

Clinical Evidence for the Activity of Tetrahydroxypropyl Ethylenediamine (THPE), a New Anti-Aging Active Cosmetic

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ABSTRACT

Background/ Objectives: The cellular surface modification of superficial epidermal keratinocytes can induce immediate skin tensioning effects and may improve signs of skin aging. Tetrahydroxypropyl ethylenediamine (THPE) is an active that has been described to induce keratinocytes' morphological changes in vitro. We conducted an in vivo study to assess anti-aging clinical benefits of a THPE-containing product.

Methods: An eight-week double-blind, randomized intra-individual placebo controlled clinical study was performed to evaluate the clinical benefits of a 2.5% THPE-containing cream. This study included 41 Caucasian women who received the THPE cream product on one side of the face and a placebo cream on the other side daily. Evaluations were performed at baseline, 45 minutes after first application, week 4 and week 8 and included clinical examination and digital photography.

Results: The study demonstrated the immediate lifting effect of a 2.5% THPE-containing cream. Forty-five minutes after a single application on the face, as the skin surface smoothed out, light reflection was modified: healthy glow and radiance of the skin were significantly improved (respectively 22.9% and 40% of improvement) and skin yellowishness was reduced (7.1%). Notably, the THPE-treated side was significantly lifted, both immediately after product application (8.1%) and after 8 weeks of application (14%), compared to the placebo-treated side.

Conclusion: This clinical study demonstrated that the effect of a 2.5% THPE-containing cream on the keratinocytes cells leads to an immediate and long-term clinical improvement of the skin appearance (radiance and skin firmness, skin lifting) and can therefore be considered as a new anti-aging cosmetic active.

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INTRODUCTION

In 2040, one out of four people will be at least 65-years-old, as a consequence of the "baby boom" generation (1946 to 1964 in the U.S.; 1946 to 1974 in France and the United Kingdom, for example). The aging population in conjunction with the desire to maintain a youthful appearance has led to a nearly simultaneous growth in the anti-aging cosmetic market. Facial skin is particularly vulnerable to the effects of aging, which take place gradually often being recognized by the emergence of furrows and wrinkles together with a loss of tonicity.¹ The loss of skin firmness can lead to facial sagging which has been reported to strongly affect the perceived age.

To meet the increasing demand for anti-aging treatments, various cosmetic active ingredients have been investigated. However, most of them require long-term use to perceive benefit. Consumers desire more instantaneous benefits leading to an immediate smoothing of the cutaneous microrelief, a lifting of the skin and an

improvement of its viscoelastic properties. High molecular weight polymers, such as proteins and polysaccharides well known as skin tightening agents, are able to form a film leading to the stratum corneum retraction have been commonly used in cosmetic practice.

It was reported that modulation of superficial epidermal keratinocytes could induce rapid skin tensioning effects and affect skin appearance.²⁻⁴ Tetrahydroxypropyl ethylenediamine (THPE) acts through this novel mechanism of action of keratinocyte contraction. Toxicological in vitro studies have demonstrated that THPE did not induce vacuolization and had no effect on keratinocyte or fibroblast proliferation (Data not shown). Using real-time visual microscopy and electric cell-substrate impedance measurements of primary human keratinocytes,⁵ THPE was found to induce a dose-dependent in vitro keratinocyte contraction, decreasing significantly cell area by up to 44.5 percent in five hours.⁶

We performed a double-blind, randomized, placebo-controlled study to investigate the anti aging/lifting benefit of a 2.5% THPE-containing cream compared to a placebo.

METHODS

Subjects

Forty-one Caucasian female subjects between the ages 45 and 66-years-old were included. Exclusion criteria were: subjects presenting serious illness or under medical treatment, subjects presenting skin diseases on the face and subjects having used an anti-aging cosmetic product up to four weeks prior to the start of the study. All subjects signed an informed and express consent form and accepted to not apply any other product to their face three days before starting until the end of study.

Tested products

The active cream contained glycerin, THPE 2.5%, glycine soy, Mg aspartate, Zn gluconate and Cu gluconate. The placebo had the same color and texture as the product, but contained no active ingredients.

Protocol

As noted, this was a double-blind, randomized, split-face, placebo-controlled study. The assignment of the active product or the placebo to left/right side of the face was determined by randomization.

At baseline (T0), a clinical assessment on each half-face was performed by a trained reliable investigator and standardized photographs of the face were performed to assess the signs of aging. For each side of the face, nine parameters linked to skin aging⁵ were scored on a 12 cm visual analogical scale (from 0 = not at all to 12 = very severe): lifted skin, wrinkles under the eye, jaw line definition, skin neck firmness, skin face elasticity, nasolabial fold, healthy glow, skin color, radiance. Then, a blinded controlled application of each product (placebo/active, according to the randomization) was applied to the respective half-face by the trained technician. Forty-five minutes after application, clinical evaluation was performed again, on each side of the face, by the blinded investigator.

After that, both products, labelled as "right" and "left" were applied by the subject on each side of the face once daily (in the morning) for eight consecutive weeks.

At week 4 (T4) and week 8 (T8), subjects were instructed to come to the study site without having applied any product since the morning of the previous day. Clinical assessment and photographs were performed on each side of the face.

Statistical analysis

The mean value and standard deviation of each clinical parameter were determined at each time point of the study and for each half-face. The variations of the parameter relative to control were expressed as a percentage.

The paired Student's "t" test ("two-tail", $P < 0.05$) was used to compare the values obtained 45 minutes after the first application, then T4 and T8 weeks of the product application to the initial values (T0 before the first application), for each half-face application. This test was also used to compare the active versus the placebo.

RESULTS

Forty-five minutes after the first application, a significant lifting effect triggered by the THPE product (+8.1%) was observed versus baseline, coupled with an improvement of under eye wrinkles (+12.5%) and skin face elasticity (+6.5%) ($P < 0.05$; paired Student's *t* test) (Figure 1). The jaw line was redrawn (+7.4%), skin glow (+22.9%), skin color (+7.1%) and radiance (+40.3%) were also significantly improved ($P < 0.05$; paired Student's *t* test). A comparison of the effect of active versus placebo highlighted a significant improvement of the THPE containing product on under eye wrinkles, healthy glow and skin radiance ($P < 0.05$; paired Student's *t* test).

After four weeks of daily application of the THPE-containing product, eight out of nine parameters were significantly improved ($P < 0.05$; paired Student's *t* test) (Figure 2). Compared to the placebo, significant improvement on the lifted aspect of the skin, the jaw line and the wrinkles under the eyes were noticed ($P < 0.05$; paired Student's *t* test) on the side treated with the THPE-containing product.

FIGURE 1. Clinical assessment of the signs of aging on the volunteers, 45 minutes after the first application on day 0 (mean percentage change versus baseline; n=41 subjects).

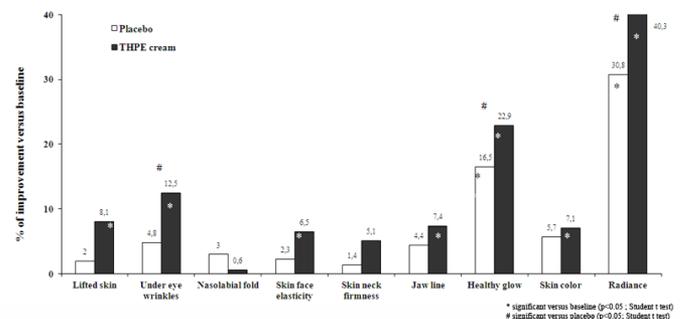
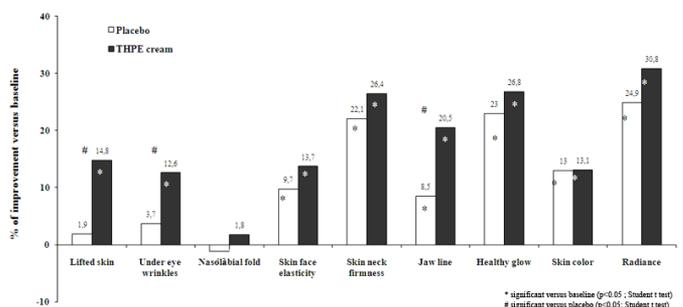


FIGURE 2. Clinical assessment of the signs of aging on the volunteers, 4 weeks after the beginning the study (mean percentage change versus baseline; n=41 subjects).



After eight weeks of application, all the nine skin parameters were significantly improved versus baseline ($P<0.05$; paired Student's *t* test) on the THPE-treated side (data not shown). Compared to placebo, the THPE-containing product significantly lifted the skin and improved skin elasticity ($P<0.05$; paired Student's *t* test).

These observations evidenced a more global anti-ageing property of the THPE product over time. Figure 3 shows an example of change observed on the nasolabial fold and the jaw line.

FIGURE 3. Visible improvement of the sloping upper eyelid (3a) and the lower face (3b) after eight weeks of product application (Representative subjects).



DISCUSSION

The loss of skin elasticity and firmness negatively influences self-perception and self-esteem since the entire shape of the face is affected. This paper describes the results of a double-blind, randomized, placebo-controlled clinical study performed on healthy Caucasian women to assess the immediate and long term benefits of a THPE-containing cream on their facial appearance. A trained clinical expert blindly evaluated the facial skin features clinically and reported them on analogical visual grading scales. The accuracy and the reproducibility of

the observer to evaluate the skin facial attributes were initially validated and a good agreement was found with other experts (data not shown). The clinical evaluation was preferred to any instrumental measurement in order to focus on changes that can be perceived by a human observer, visible benefits being more relevant from a consumer point of view. An objective method such as the 3D fringe projection would have enabled to capture the shape transformations, but the documented changes may not have been related to the human perception. Moreover, such a method would not have properly documented the improvement related to the skin tone.

Previous *in vitro* and *in vivo* studies have demonstrated that THPE induces keratinocyte contraction and increases skin firmness.⁷ Thus, the expected clinical benefit was a lifting effect of the skin. Several articles have already demonstrated the benefits of cosmetics formulation on wrinkles.^{8,9} However, to the best of our knowledge, very few double-blind, placebo controlled studies investigating the benefits of an active ingredient against skin sagging have been published. Most of the cosmetic products which claim a lifting benefit rely on the tensioning properties of a polymer topically applied on the skin^{2,3} to form a tightening film. Thus, lifting products generally deliver an immediate benefit, however there is no long-term efficacy. On the contrary, the mechanism of action of THPE relies on the contractile ability of the cells, which may result in a more natural, physiologic and lasting effect. This clinical study revealed that the contractile effect which occurs at a cellular/microscopic level leads to a visible/macroscopic change of the skin appearance.

CONCLUSION

Our results demonstrate that 45 minutes after application of a 2.5% THPE-containing product on face, we can visually detect a significant improvement of the skin tone and radiance when compared to the placebo. The face is also significantly lifted after four and eight weeks of daily application, this lifting effect being statistically significant on the product side in comparison to the placebo treated side ($P<0.05$, Student's *t* test). Moreover, the under-eye wrinkles were significantly reduced compared to the placebo treated side. As the skin surface smoothed out, light reflection was modified: healthy glow and radiance of the skin were significantly improved compared to the placebo treated side ($P<0.05$, Student's *t* test). Notably, an eight-week treatment with THPE-containing product provided benefits in all measured parameters. The skin tone was still significantly improved versus baseline, despite the product being applied at least 24 hours before the measurements.

This clinical study demonstrated that a THPE-containing cream significantly improves the skin tone, elasticity and lifts the skin in a perceptible way. This means that visible rejuvenation of the skin appearance can be achieved by modifying the geometry of epidermal layers' cells.

DISCLOSURES

Christiane Bertin, Alex Nkengne, Arlette Da Cunha and Nathalie Issachar were employees of Johnson & Johnson Consumer France, who provided financial support for this study.

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