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The Effect of a Ceramide-Containing Product on Stratum Corneum Lipid Levels in Dry Legs

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ABSTRACT

Roughly equimolar concentrations of ceramides, cholesterol, and free fatty acids arranged in lamellar sheets form the intercellular lipid barrier in the stratum corneum (SC). Intercellular lipid deficiencies, specifically ceramides, and barrier disruption are associated with many dermatologic conditions, including dry skin. This study explored the relationship between the improvement in the signs of dry skin and the amounts of ceramides in the SC by combining clinical observations with a biochemical analysis to quantify the level of SC intercellular lipids. The efficacy of a multilamellar vesicular emulsion (MVE), ceramide-containing moisturizing cream was evaluated in a randomized, investigator-blinded, split-leg study on female subjects with dry, itchy skin. The cream increased skin hydration and demonstrated an immediate and sustained reduction in the visible signs of dry skin and subject perceived sensory discomfort. Additionally, ceramide, cholesterol and free fatty acid levels in the SC significantly increased after 4 weeks of moisturizer application. Thus, the clinical effect of the ceramide-containing moisturizing cream on dry, itchy skin was accompanied by an increase in SC intercellular lipid levels.

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INTRODUCTION

Ceramides have become important ingredients in moisturizers since they were first synthetically introduced 15 years ago. The addition of ceramides to moisturizer formulations was built on the concept that ceramide synthesis is the initiating event for barrier repair following damage.[1] Barrier disruption characterizes many dermatologic conditions, including psoriasis, acne, eczema, atopic dermatitis, and rosacea, where the intercellular lipids have been either removed or poorly formed. These intercellular lipids are composed of roughly equimolar concentrations of ceramides, cholesterol, and free fatty acids arranged in lamellar sheets accounting for the barrier property of the epidermis.[2]

Ceramides are a complex group of sphingolipids composed of sphingosine bases in amide linkages with fatty acids.[3] There are 9 major classes of free ceramides (Cer 1-9) and 2 major protein-bound ceramides covalently bonded to corneocyte protein envelopes (Cer A, Cer B).[4] However, the nomenclature for synthetically derived ceramides has been updated for proper ingredient disclosure. The new INCI nomenclature for Ceramide 1, 3, and 6-II will be Ceramide EOP, NP, and AP. The "P" indicates the ceramide contains phytosphingosine, while the EO, N and A distinguish the type of fatty acid. Phytosphingosine-containing ceramides are often called phytoceramides since they can be derived from plants.

It has been demonstrated in atopic dermatitis, there is a decrease in ceramides 1 and 3, which has been associated with an increased skin susceptibility to irritants and increased transepidermal water loss (TEWL).[5] These observations have been found in both lesional and nonlesional skin.[6] In addition, it has been demonstrated that treatment with ceramide 1 increases skin barrier resistance against sodium lauryl sulfate induced damage.[7]

Traditional moisturizers create an environment for barrier repair by decreasing TEWL through occlusive agents, such as petrolatum, dimethicone, mineral oil, and botanical oils, in combination with humectants attracting water to the skin, such as glycerin, propylene glycol, and sodium PCA. However, therapeutic moisturizers attempt to deliver more benefit by addressing additional physiologic needs with the addition of ceramides, cholesterol, and fatty acids. In addition, synthetic skin identical ceramides 1, 3, and 6-II can be topically applied to attempt to reduce the skin susceptibility to irritants.[8]

However, dermatologists have questioned whether topically applied skin identical ceramides can be incorporated into the stratum corneum or whether they reside only on the skin surface, providing minimal physiologic benefit. This research attempted to understand the relationship between improvement in the signs and symptoms of dry skin and the amounts of ceramides in the stratum corneum of the lower leg. This was accomplished by combining clinical observations with a biochemical analysis attempting to document the presence of ceramides within the stratum corneum.

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METHODS

Clinical Methods

This single site split body investigator blinded study enrolled 53 healthy women of Fitzpatrick skin types I-VI who signed informed consent (Concordia Institutional Review Board, Beach Haven, NJ). Twenty-six subjects aged 30-50 years were enrolled and twenty-seven subjects aged 51-65 years were enrolled with dry to very dry skin, skin texture roughness (visual/tactile), desquamation/flakiness, lack of radiance/dull skin, and erythema on the lower legs. Following the completion of informed consent and photography consent, the subjects completed an itch assessment on an ordinal scale. Twenty-nine subjects were enrolled with mild to moderate self-perceived itchy skin on both legs with a score of 2 to 3 on a 5-point ordinal scale (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe). Subjects were dispensed a diary and asked to follow their regular hair removal routine to include consistency in type of products (waxing, shaving, depilatory, etc.) used and the frequency of hair removal. Subjects recorded their hair removal activities in the diary. Subjects were dispensed a glycerin cleansing bar (Neutrogena®, Johnson & Johnson™, Skillman, NJ) for cleansing of the legs and body to replace their usual body soap and asked to return to the research center in 2 weeks. No other cleansers were allowed for the duration of the study.

Subjects were re-qualified after 2 weeks and asked to shower in the morning, apply no moisturizers, and present to the clinic between 3pm to 5pm. Subjects underwent dermatologist investigator clinical grading for skin dryness, skin texture/roughness (tactile), skin texture/roughness (visual), desquamation/flakiness, luminosity/radiance, erythema, and overall appearance of healthy skin for each leg separately. The investigator also assessed skin itching, stinging, and burning separately for each lower leg, defined as the subjective sensory assessment. All investigator assessments were on a 5-point ordinal scale (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe). A noninvasive instrumental assessment of corneometry was obtained for each lower leg using a template to locate the same site for sampling at each visit. A lower leg target site was identified on each leg for standard visible light photography with a Slue Nikon D90 camera. Finally, surface biomarkers were collected using the D-Squame® technique separately for each lower leg.

The D-Squame technique involved placing a transparent sticky tape with clean forceps holding the side tab onto the templated location and pressing with a constant pressure plunger onto the skin for 10 seconds. The side tab was again grasped with the clean forceps and placed into a specimen bottle for transport to the laboratory (Synelvia, Labège, France). The D-Squames were only handled by the side tab to avoid contamination. The first tape strip was discarded and the 2 subsequent tapes collected from the same location were submitted for analysis.

Subjects were dispensed the study product (Moisturizing Cream, CeraVe, New York, NY) and asked to apply it to one randomized lower leg, between the knee and ankle. Immediately after application dermatologist expert grading, subject self-assessment questionnaire, and subjective sensory assessment were performed along with target site photography separately for each lower leg.

Subjects were asked to apply the study product twice daily to the randomized leg from the knee down and to shower with the provided cleanser the morning of their return visit on day 3 between 3pm to 5pm. No study product or other products were applied to the legs following showering. At day 3, subjects underwent clinical expert grading, subject self-assessment questionnaire, subjective sensory assessment, and corneometry instrumental assessment separately for each lower leg. The lower leg target site on each leg was photographed and subjects returned to the research center for the same activities at week 4. Again, subjects were asked to shower with the provided cleanser the morning of their visit between 3pm to 5pm and apply no study product or other products to the legs. The target site selected on each leg underwent D-Squame collection of skin surface biomarkers with the first tape discarded and subsequent 2 tapes submitted for analysis.

Subjects discontinued study product use at this time and returned to the clinic at 48 hours for a regression analysis to determine the level of ceramides present in the skin after product use ceased. Dermatologist expert grading, D-Squame collection, subject self-assessment questionnaires, and subjective sensory assessment were performed along with target site photography separately for each lower leg. An instrumental assessment of corneometry was obtained separately for each lower leg.

A Mann-Whitney two-tailed t-test was used to analyze the nonparametric investigator and subject data. A t-test was used to analyze the numerical noninvasive data.

Lipid D-Squame Analytical Methods

Lipids were extracted from each D-Squame by a multi-step method using chloroform and methanol solvents to collect the neutral lipid, fatty acid and ceramide fractions. Cholesterol and free fatty acid levels in these extraction samples were analyzed using gas chromatography system coupled with mass spectrometry. Ceramide content was analyzed using a liquid chromatography-mass spectrometry method (LC-MS).

RESULTS

Fifty of fifty-three subjects successfully completed the study with 3 subjects discontinuing for scheduling conflicts. The study yielded four data sets: instrumental, investigator, subject, and D-Squame lipid content.

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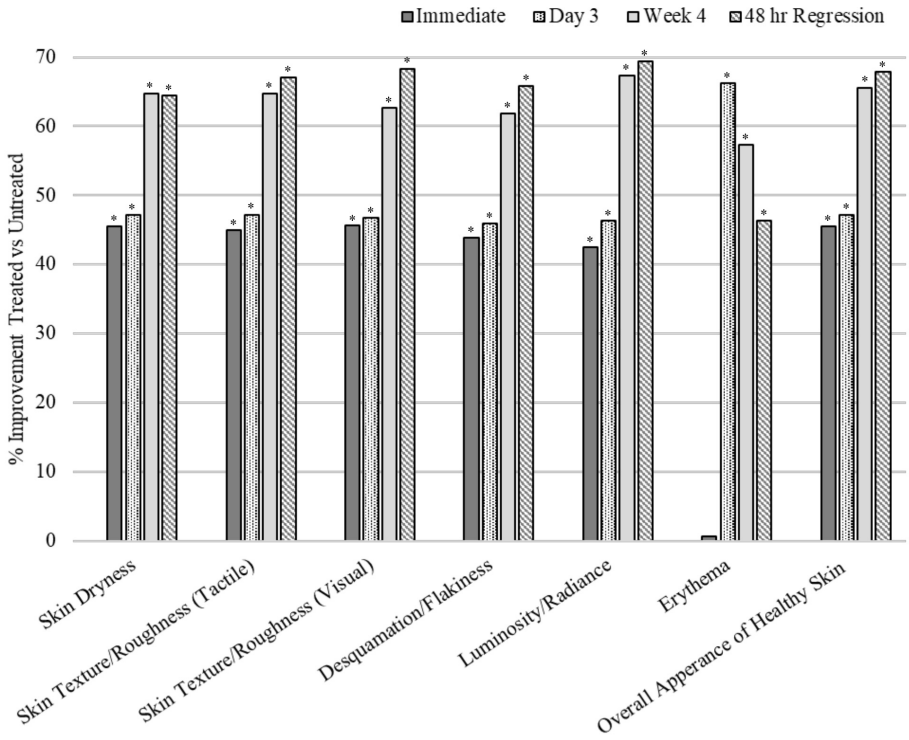
FIGURE 1. Mean percent (%) improvement in the investigator-assessed attributes comparing the treated leg to the untreated leg. Asterisk indicates *P*<0.001.



Instrumental Results

Corneometry was used to assess skin water content. The water content of the skin increased after 3 days of study product use with a statistically significant 30.6% increase in the treated leg as compared to the control leg (*P*<.001). This improvement continued into week 4 with a 38% water content increase in the treated leg as compared to the untreated leg (*P*<.001). The increased water content was even maintained into the 48-hour regression phase where the skin had no moisturizer applied with a statistically significant 21.85% increase in water content comparing the treated to untreated leg (*P*<.01).

Investigator Results

Immediately after application, the blinded investigator assessed

FIGURE 2. Mean percent (%) increase in the measured ceramide, cholesterol and free fatty acid content comparing the treated leg to the untreated leg. Asterisk indicates *P*<0.05.

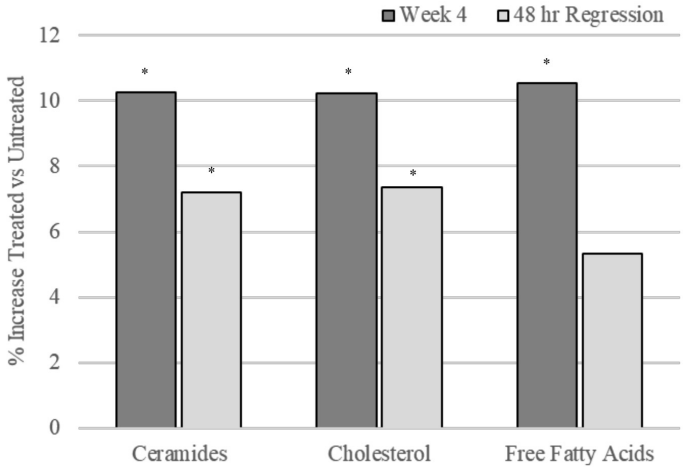


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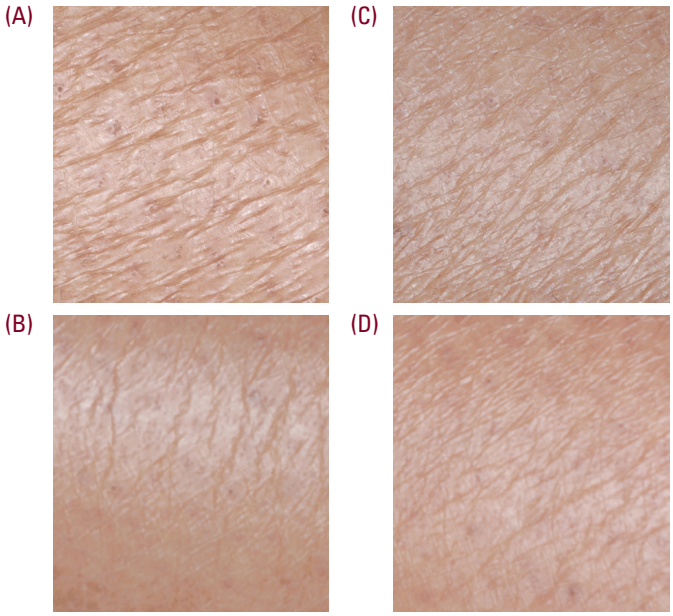
FIGURE 3. Photos of subject with average results for investor assessed dryness, visual skin texture/roughness, desquamation/flakiness and radiance at (A) baseline, (B) day 3, (C) week 4, and (D) 48-hour regression time points.



highly statistically significant improvement in skin dryness, skin texture/roughness (tactile), skin texture/roughness (visual), desquamation/flakiness, luminosity/radiance, and overall appearance of healthy skin comparing the treated leg to the untreated leg (P<.001; Figure 1). At the remaining time points, day 3 and week 4, highly statistically improvement was seen in all parameters assessed comparing the treated to the untreated leg (P<.001). This improvement was maintained during the 48-hour regression period in all parameters (P<.001). The investigator captured the improvement with photographs at each time point (Figure 2).

Subject Results

In the Subjective Sensory Assessment, the subjects were asked to assess itching, stinging, and burning at baseline, immediately following application, day 3, week 4, and 48-hour regression separately for each lower leg. Immediately following application, the subjects noted a statistically significant reduction in stinging (P=.008), burning (P=.001), and itching (P<.001) for the leg receiving the study product. This represented a 60% reduction in itching, 55% reduction in burning, and a 42% reduction in stinging. This improvement continued throughout the 4-week study and even into the 48-hour regression period.

D-Squame Lipid Results

After 4 weeks of moisturizer application, the D-Squame analysis revealed a 10% increase in total skin ceramide content comparing the treated to the untreated leg (Figure 3). In addition, there was a 10% increase in cholesterol levels and increase in free fatty acids levels. This reflects a significant increase (P<.01) in skin barrier lipid content at 4 weeks. This was maintained for the total skin ceramide content and cholesterol levels after discontinuation for 48 hours (P=.025).

DISCUSSION

Many ingredients are incorporated into moisturizer formulations for a variety of purposes. The studied moisturizer contained glycerin and hyaluronic acid to function as humectants to increase skin water content and dimethicone as an occlusive agent to maintain the increased water content in the skin. Emollients, such as caprylic/capric triglyceride, cetearyl alcohol, and ceteareth-20, were included to smooth rough skin created by improperly desquamating corneocytes. Most moisturizers contain ingredients to meet these basic formulation functions; however, the study moisturizer contained a variety of ingredients to bring additional skin barrier benefits. These included cholesterol, phytosphingosine, and ceramides 1, 3, and 6-II, which are all components of the intercellular barrier lipids. The phytosphingosine-containing ceramides utilized in the formulation are plant derived using a bio-fermentation process to produce skin-identical configurations. These ingredients are organized within a multivesicular emulsion (MVE) designed to time-release concentric spheres of oil and water into the skin. The MVE contains concentric spheres of moisturizing ingredients that are released onto the skin surface layer by layer to create the extended ingredient delivery mechanism.

Of the ingredients incorporated into the barrier lipids, ceramides are one of the most important in providing for the waterproof characteristics of the skin barrier. In order to prevent skin surface ceramide contamination, the top tape strip from all samplings was discarded and only the lower tape strips analyzed. This provided for documentation that the measured ceramides were actually present within the stratum corneum and not only onto the surface. The study moisturizer significantly increased skin barrier lipid levels after 4 weeks of regular use. This increase in total skin ceramide content was sustained even after moisturizer was discontinued for 48 hours.

Rather than running the study during the winter months with low ambient humidity, the study was run with daily rain from weeks 2 to 4, providing an opportunity to understand the ability of a moisturizer to increase skin water content despite high humidity conditions. The studied moisturizer increased skin hydration after only 3 days compared to the untreated leg. This improvement was maintained at week 4 and was still significant after the 48-hour regression phase. The increase in skin hydration was accompanied by a significant and sustained visual improvement in dry skin symptoms in the treated leg. Despite the high humidity and warmer temperatures during the study, the test product was very well perceived for both texture and

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efficacy by the study participants with >93% expressing overall satisfaction at all time points. These results and those from the stratum corneum lipid analysis are evidence for the benefits of using the studied ceramide-containing moisturizer for dry skin even in warmer, high-humidity conditions.

CONCLUSION

This 4-week, ceramide-containing moisturizer study was able to demonstrate an improved skin water content through corneometry, a reduction in subject perceived sensory discomfort, and dermatologist investigator assessed resolution of the signs of dry skin. Improvement continued for 48 hours after moisturizer withdrawal.

DISCLOSURES

The study was funded by the L'Oréal Research & Innovation Dpt. Dr. Zoe Draelos received funding from L'Oréal to conduct the research presented in this manuscript. Nada Baalbaki, Shelby Cook, Susana Raab are employees of L'Oréal Research and Innovation. Gene Colón is an employee of L'Oréal USA, Inc. (CeraVe).

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