Correlation of *In Vitro* Gene Expression Analysis with *In Vivo* Efficacy

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Abstract

The objective of this research was to conduct *in vitro* gene expression testing of hydrolyzed jojoba esters and correlate these results with randomized, double-blind, vehicle-controlled *in vivo* efficacy studies. Gene expression testing of 1% hydrolyzed jojoba esters in glycerin produced the following statistically significant gene expression changes over the vehicle: up regulation in AQP3, AQP5, KLK5, KLK6, KLK7, TXN, TXNRD, and CAT; and down regulation in TNF, MKI67, and EDN1.[1] Current literature shows associations between AQP3 and APQ5 and skin hydration, as well as associations between TNF and an inflammatory response. Three small IRB approved studies were carried out to obtain efficacy data. Study 1: An oil in water emulsion containing 1% hydrolyzed jojoba esters produced statistically significant ($p<0.05$) increases in skin hydration compared to the vehicle, one and two hours post application (43% and 67%, respectively, n=17). Study 2: A water-based toner containing 0.2% hydrolyzed jojoba esters produced statistically significant ($p<0.05$) increases in skin hydration compared to the vehicle toner, one and two hours post application (22% and 16%, respectively, n=15). Study 3: Inflammation was studied *in vivo* by evaluating erythema and barrier function of insulted (*i.e.* dry shaved) skin. The addition of 0.2% hydrolyzed jojoba esters to a baby wipe formulation produced statistically significant decreases in erythema ($p<0.05$) and increases in barrier function ($p<0.05$) as compared to the vehicle baby wipe (n=14). These studies demonstrate the correlation between *in vitro* gene expression data and *in vivo* efficacy. Additional *in vivo* studies will be performed to evaluate desquammation, antioxidant responses, proliferation, and pigmentation as indicated by the remaining genes that were impacted by hydrolyzed jojoba esters.

Introduction / Background

Hydrolyzed jojoba esters are multifunctional ingredients that have been utilized and/or tested in a variety of cosmetic and personal care formulations such as creams/lotions, hand sanitizers, nonwoven wipes, sunscreens, sunless tanners, shampoos / conditioners, toners / astringents, face washes, face (sheet) masks, and oil-free formulations. Its film-forming properties make it ideal for rinse-off products and products that require water resistance or to extend the period of residence time on the skin.

Hydrolyzed jojoba esters are made by the hydrolysis of jojoba oil, and are available in two forms: high concentration (HC HJE) [INCI: Hydrolyzed Jojoba Esters (and) Jojoba Esters (and) Water (Aqua)] and low concentration (LC HJE) [INCI: Hydrolyzed Jojoba Esters (and) Water (Aqua)].

Skin Hydration

Objective: *In vivo* evaluation of LC HJE in both a sunscreen, and a clear, alcohol-free, and PEG-free toner, for its potential to increase skin hydration.

Method: Sunscreens and toners with and without LC HJE were applied to dry skin on the lower legs of the subjects. Skin hydration measurements (using the Corneometer[2]) were taken at baseline, and one and two hours post-test article application.

Results: **Figure 1** shows the test article containing 5% LC HJE increased skin hydration up to 67% compared to the vehicle sunscreen without LC HJE. **Figure 2** shows LC HJE added to clear, alcohol-free, and PEG-free toners, in conjunction with either glycerin or butylene glycol, increased skin hydration at least 100% as compared to each test article without LC HJE.

Figure 1: Skin Hydration (Sunscreen)

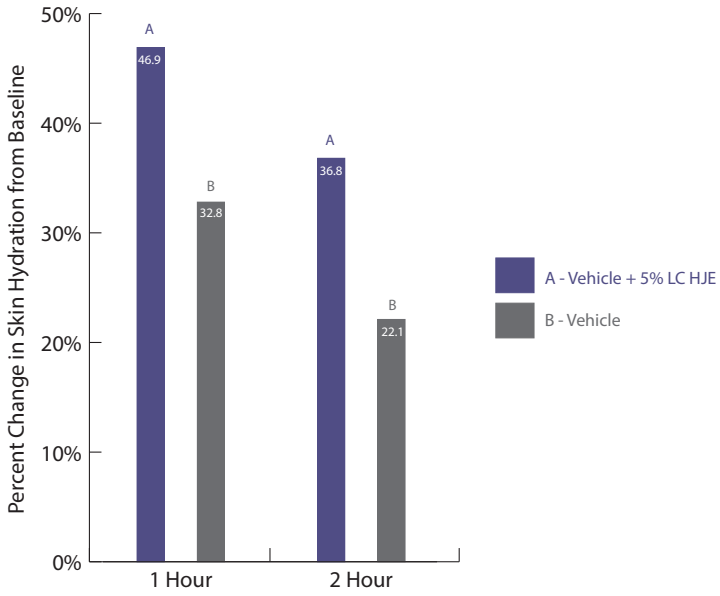
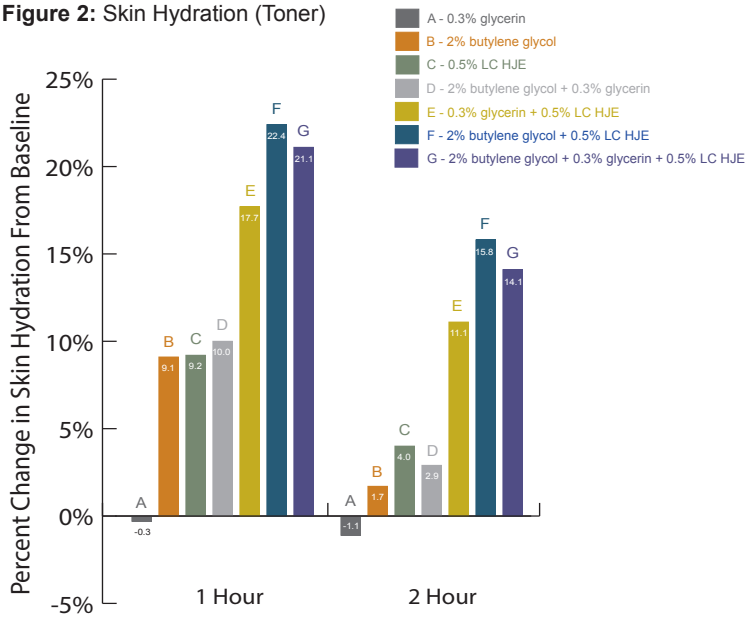


Figure 2: Skin Hydration (Toner)



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Erythema Reduction and Barrier Recovery

Objective: *In vivo* evaluation of HC HJE and LC HJE in a baby wipe solution to decrease erythema and restore barrier function in insulted (*i.e.* dry shaved) skin.

Method: Forearms were dry shaved. Baby wipes with and without 1% LC HJE or 0.2% HC HJE were then applied to the forearms. Skin erythema measurements (using the Mexameter[2]) were taken at baseline, and four hours and twenty-four hours post-test article application. TEWL (transepidermal water loss) measurements (using the Tewameter[2]) were taken at baseline, thirty minutes post-shave, and twenty-four hours post-test article application. An additional baby wipe application was made following the four hour measurement.

Results: Peak erythema measurements were obtained at 4 hours. **Figure 3** shows the decrease in erythema from the 4 hour measurement to the 24 hour measurement. LC HJE, HC HJE, and bisabolol produced statistically significant ($p<0.05$) decreases in erythema over the vehicle. **Figure 4** shows that HC HJE and LC HJE produced statistically significantly ($p<0.05$) more effective barrier recovery than the vehicle, and LC HJE produced statistically significantly ($p<0.05$) more effective barrier recovery than bisabolol.

Figure 3: Erythema Reduction

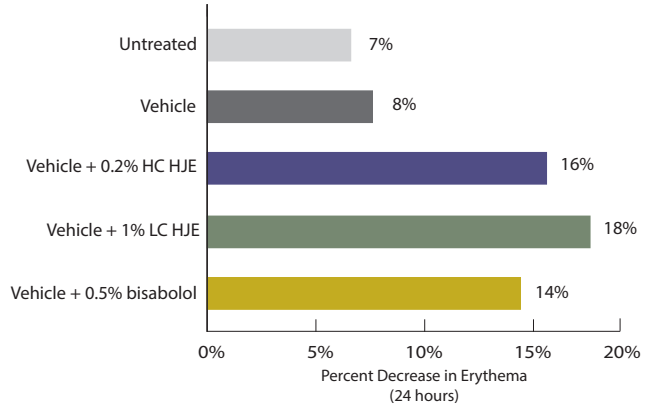
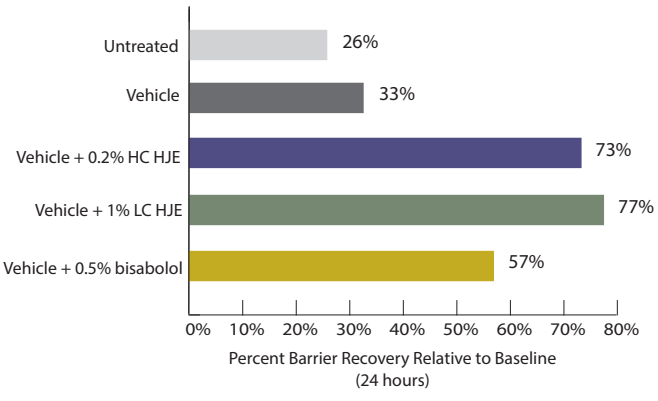


Figure 4: Barrier Recovery



Gene Expression

Objective: To determine the effect of LC HJE on gene expression *in vitro* using full thickness skin.

Method: Gene-expression testing of hydrolyzed jojoba esters (LC HJE)[3] was conducted by Genemarkers, LLC (Kalamazoo, MI) using quantitative PCR to measure changes in gene expression using the MatTek full thickness skin system (EpiDerm FT).

Results: The data indicates statistically significant change over the vehicle in gene expression for genes[1] related to the following biological functions.

- Up regulation of **AQP3** and **AQP5** suggests an increase in **skin hydration**.
- Down regulation of **TNF** suggests a reduction in the **inflammatory response**.
- Up regulation of **KLK5**, **KLK6**, and **KLK7** suggests an increase in stratum corneum shedding and keratinocyte turnover (**desquamation**).
- Up regulation of **TXN**, **TXNRD1**, and **CAT** suggests an increase in **antioxidant response**.
- Down regulation of **MKI67** is in agreement with anti-aging literature (**proliferation**).
- Down regulation of **EDN1** suggests a reduction in **pigmentation / brightening effect**.

Conclusions

- Hydrolyzed jojoba esters increased skin hydration *in vivo*, and produced up regulation of biomarkers associated with skin hydration (AQP3 and AQP5) *in vitro*.
- Hydrolyzed jojoba esters reduced erythema and increased barrier recovery *in vivo*, and produced down regulation of biomarkers associated with an inflammatory response (TNF) *in vitro*.

References

1. AQP (aquaporin), TNF (Tumor Necrosis Factor), KLK (kallikrein), TXN (Thioredoxin), TXNRD (Thioredoxin Reductase), CAT (Catalase), MKI (Marker of proliferation Ki-67), amd END (Endothelin 1).
2. Corneometer® CM 825, Tewameter TM 300, and Mexameter MX 18 are products of Courage + Khazaka Electronic GmbH, (Köln, Germany).
3. Study was conducted on a 1% hydrolyzed jojoba esters (*i.e.* 5% LC HJE solution in glycerin. Final Report available upon request.

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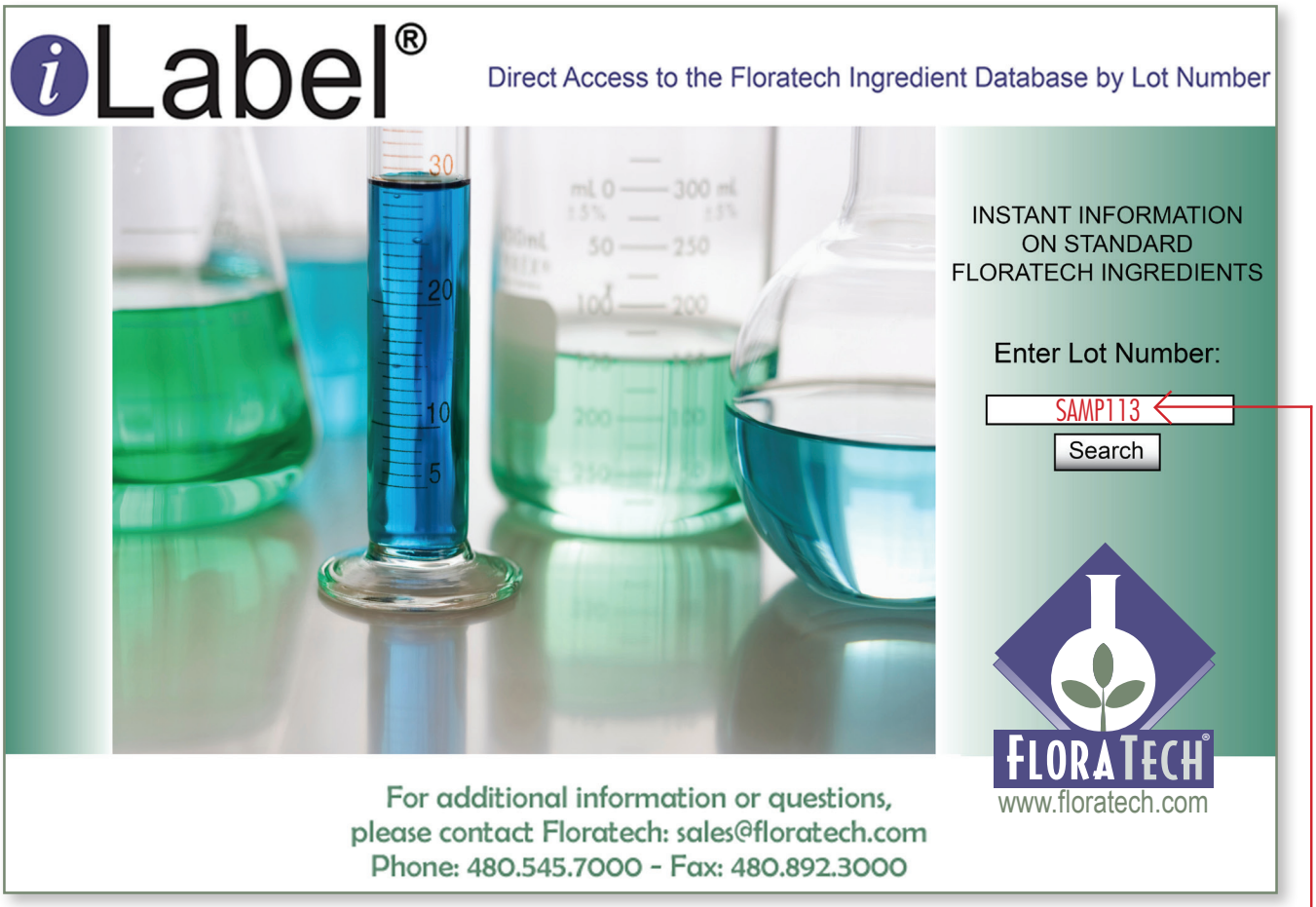

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