydration level of 42 ± 10 AU) and a dry skin group composed of 15 volunteers (mean age of 53 ± 10 years old with a mean TEWL of 7.1 ± 1.8 g/h/m² and a mean hydration level of 24 ± 6 AU). Epidermal lipids were sampled for LC-MS/MS investigation and Raman microspectroscopy measurements were conducted on the calves of each volunteer (Figure 2).

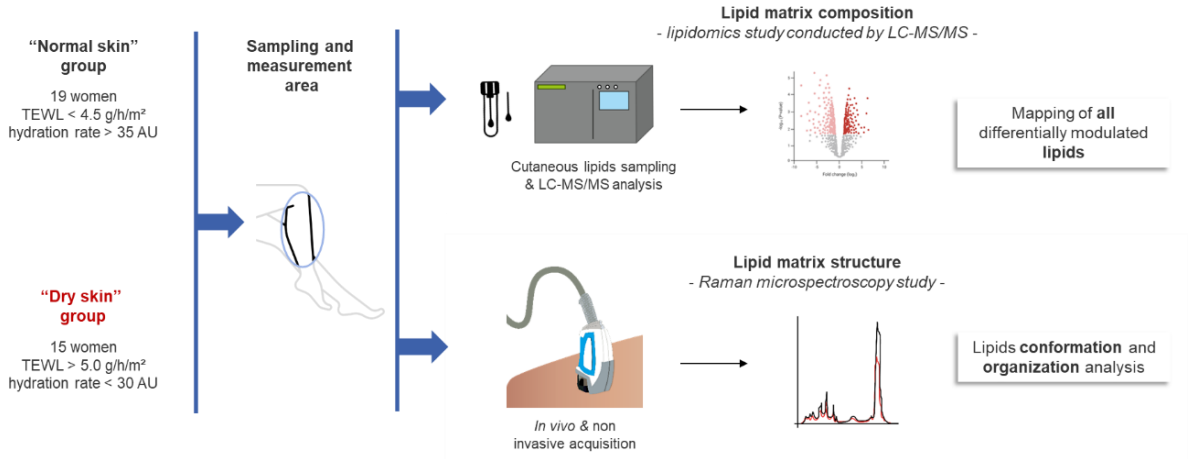

Figure 2. Schematic representation of the design of the modeling study.

Study of the lipid matrix composition

Epidermal lipids were sampled from the external side of the calves using swabs previously imbibed with a mixture of ethyl acetate. After sampling, lipids were extracted in a mixture of solvents (tert-butyl methyl ether/methanol (70/30)) containing three internal standards (C17 ceramide at 0.1 µg/mL, cholesterol-d7 at 4 µg/mL and heptadecanoic acid at 15 µg/mL). Samples were then injected in the LC-MS/MS system on the day of analysis. A pool of all samples was prepared and injected throughout the analysis as quality control. Lipids were separated on a CSH C18 column and detected by mass spectrometry in MSe mode using separation and detection conditions that were optimized with lipid standards. Areas under the curve of lipids were then calculated with WATERS CONNECT software of the instrument. Progenesis QI software was used for alignment of chromatographic peaks, normalization and the integration of ions detected.

All data were analyzed with statistical methods to identify lipids that were significantly modulated between normal and dry skin. Study results were visualized as a cloud of point in which the X axis represented the variation between dry and normal skins and the Y axis represented the significance of the statistical tests.

Study of the lipid matrix structure (conformation and organization)

The system is composed of a confocal Raman probe (Horiba Jobin Yvon) coupled with a dispersive Raman spectrometer (LabRam HR Evolution, Horiba Jobin Yvon). A piezoelectric system (Physics Instruments) was used to collect Raman profiles in the Z axis, from the surface down to a defined depth in the skin. Axial resolution of the system is about 3 µm. The acquisition system was controlled by Labspec 5 software (Horiba Jobin Yvon). Raman profiles were recorded by collecting spectra from -6 µm above the surface of the skin and down to a depth of 30 µm. Four spectral profiles were obtained for each volunteer. Spectral data were preprocessed with MATLAB® 7.2 software (The MathWorks). Aberrant spectra differing by more than two standard deviations from the profile were excluded from the data. Profiles selected underwent a series of corrections to clean up the Raman signal from the skin. The following descriptors were studied to determine changes of data relating to lipids:

- The νCC trans/νCC gauche ratio shows information on the intramolecular conformation of lipids (sum of the bands 1,130 cm^{-1} and 1,060 cm^{-1}, normalized by the pic at 1,080 cm^{-1}). The predominance of the trans conformation results in an upper compactness of the cutaneous barrier. An upper quantity of gauche conformers, on the other hand, shows a weakened compactness of the cutaneous lipid structures.
- The ν_{asym} CH2/ν_{sym} CH2 ratio is an indicator of the lateral organization of SC lipids (pics at 2,885 cm^{-1} normalized by the one at 2,850 cm^{-1}). High values of this ratio are related to an ordered organization. A reduction of this ratio is related to a loss of organization and therefore a decrease of the barrier function.

Study of the quality of the cutaneous barrier

The TEWL was studied with a TM 300 Tewameter® (Courage & Khazaka). The instrument is equipped with a probe that measures the water vapor gradient installed between the skin surface and surrounding air, providing information on the quality of the barrier function of the SC. A decrease in TEWL is characteristic of an improved barrier function of the skin.

Efficacy study of a natural active ingredient

A natural active ingredient (INCI: *Centaurea cyanus* flower extract) was then developed to answer the needs of dry skin. This restructuring natural active ingredient is obtained from cornflowers and its efficacy is conferred by its proline-rich peptide fraction. A placebo-controlled study was conducted *in vivo* to determine the effect of the *Centaurea cyanus* flower extract. The study was conducted on 18 healthy Caucasian women between 33 and 64 years of age (mean age 53 ± 10 years) with dry skin on the calves. In this study, volunteers applied twice daily the placebo or the active ingredient formulated at 1% in an emulsion for 14 days on the external side of the calves, according to predefined randomization.

The following methods were used to evaluate the biological activities of the *Centaurea cyanus* flower extract:

- study of the lipid matrix (targeted lipidomics study by LC-MS/MS, conducted on 15 volunteers among the 18 of the clinical study) and analysis of the lipid matrix structure by Raman microspectroscopy;
- study of the quality of the cutaneous barrier (Tewameter®);
- study of skin hydration (Corneometer® and Raman microspectroscopy).

3. Results

1. Comparative study of normal and dry skins

The two panels selected for this study have the relative characteristics of normal and dry skins (TEWL and hydration rate) while their mean age was not significantly different (Figure 3).

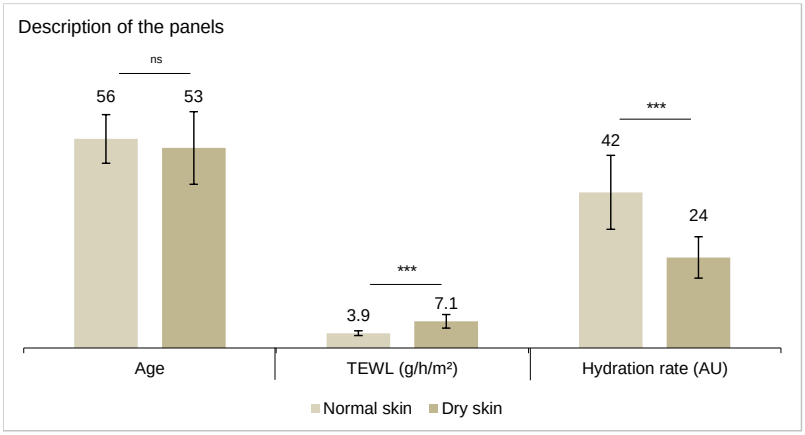

Figure 3. Description of the panels. *Result(s)/normal skin: ***: P < 0.001 --- ^{ns}: non-significant*.

First, the lipid matrix composition was investigated by LC-MS/MS. Figure 4 shows a map of all the lipids that are differentially modulated between dry and normal skins.

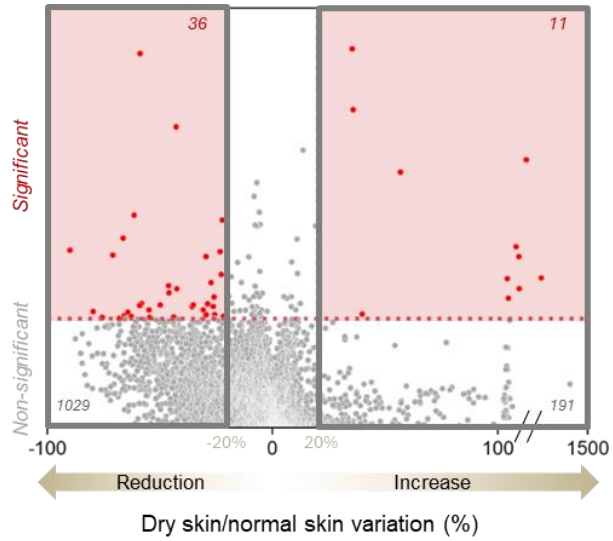

Figure 4. Comparison of lipids between the normal and dry skin groups.

The x axis represents the variation between dry skin and normal skin. A lipid is considered modulated when this variation exceeds an absolute threshold of 20%. The y axis indicates the significance of statistical tests. This lipidomics study showed that the syntheses of 47 lipids are significantly modulated between normal and dry skin groups, revealing a modification of the lipid matrix composition in dry skin.

Then, the lipid matrix structure was investigated by Raman microspectroscopy. Figure 5 reveals a modulation of the conformation and the organization of lipids.

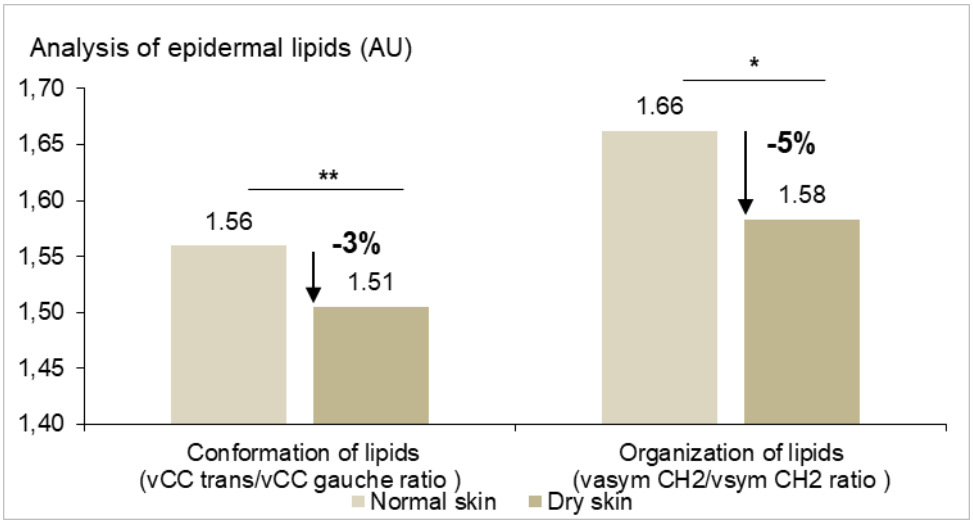

Figure 5. Raman microspectroscopy analysis of the conformation and organization of lipids between the normal skin and dry skin groups. *Result(s)/normal skin: **: P < 0.01 --- *: P < 0.05.*

2. Efficacy study of a natural active ingredient

The targeted lipidomic study, consisting in the quantification of lipids of interest selected beforehand, was thus conducted to determine the effect of *Centaurea cyanus* flower extract formulated at 1% in an emulsion and to compare its efficacy to that of the placebo. Figure 6 reveals that after 14 days of twice daily application to the calves and compared to the placebo formula, the active ingredient restores the synthesis of 33% of lipids whose synthesis was found to be reduced in dry skin. Indeed, Among the 36 lipids with a decreased synthesis in dry skin, this active ingredient restores the synthesis of 12 of them (7 ceramides and 5 free fatty acids).

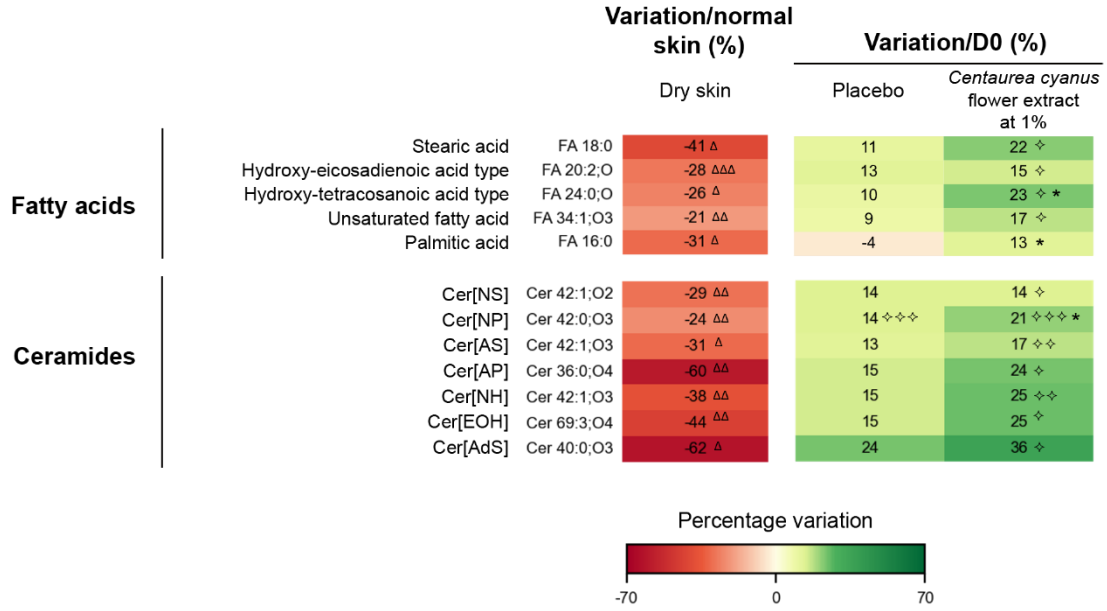

Figure 6. Effect of the *Centaurea cyanus* flower extract on the lipid profile of dry skin, compared to the placebo formula.
*Result(s)/normal skin: $^{\Delta\Delta\Delta}$: P < 0.001 --- $^{\Delta\Delta}$: P < 0.01 --- $^{\Delta}$: P < 0.05; Result(s)/D0: $^{\text{✧✧✧}}$: P < 0.001 --- $^{\text{✧✧}}$: P < 0.01 --- $^{\text{✧}}$: P < 0.05; Result(s)/placebo: *: P < 0.05.*

Figure 7 summarizes the results of the effect of the active ingredient formulated at 1% in an emulsion on the conformation and organization of lipids after 14 days of twice daily application.

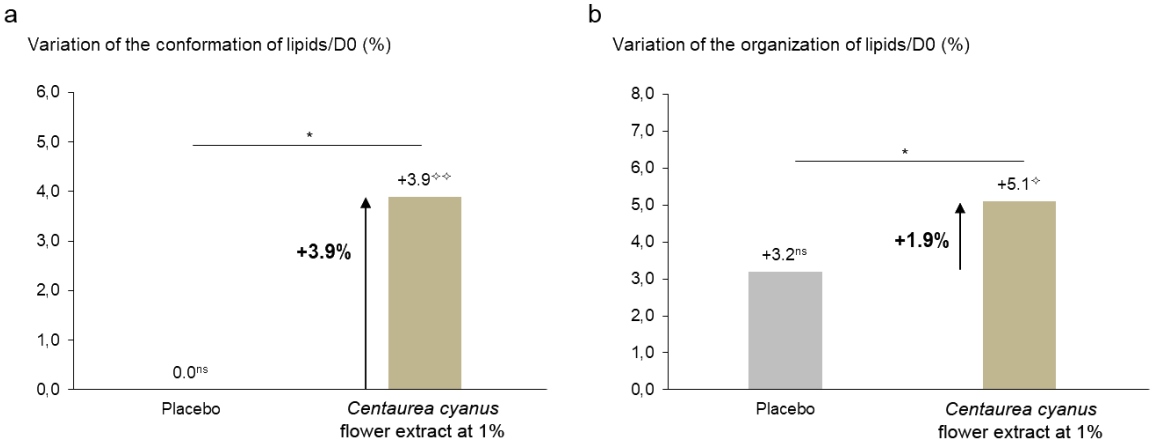

Figure 7. Effect of the *Centaurea cyanus* flower extract on the lipid conformation (a) or lipid organization (b), compared to the placebo formula. *Result(s)/D0: ✧✧: P < 0.01 --- ✧: P < 0.05 --- ns: non-significant; Result(s)/placebo: *: P < 0.05.*

Results obtained by Raman microspectroscopy reveal that after 14 days of twice daily application, the formulas containing the natural active ingredient significantly improve the conformation and organization of lipids. Hence, the *Centaurea cyanus* flower extract improves the lipid matrix structure of dry skin.

In addition, TEWL is significantly reduced by 19.3% after 14 days of daily use of this active ingredient (Figure 8a). Moreover, the water content is significantly improved as revealed by Raman microspectroscopy and Corneometer® measurements (Figures 8b and c, respectively).

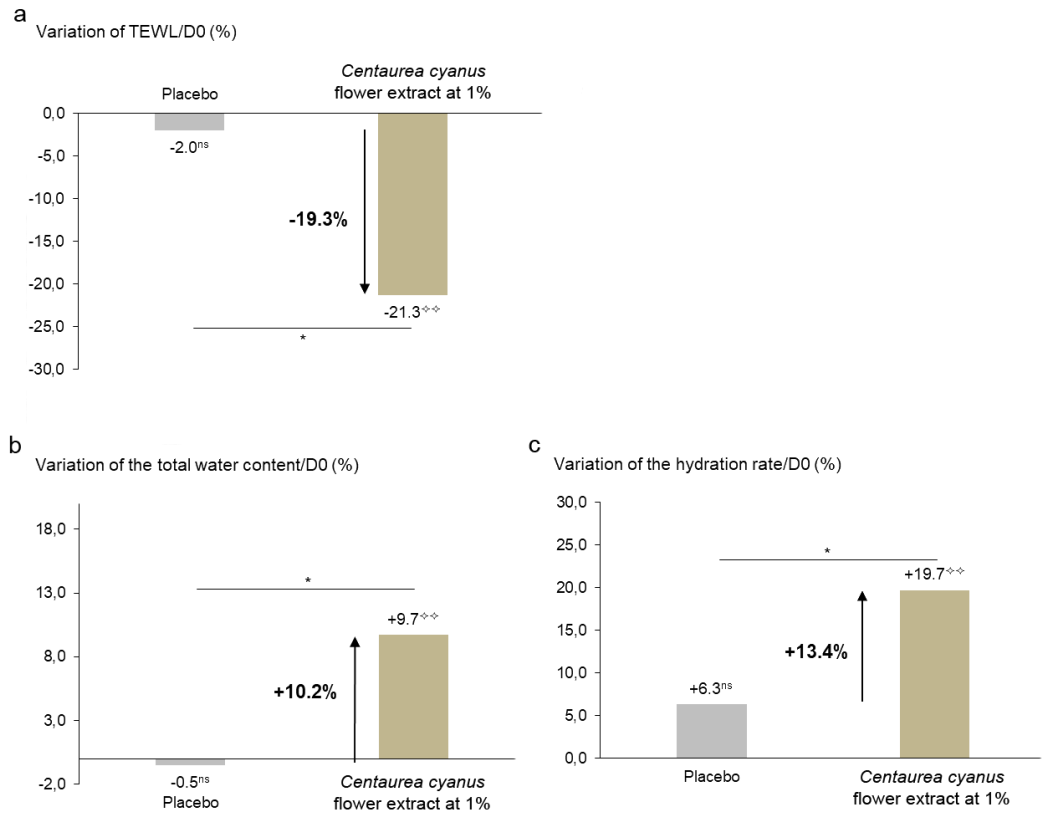

Figure 8. Effect of the *Centaurea cyanus* flower extract on the skin barrier (a) and on the skin hydration measured by Raman microspectroscopy (b) and corneometry (c).
*Result(s)/D0: ✧✧: P < 0.01 --- ns: non-significant; Result(s)/placebo: *: P < 0.05*

4. Discussion

This study, combining mass spectrometry-based lipidomics and Raman microspectroscopy highlights for the first time the modification of the SC lipid matrix composition and structure occurring in dry skin. The natural active ingredient, *Centaurea cyanus* flower extract, is able to target disorders of dry skin. At the molecular level, this natural active ingredient re-equilibrates the lipid matrix by restoring the synthesis of epidermal lipids and optimizing its structure. At the level of the skin, the barrier function is restored, providing hydrating and smoothing effects. The efficacy of this lipid restructuring agent was also demonstrated on Asian skin.

5. Conclusion

This study paves the way to the development of innovative cosmetic solutions dedicated to the care of dry skin. Its efficacy and kinetics of action were compared to those of niacinamide, revealing a niacinamide-like action of this *Centaurea cyanus* flower extract.

The restructuring benefits of this active ingredient were also shown in combination with retinol. In spite of its anti-aging benefits, this reference molecule can also cause adverse effects such as drying of the skin. When formulated with retinol, the *Centaurea cyanus* flower extract limits its adverse effects, without altering its anti-wrinkle action. As a natural partner of retinol, it also improves its radiance-boosting effect.

6. References

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[3] Fluhr JW, Moore DJ, Lane ME, Lachmann N, Rawlings AV. Epidermal barrier function in dry, flaky and sensitive skin: A narrative review. J Eur Acad Dermatol Venereol. May 2024 38(5): 812-820. doi: 10.1111/jdv.19745.