

Tradename: AC ExoCalm

Code: 60192

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Test Request Form #: 11948

Lot #: 9400822

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

Study Director: Daniel Shill

Principal Investigator: Kayla Patterson

Test Performed:

Transepidermal Water Loss (TEWL) Study

Introduction

As the largest human organ, the skin's integrity is critical to properly function as a physical barrier and maintenance of a healthy appearance for aesthetics. Moisture retention is a fundamental component to the preservation of the skin's protective barrier function. Transepidermal water loss (TEWL) is the passive evaporation of water across the stratum corneum to the external environment because of the water vapor pressure gradient on both sides of the skin barrier. In healthy skin, TEWL is inversely proportional to skin hydration (i.e., decreased TEWL indicates properly hydrated skin). However, when the skin's protective barrier is compromised, TEWL levels are high and the skin feels dry, flaky, and rough. High TEWL levels, and reduced skin hydration, are correlated with skin aging and seen in many skin diseases. Consequently, moderating excessive TEWL improves the skin's protective barrier function and contributes to the appearance of healthier looking skin.

Accordingly, a Transepidermal Water Loss Study was conducted to evaluate the immediate and short-term moisture retention properties of **AC ExoCalm**. The key active ingredient in **AC ExoCalm**, *Glycyrrhiza glabra* (Licorice) Root Extract, was tested to demonstrate the superior nature of Bioauthentic Exosomes as a delivery system.

Study Principle

TEWL measurements are made by placing a probe on the skin of preidentified test sites. By assessing changes in local humidity above ambient values the TEWL probe measures changes in water vapor density in a defined area over time. The controls and test materials are applied to the skin test sites once and TEWL is measured at four-time increments within a 24-hour period.

Materials

- A. **Equipment:** DermaLab Skin Combo (Transepidermal Water Loss Probe)
- B. **Products:** Base Lotion (Cetaphil® Moisturizing Cream for All Skin Types)
- C. **Software:** Excel Analysis ToolPak (Microsoft)

Methods

20 volunteers between the ages of 23 and 54, who were known to be free of any skin pathologies with Fitzpatrick skin types I to IV, participated in this study (Table 1).

Table 1. The Fitzpatrick Classification of Skin Types Chart¹

Fitzpatrick Skin Type Descriptions*	
Skin Type	Description
I	Always burns, never tans
II	Burns easily, tans minimally
III	Burns moderately, tans to light brown
IV	Burns minimally, tans to moderate brown
V	Rarely burns, tans to dark
VI	Never burns, least sensitive to changes

*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer

Four randomly assigned test sites were identified on the volar forearm of participants and baseline TEWL measurements were recorded. Following baseline measurements, participants applied 0.2 g of each test material on their volar forearms once during the 24-hour study. A dry down phase was not incorporated into the study design, prior to baseline measurements, to resemble a real-world consumer application experience. TEWL measurements were recorded at four time increments within a 24-hour period. The skin test site conditions and treatments are described below (Table 2). The Base Lotion utilized in this study was Cetaphil® Moisturizing Cream for All Skin Types.

Table 2. Descriptions of the Conditions and Treatments for each Skin Test Site

Skin Test Site	Condition	Treatment / Test Article Application Description
1	Untreated Control	None
2	Base Lotion	Base Lotion
3	0.4% <i>Glycyrrhiza glabra</i> (Licorice) Root Extract	0.4% <i>Glycyrrhiza glabra</i> (Licorice) Root Extract in Base Lotion [#]
4	2.0% AC ExoCalm	2.0% AC ExoCalm in Base Lotion

[#]This concentration of *Glycyrrhiza glabra* (Licorice) Root Extract is equivalent to the amount present in 2.0% AC ExoCalm

An average of three consecutive TEWL measurements per condition at each time point was recorded and expressed as g/m²/h for each volunteer. Data are displayed as averages from all volunteers and analyzed using t-tests with statistical significance accepted at p ≤ 0.05. The percent change in TEWL values was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

$$\text{Percent Change (\%)} = \frac{TEWL_{\text{Measurement Time}} - TEWL_{\text{Baseline}}}{TEWL_{\text{Baseline}}} \times 100$$

Results

The data obtained met criteria for a valid study as the Untreated Control and Base Lotion performed as anticipated. Application of 2.0% AC ExoCalm once during a 24-hour period demonstrated effective immediate and short-term moisture retention properties by reducing TEWL throughout the study duration.

Transepidermal Water Loss AC ExoCalm

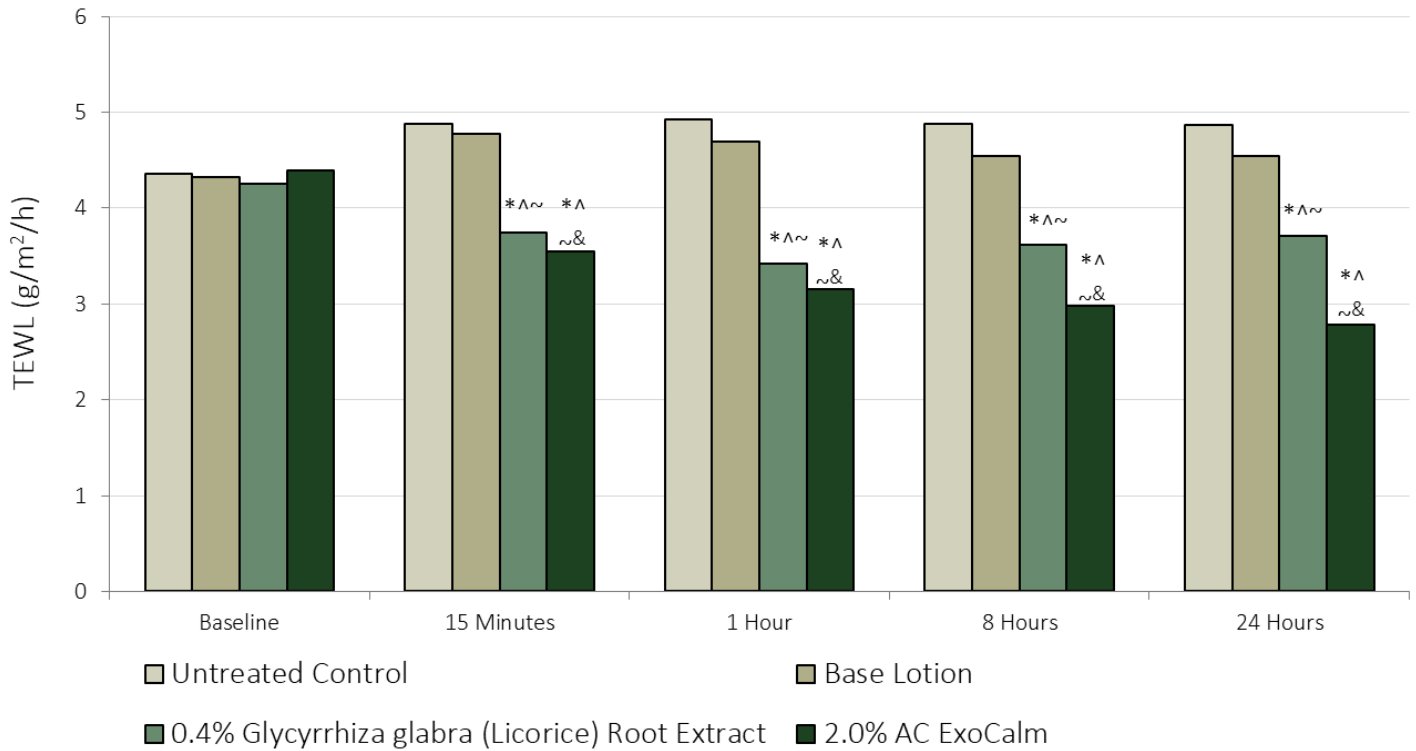


Figure 1. TEWL Measurements Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values. ^ indicates significance ($p \leq 0.05$) compared to Untreated Control within the same timepoint. ~ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint. & indicates significance ($p \leq 0.05$) compared to *Glycyrrhiza glabra* (Licorice) Root Extract within the same timepoint.

Table 3. P-values from t-test Analyses of TEWL Values from Baseline to 8 Hours and 24 Hours After Application. * indicates significance ($p \leq 0.05$) compared to Baseline values.

	Untreated Control	Base Lotion	0.4% <i>Glycyrrhiza glabra</i> (Licorice) Root Extract	2.0% AC ExoCalm
8 Hours After Application	0.133	0.588	0.049*	< 0.001*
24 Hours After Application	0.163	0.591	0.045*	< 0.001*

Table 4. T-test Analysis of TEWL Values Eight Hours After Application. ^ indicates significance ($p \leq 0.05$) compared to Untreated Control. ~ indicates significance ($p \leq 0.05$) compared to Base Lotion. & indicates significance ($p \leq 0.05$) compared to *Glycyrrhiza glabra* (Licorice) Root Extract

	Untreated Control	Base Lotion	0.4% <i>Glycyrrhiza glabra</i> (Licorice) Root Extract	2.0% AC ExoCalm
Untreated Control	----	0.366	< 0.001 [^]	< 0.001 [^]
Base Lotion	0.366	----	0.016 [~]	< 0.001 [~]
0.4% <i>Glycyrrhiza glabra</i> (Licorice) Root Extract	< 0.001 [^]	0.016 [~]	----	0.001 ^{&}
2.0% AC ExoCalm	< 0.001 [^]	< 0.001 [~]	0.001 ^{&}	----

Change in Transepidermal Water Loss AC ExoCalm

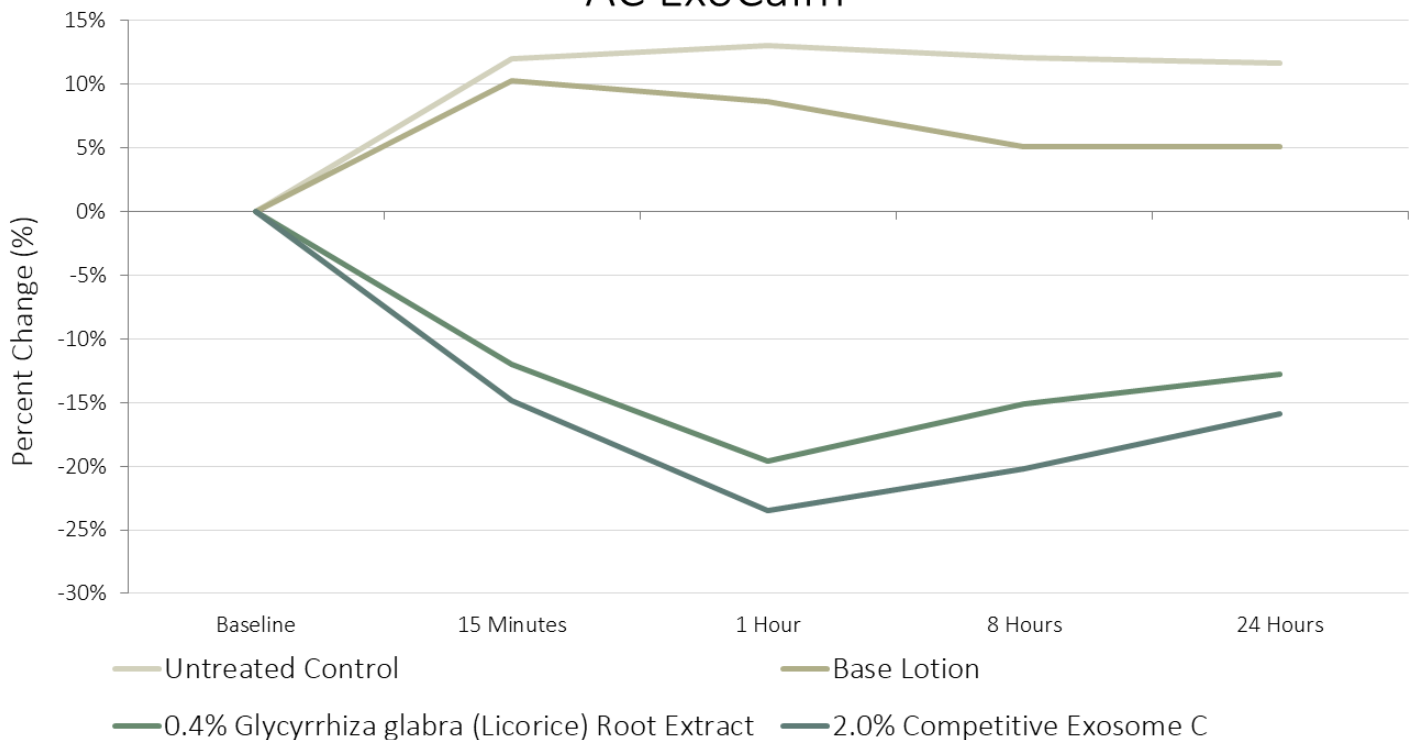


Figure 2. Percent Change in Transepidermal Water Loss Relative to Baseline Values

Discussion

The ability of **AC ExoCalm** to retain skin moisture was assessed via TEWL throughout 24 hours with one initial application. As shown in Figure 1 and 2, TEWL did not significantly change throughout the study with the Untreated Control test site, indicating consistent skin moisturization over 24 hours (Table 3). Similarly, TEWL was not significantly altered throughout the study with Base Lotion application, indicating the Base Lotion does not exert significant moisture retention on the skin (Figures 1, 2; Table 3). Conversely, 0.4% *Glycyrrhiza glabra* (Licorice) Root Extract significantly reduced TEWL compared to baseline, by 13% eight hours after application (Figures 1, 2; Table 3). However, applying 2.0% **AC ExoCalm** once in a 24-hour period significantly reduced TEWL by 36% eight hours after application, and remained suppressed 24 hours after application (Figures 1, 2; Table 3). These results demonstrate **AC ExoCalm** has effective immediate and short-term moisture retention properties and outperforms the active ingredient, *Glycyrrhiza glabra* (Licorice) Root Extract, alone.

Similar results are shown when examining the collective effect between each condition. There is no difference in TEWL between the Untreated Control and Base Lotion eight hours after application (Figure 1; Table 4). However, applying 2.0% **AC ExoCalm** significantly reduced TEWL compared to the Untreated Control, Base Lotion, and 0.4% *Glycyrrhiza glabra* (Licorice) Root Extract (Figure 1; Table 4). These results demonstrate **AC ExoCalm** elicits acute moisture retention in the skin with just one application.

Taken together, these results indicate **AC ExoCalm** reduces TEWL immediately when added to personal care applications at recommended use levels. Importantly, the absence of a dry down phase prior to baseline measurements emphasizes these results and is more reflective of a real-world consumer application experience. Collectively, **AC ExoCalm** demonstrates immediate and short-term moisture retention properties which improves the skin's protective barrier function and contributes to the appearance of healthier looking skin.

References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557626/>