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An *In vivo* Comparative Study on Ageing Skin: a Bio-Mimetic versus a Traditional Approach to Skin Moisturisation

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**Abstract**

Skin dryness is a common condition in elderly individuals. The two main mechanisms to cosmetically alleviate this state rely on the effects of humectants and occlusive substances, mostly in combination. A relatively recent approach for skin moisturisation is the biomimetic mechanism, i.e. using active ingredients with lamellar structure containing skin-identical lipids. The aim of this study was to determine whether a bio-mimetic cream can deliver superior moisturisation to the skin of human volunteers aged over 60, compared to the effects of a conventional moisturiser containing high levels of petrolatum and mineral oil.

The study design included a 4-hour skin hydration trial, a mini-regression study and a self-evaluation study. The instrumental methods used were skin hydration measured by corneometer and skin pH evaluation. The results have shown that providing the elderly skin with lipids that make up the natural skin barrier had a significantly higher effect on the skin hydration levels than the treatment with a commercial product containing standard occlusive agents.

**1. Introduction**

The stratum corneum’s functional status depends on being in a plasticised state (Menon and Norlen, 2002) which relies on having adequate water-holding and waterproofing abilities. These abilities depend on the state of the skin barrier, crucial to human survival. Daily life presents a number of challenges for the protective layer of the body, including the use of simple cleansers (Polefka, 1999). Other factors contributing to the decline of the skin barrier functions include UV damage, environmental conditions, ageing and skin diseases. Out of these, the effects of the ageing process and environmental aspects on the skin condition will be the main interest of this study, conducted over the winter months in a Nordic country and employing elderly female volunteers.

Many of the skin functions decline with age such as cell regeneration, injury response, barrier function, sweat and sebum production (Gilchrest and Krutmann, 2006). Dry and flaky skin is a common condition in elderly people due to reduced water binding capacity of the stratum corneum and a decline in epidermal regeneration (Proksch, 2006). The process of renewing the epidermal layer, which would typically take about 28 days in young adult skin, may increase to 40 to 60 days with age (Kligman, 1979). A significant correlation has also been found between the hydration state of the stratum corneum and its amino acid content in
elderly individuals with dry skin (Horii et al., 1989). Aged skin is characterised by a decline in the water barrier repair which can lead to a loss of the water-soluble NMF compounds from the surface layers (Zettersten et al., 1997).

According to Luebberding et al. (2013), only some aspects of the skin barrier function change with ageing. Sebum production has been confirmed to decrease significantly with age, but stratum corneum hydration has either remained unchanged (cheek, hand) or increased (forehead, neck, forearm). This information corresponds with some previous studies in the area (Agarwal et al., 2007; Wilhelm, Cua and Maibach, 1991, but is in conflict with others (Shlivko et al., 2013; Thune et al., 1988) and the human perception that aged skin has an increased need for moisturisation (Proksch, 2006).

Even though dry skin has been considered to have a genetic predisposition, people with different skin types could experience it at given moments of their life due to various factors such as climatic conditions (Couturaud, 2009). In studies conducted in the summer and winter months in the UK, Rogers et al. (1996) demonstrated that there was a significant reduction in the levels of stratum corneum ceramides and fatty acids in subjects during the winter. This is why the weather has a key role in dry skin evaluation (Nole, 2002).

The skin mechanisms to retain moisture are related to the activity of NMF, the lamellar lipid bilayers and the maturation of the corneodesmosome-bound corneocytes (Rawlings and Matts, 2005). Moisturising products arose from a consumer need to treat and prevent dry skin conditions. The mass market for moisturisers has started over 150 years ago with products such as Vaseline Petroleum Jelly and Pond’s Cold Cream (Johnson, 2002).

The majority of products are still designed around the two traditional cosmetic methods for skin moisturisation, i.e. employing the technology of humectants or occlusive agents (Chavan et al., 2012). An impaired skin barrier would cause the loss of water-soluble natural moisturising factor (NMF), so the humectancy mechanism relies on the application of hygroscopic ingredients which would act in a similar way to NMF. In fact, some humectants used in moisturising formulations, such as lactic acid and urea, are components of the skin NMF (Johnson, 2002). On the other hand, a deposition of oily materials to the skin surface creates an artificial barrier that restricts evaporation of water from the skin, i.e. they act as occlusive materials (Johnson, 2002).

Nowadays, moisturising products are aiming to go beyond the mechanisms of humectancy and occlusion in order to treat the underlying causes of skin dryness (Nole, 2002), e.g. by delivering skin lipids, NMF, lipid precursors, peptides, amino acids. The ‘bio-mimetic’ approach involves application of multi-lamellar lipid structures similar to the skin surface lipids which would promote the reconstruction of the lamellar structures of the stratum corneum and the restoration of the skin barrier function (Jung et al., 2007). As defined by the Centre for Biomimetics at the University of Reading (2007), biomimicry is the abstraction of good design from nature and its use as inspiration for research.

Using infrared spectroscopy methods, Prasch et al. (2000) have studied the mechanisms of strengthening the skin lipid film by means of creams. They have compared a conventional
water-in-oil (w/o) emulsion versus a lamellar cream containing lipids with a structure that resembles the lipid film in the stratum corneum. The lamellar cream was shown to increase the degree of order of the alkyl chains in the SC in a biomimetic manner, while a standard w/o emulsion has caused a reduction in the degree of order. This is also confirmed by the changes in skin moisture, where 6 hours after application of the two products, the lamellar cream has still shown a significant increase. Other studies on the topic have suggested that while mineral oil causes an immediate partial restoration of the skin barrier properties of the stratum corneum, physiological lipid mixtures can have a biological effect on the metabolism of the living epidermis. They slowly penetrate into the epidermis and rebuild the barrier in a natural way by accelerated metabolism in the lamellar bodies (Mao-Qiang et al., 1995) and play a part in the recovery of squamous skin following surfactant damage (Imokava et al., 1989). A more recent study by Pennick et al. (2012) has also compared the effects of a lamellar cream versus a mineral oil-containing vehicle in a regression trial employing expert visual grading. The results showed that the lamellar cream has improved the visible signs of skin dryness to a significantly greater extent and maintained a better skin condition during the regression phase.

The aim of this project was to compare two different approaches to skin moisturisation of women over 60 - the ‘traditional’ occlusive technique and the biomimetic method. The data could give an insight into the best way to approach dry skin re-hydration in older people.

2. Materials and Methods

2.1 Test Products

An essential factor for the selection of skin identical lipids for a moisturisation study is their composition. Studies by Thornfeldt (2000) and Mao-Qiang et al. (1995) have shown the importance of lipid ratios and how topical applications of individual lipids or incomplete mixtures of lipids could interfere with barrier recovery instead of promoting it. Two commercially available bio-mimetic active ingredients containing approximately 2.5% active content of skin-identical lipids were selected for the formulation of test products. Active I is a ready-to-use emulsified lamellar system and Active II is a concentrated lipid paste. Both of the blends claim to have a composition similar to the skin surface lipids.

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<tr>
<th>Skin Lipids</th>
<th>Active I</th>
<th>Active II</th>
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<tr>
<td>Triglycerides</td>
<td>Caprylic/ Capric Triglyceride</td>
<td>Macadamia Triglyceride</td>
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<tr>
<td>Fatty Acids</td>
<td>Palmitic Acid</td>
<td>Vegetable Fatty Acids</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Phytosterols</td>
<td>Soja Bean Sterols</td>
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<tr>
<td>Ceramides</td>
<td>Ceramide 3</td>
<td>Safflower Ceramides</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Phospholipids</td>
<td>Soja Bean Phospholipids</td>
</tr>
<tr>
<td>Squalane</td>
<td>Squalane</td>
<td>Olive Squalane</td>
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Table 1. Comparison of the components of the active Ingredients and the skin lipids
Both actives claim to work by forming a protective barrier on the skin and restoring the missing lipids, thus providing care to dry and sensitive skin. They were formulated into an emulsion vehicle containing glycerine, carbomer, caprylic/capric triglyceride, preserved water, sodium hydroxide, disodium EDTA andtocopheryl acetate, stabilised with an alkyl polyglucoside emulsifier system.

2.2 Study Design and Considerations

This *in vivo* study was carried out in two stages: 1) a 4-hour moisturisation study on the inner lower legs and 2) a 3-week regression study on the inner lower legs and the back of hands, in combination with self-assessment at baseline and 3 weeks. The testing equipment consisted of Corneometer CM825 (Courage + Khazaka electronic GmbH, Germany) for the first stage and a Corneometer CM825 and Skin-pH-Meter PH905 (Courage + Khazaka electronic GmbH, Germany) for the second stage.

In the first stage of the study, the performance of the two bio-mimetic prototypes was evaluated against a no-application site and a commercial product containing petrolatum and mineral oil as moisturising ingredients. Sixteen volunteers from the target age group were recruited for a 4-hour blind skin hydration trial on the lower legs, using randomised intra-individual left-right comparison. The study was conducted after a one-week wash-out phase of no moisturiser application on the test sites. The better performing bio-mimetic prototype was chosen for the testing against the commercial product in the second stage of the study.

A long-term study was designed as a mini-regression test with 2 weeks (Monday to Friday) product application twice daily and a one-week regression phase. This included application of a bio-mimetic prototype on one lower leg (shin) and the back of hand (dorsal part), and a commercial product on the other lower leg and hand. A panel of 20 participants aged over 60 were provided with colour-coded test products to use at home, usage instructions and a basic hand and body wash in order to standardise their cleansing routines. They were required to come for the corneometer and skin pH measurements at baseline, after one and two weeks of product application, and at the end of the one-week regression stage.

In order to evaluate whether the results observed in the instrumental studies correspond to the consumers’ impression of the two products, the volunteers were given a self-assessment questionnaire to complete. They were instructed to observe any changes in their skin condition on the test sites during the study. The questionnaire was designed to be very simple and easily understood by the volunteers but also to give information of their moisturising habits, the performance of the test products regarding skin moisturisation and softness, and their preference between the two.

The data obtained from the two trials were evaluated using the “R” software package for statistical computing and graphics, using the median of three consecutive measurements. The results were checked for normality and the methods used consisted of 2-way analysis of variance (ANOVA), Student’s t-test and binomial tests.
3. Results and Discussion

3.1 Short-term Study

The aim of this trial was to evaluate and compare the 4-hour effects of the 3 test products and select a biomimetic prototype for the next stage.

Firstly, a one-way ANOVA showed that there was no significant difference in the baseline measurements of the test sites, even though the skin on the lower legs was shown to be quite patchy and with variations in the measurements. Therefore, a two-way ANOVA test with replications was employed on all the hydration measurements for the short-term study to compare the different test products and check how the two factor variables Treatment (i.e. test product) and Time (i.e. after application) match up with the response variable Hydration. The resulting p-values show a very significant difference (p = 1.274e-07) between the treatments with different test products and a significant difference (p = 0.00861) between the levels of hydration at different time points. This is visually demonstrated on Figure 1, showing the results of Tukey post-hoc test, which revealed where exactly the significant changes in hydration were.

![Figure 1](image)

**Figure 1.** Tukey HSD test results for statistical significance between different test products and the different time points; A- Active I; B- Active II; C- Commercial product; D- No application; H0- Baseline; H1- 1 hour after application; H2- 2 hours; H3- 3 hours; H4- 4 hours

Figure 1 shows that the two prototypes have performed equally well and as the B-A segment goes right across the vertical 95% confidence line, there is no significant difference between the effects they have delivered. Both prototypes have delivered significantly higher results compared to the benchmark and the no-application control. The confidence levels for prototype A (the C-A and the D-A interactions) are slightly higher than the ones delivered by
prototype B (C-B and D-B), which indicates (but does not prove) a better performance. What is interesting, however, is that no significant change was found in the effects provided by the commercial lotion compared to the no-application control. This could be explained by the fact that the occlusive mechanism relying on materials such as petrolatum provides better effects over time and with repeated applications (Nole, 2002). On the other hand, the changes in skin hydration delivered by the test products have been found to be significant up to 2 hours after application compared to the baseline values. No other significant differences were found regarding the time after application.

A representation of the variations of skin hydration responses for the three different treatments and a no-treatment control over the course of the study is presented in Figure 2.

![Figure 2. Changes in the SC hydration during the 4-hour study for three test products and a no-treatment control (n=16).](image)

Figure 2 shows that the skin hydration values for all test products peak at 1 hour after application. There is an identical pattern observed for the two biomimetic prototypes and the results for both of them are much higher compared to the occlusive commercial product. The small drop in the no-application control readings at 3 hours after application was analysed using two-sample t-test for hours 2-3 and 3-4 to see if there are any significant variations which could mean a variation in the test conditions. The tests showed no statistical significance.

As the results of this trial suggest, both Active I and Active II prototypes showed superior performance to the commercial product. These findings are in accordance with the above mentioned 6-hours study by Prasch et al. (2000). This test, however, is not conclusive and it is clear that further investigation is needed to fully study the effects of the products in a multiple application trial. Active I prototype was chosen for further study due to slightly higher confidence levels compared to the commercial product and the no-application control (Figure 1).
3.2 Long-term Study
This study was performed to assess the changes in skin hydration and pH levels over time during a 2-week application and after a week of no product application. The consumers’ opinions are also taken into consideration and compared to the instrumental results.

3.2.1 Stratum Corneum Hydration

The two-way ANOVA analysis has revealed that there is a significant difference between the two treatments, both on hands (p= 1.224e-05) and on lower legs (p= 5.466e-05). No significance was found between the different weeks of product application. The results are shown in Figure 3.

![Figure 3](image.png)

**Figure 3.** Changes in skin hydration during a 3-week regression study on the back of hands (n=20)
* Statistical significance between the baseline and the end of regression (week 3) values
✓ Statistical significance between week 2 and end of regression (week 3) values

The results for different weeks were isolated from the set of data and compared using two-sample paired data t-test. The significant results were marked on the interaction plot using symbols. As Figure 3 shows, there is an increase in the hydration values on the hands for Active I prototype after one week of application. This, however, showed no statistical significance compared to the baseline values. In the second week of application, the results increased very slightly, but continued to rise in the regression stage. Even though no statistical significance was found for the increase between week 2 and the end of regression phase (week 3), the comparison of baseline and end of regression has shown to be highly significant (p= 0.002229). On the contrary, the commercial benchmark has shown no improvement during the course of application and a statistically significant p (p= 0.0008) drop in hydration from week 2 to the end of regression phase. This decrease was also significant (p= 0.003) in comparison to the baseline.
The skin hydration results of the regression study on lower legs, shown in Figure 4, have revealed similar trends to those obtained for the back of hand. For this test site the prototype has shown no increase during the first week of application but a statistically significant rise ($p= 6.606 \times 10^{-05}$) during the second week. Corresponding to the findings for the hands, the hydration has continued to increase in the regression stage, with no significance compared to week 2, but significantly higher to the baseline values ($p=0.001$). Similarly to the back of hand results, the benchmark has shown a significant decrease during the regression stage compared to week 2 ($p=0.006$) and to the baseline ($p=0.019$).

![Figure 4](image)

**Figure 4.** Changes in skin hydration during a 3-week regression study on the lower legs (n=20)

- * Statistical significance between the baseline and the end of regression (week 3) values
- + Statistical significance between week 1 and week 2 weeks values
- ✓ Statistical significance between week 2 and the end of regression (week 3) values

Overall, the results of both test sites have confirmed a superior performance of the biomimetic prototype to the standard commercial o/w lotion. This data correspond to the findings of Prasch *et al.* (2000) in terms of higher skin hydration obtained from the lamellar-structured cream. A regression test by Pennick *et al.* (2012) employing visual grading has also shown positive effects of application of lamellar structures. Because the reasons behind the increase of skin hydration during the regression stage of the prototype are not fully understood, further research could benefit from a longer regression phase to assess at which time point the skin would return to its initial state. A possible explanation for the continuous improvement could be referred back to the work of Mao-Qiang *et al.* (1995), suggesting that a physiological lipid mixture could influence the metabolisation of lamellar bodies in the epidermis and thus improve the skin barrier function. The low results recorded for the benchmark might be due to the fact that measurements were taken 3 days after the last product application. This suggests that while the biomimetic prototype might be able to deliver increased skin moisturisation up to 3 days after the last treatment (as observed by...
Pennick et al., 2012), the occlusive effects of the benchmark might be limited to a shorter-term efficacy. Further investigation of these test products is needed with alternative instrumental methods such as TEWL and Confocal Raman Spectroscopy to determine changes in the water content of the stratum corneum and its thickness, as suggested by a study by Crowther et al. (2008).

3.2.2 Skin pH
The measurements of the skin surface pH can reveal important information about the state of the skin barrier. This is why pH values of the back of hands and the lower legs were recorded during the long term study. The analysis of the data using a 2-way ANOVA test showed no significant changes in the pH values for any of the test sites.

3.2.3 Self-Assessment
The self-assessment questionnaire revealed the volunteers’ moisturising habits and their impressions of using the two test products. A self-assessment of their skin type showed that 55% of the volunteers considered their skin to be dry, while 35% thought it was normal (Figure 5).

![What is your skin type?](image)

**Figure 5.** Self-assessment data of skin type (n=20).

These data fit with the findings of Luebberding et al. (2013) that ageing is not always accompanied by skin dryness. In fact, the questions regarding their moisturising habits showed that 20% of the participants do not moisturise regularly and none of them would typically use a moisturiser more than once a day. This shows that even though studies show a reduced water binding capacity, a decline of the regeneration of the stratum corneum and of the production of skin lipids (Proksch, 2006) and a significant reduction in skin hydration with age (Shlivko et al., 2013), most of the volunteers do not feel the need to moisturise every day, with the majority doing so several times a week. A comparison between the effects of the two test products in delivering skin moisturisation and softness showed significance at p<0.01 (2-sample paired data t-test) for both questions. Figures 6 and 7 illustrate the results obtained.
Figure 6. Self-Assessment of skin moisturisation
Sensory scale used: 1(not effective); 5(no change); 9(very effective)

Figure 7. Self-Assessment of skin softness

The effects of the Active I prototype have been perceived to be better than the commercial product, which was in line with the instrumental results. Interestingly, the negative effects observed for the benchmark during the instrumental trial were not detected by all consumers and the product has received scores as high as 8. This shows that computerised equipment could sometimes identify changes that might not necessarily be recognisable by the human eye and touch.

Apart from delivering superior moisturisation, the biomimetic prototypes were designed in order to provide a better texture and improved skin feel than the products based on the occlusive mechanism. The opinion of the volunteers on this matter is demonstrated in Figure 8.

Figure 8. Preference regarding the test products’ textures

These data were analysed using a two-sided binomial test and the 65% of people preferring the texture of the biomimetic prototype were found significant at the 99% confidence level.

On a whole, the self-assessment questionnaire has revealed that the Active I prototype was the preferred option to the commercial product in delivering benefits to the skin and for its sensory properties. This reflects the outcomes of the 4-hour instrumental trial and the 3-week
regression study and compliments other instrumental studies (Prasch et al., 2000; Mao-Qiang et al., 1995; Imokawa et al., 1989) and a visual grading study (Pennick et al., 2012), showing that the measured effects can also be identified by the consumers.

4. Conclusion
This study has demonstrated that providing the skin with lipids identical to those that make up the natural skin barrier can improve the skin hydration to a greater extent than a conventional o/w emulsion based on occlusive materials and humectants. In addition, it suggests that using bio-mimetic moisturisers is an effective and long-lasting method to alleviate dry skin condition in people over 60. In the future, it would be interesting to repeat this study with older participants (e.g. over 80). That group tend to suffer from unpleasant consequences of very dry skin and would greatly benefit from effective and long-lasting moisturisation.

5. References

- Centre for Biomimetics (2007)- ‘What is biomimetics?’ [Online]. University of Reading. Available at: http://www.reading.ac.uk/biomimetics/about.htm (Accessed: 28.03.13)


