

Clinical and instrumental evaluation of a food supplement in improving skin hydration

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Synopsis

Topically applied cosmetic products can be helpful in improving skin hydration. The study shows how oral supplementation could be helpful in improving and preventing the skin dehydration. A total of 32 healthy female volunteers entered the study. Of which, 16 were treated with a food supplement based on vegetable ceramides, amino acids, fish cartilage, antioxidants and essential fatty acids for 40 days and 16 with placebo. The results of the clinical and instrumental evaluations carried out in this study, have highlighted how the active treatment is effective in improving skin hydration and in reducing the cutaneous smoothness and roughness and the depth of furrows, in comparison to the placebo. In fact, concerning several important parameters, as stratum corneum hydration and skin roughness, the improvement measured exceeded 25%. We therefore suggest that a combination of treatments (oral and topical) can be more effective in improving skin hydration.

Resume

L'application topique de produits cosmétiques peut aider à l'hydratation de la peau. L'étude montre comment une supplémentation orale peut améliorer et empêcher la déshydratation de la peau. Trente deux femmes volontaires en bonne santé ont participé à cette étude. Seize ont été traitées

pendant quarante jours avec un supplément alimentaire contenant des céramides végétaux, des aminoacides, du cartilage de poisson, des antioxydants et des acides gras essentiels, seize autres ont reçu un placebo. Les résultats des évaluations cliniques et expérimentales menées dans cette étude ont montré comment le traitement actif est efficace pour améliorer, par rapport au placebo, l'hydratation de la peau et réduire la douceur, la rugosité cutanée et la profondeur des rides. En fait, si l'on considère des paramètres importants comme l'hydratation du stratum corneum et la rugosité de la peau, l'amélioration mesurée dépasse 25%. Nous suggérons également qu'une combinaison de traitements (oral et topique) peut être encore plus efficace.

Introduction

Stratum corneum (SC) hydration is of primary importance in keeping the skin supple and flexible and in preventing irritations and dermatitis. In its natural state the SC is poor in water, in fact it is one of the tissues in the human body with lowest water content [1]. The cosmetic products based on different active principles are very active in treating and preventing skin dehydration after topical application, but they may affect mainly epidermal structures. Dermal effects of such treatments could be visible after several months. On the contrary, cosmetic treatment as food supplements or dietary products has been proved to have a little effect in the short-term [2]. The aim of this study is to evaluate the improvement of skin hydration with particular regard to the skin associated changes,

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such as wrinkles and furrows, after the use of a food supplement containing pro-hydrating actives.

Materials and methods

A double blind versus placebo study was conducted on 32 healthy female volunteers for 40 days. All subjects underwent a washout period of 2 weeks for oral and topical products, which could interfere with the study. All subjects were also provided with a syndet bar for routine skin care. The treatment consisted in two capsules (product or placebo) taken orally per day. The formulation of the active product was based on vegetable ceramides, amino acids, sea fish cartilage, antioxidants and essential fatty acids (sea fish cartilage, ceramides, L-proline, L-lysine, L-valine, L-cysteine, Vitamin E, lycopene, borage oil, fish oil – Medestea International, Turin, Italy). All these agents and in particular ceramides and fish cartilage have been described to improve skin hydration after oral use [3]. Placebo was a similar pill (color and size) containing soybean oil only. Patients were monitored at baseline, after 20 days and at the end of the study.

They were selected according to the following inclusion and exclusion criteria:

Inclusion criteria

- Female;
- Age range 35–60 years;
- Subjects willing to refrain from using other topical products on test areas during the study;
- Subjects willing to refrain from ingesting drugs and/or vitamins that could interfere with the study results;
- Subjects who did not assume oral contraceptive;
- Subjects who have signed the written informed consent;
- Subjects willing to avoid the sun exposure on test areas during the study.

Exclusion criteria

- All the conditions that are not included in the inclusion criteria;
- Pregnant or nursing subjects;
- Subjects with significant history or current evidence of any medical, psychological or other disorder that in the investigator's opinion would preclude enrolment into the study.

Assessments

Instrumental assessment

Several parameters related to skin hydration have been monitored using the face as test area: *Stratum corneum hydration*: it is assessed by capacitance measurements (Corneometer CM 825, Courage and Khazaka, Koln, Germany), by taking four measurements in different parts and then averaging them [4]. *Skin smoothness, roughness, SC dryness, depth of furrows and skin health condition*: Special UV-A light video camera (Visioscan VC 98, Courage and Khazaka, Koln, Germany) with high resolution has been developed to study the skin surface directly. The images show the structure of the skin and the level of dryness very impressively. The camera can be connected to the computer directly via EPP-port. In addition to the image processing function, special software permits the calculation of a variety of skin surface parameters. Infect the gray level distribution of the image is used to evaluate clinical parameters to quantitatively and qualitatively describe the skin surface [5]. The following parameters were considered: surface calculates the size of the 'wavy' skin surface. Therefore is an indirect measurement of skin smoothness. High smoothness will lead to decreased surface values. Energy and Contrast parameters are indicators for the skin homogeneity. They indicate a general overview over the state of the skin. Young, highly hydrated, elastic skin has a high-energy value and low contrast value compared with older skin having many wrinkles. Variance is the roughness of the skin. Volume, R_1 – R_5 are indicators of the depth of furrows. Sesc represents the skin dryness.

Measurements have been performed at $20 \pm 2^\circ\text{C}$ of environmental temperature with a relative humidity of $45 \pm 5\%$ according to guidelines published for these techniques [4]. Subjects were acclimatized before readings for 30 min.

Clinical assessment

Subjects were asked to give a self-assessment of their visual skin appearance using a Visual Numerical Scale (0–10).

The evaluated parameters are Hydration, Tension, Desquamation, Elasticity, Make up endurance, Color, Tone, and Seborrhea.

Statistical analysis has been performed using the Wilcoxon's test and Student's *t*-test for paired data

at each time point. Values of *t*-test less than 0.05 was considered statistically significant.

Results

Instrumental assessment

A significant improvement of the following parameters has been detected in the active-treated groups: skin hydration improved significantly under treatment (+30%; $P = 0.01$), whereas the placebo group showed a decrease (−0.5%; $P > 0.05$) (Fig. 1). The skin roughness, measured with Visioscan VC 98, has significantly decreased only after the active treatment (−25%; $P = 0.0001$) (Fig. 2). Skin smoothness improved, infect the Surface parameter has significantly decreased only after the active treatment (−22%; $P = 0.0008$) (Fig. 3). The Energy values have significantly improved (Fig. 4) and the Contrast values have significantly decreased (Fig. 5)

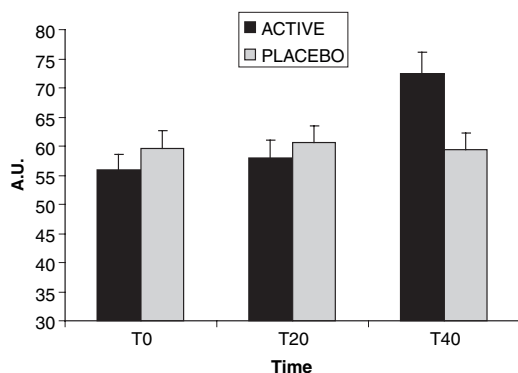


Figure 1 Skin hydration improved significantly under treatment, whereas the placebo group showed a decrease.

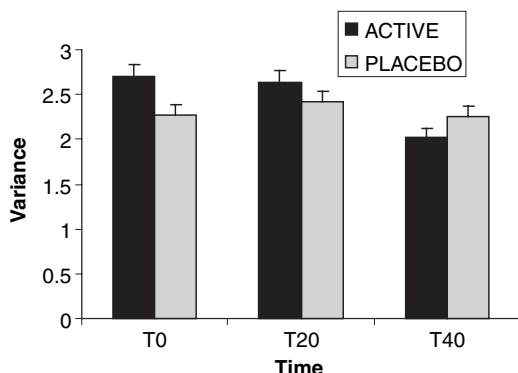


Figure 2 The skin roughness, measured with Visioscan VC 98, has significantly decreased only after the active treatment.

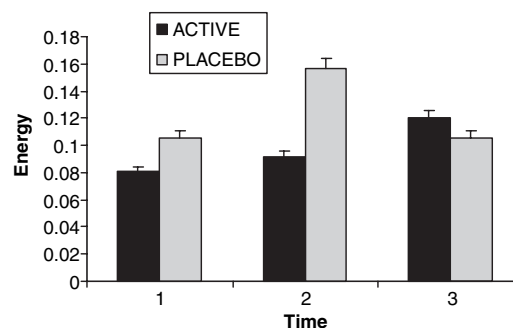


Figure 3 The Surface is a parameter measured with Visioscan VC98, which calculates the size of the 'wavy' skin surface, is an indirect measurement of skin smoothness. High smoothness will lead to decreased surface values. In this case, the surface has significantly decreased only after the active treatment. Therefore, the skin smoothness has improved only after the active treatment.

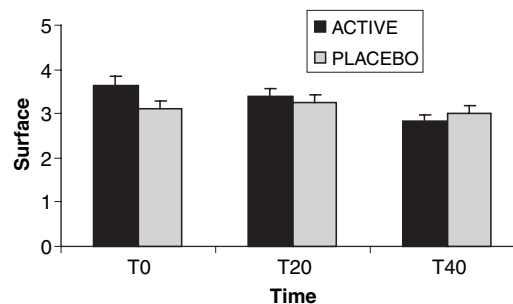


Figure 4 Energy parameter, measured with Visioscan VC98, IS indicator for the skin homogeneity. It indicates a general overview over the state of the skin. Young, highly hydrated, elastic skin has a high-energy value compared with older skin with many wrinkles. In this study, the Energy has significantly improved only with the active treatment. Therefore, the skin condition has improved significantly only after the active treatment.

only under the treatment (Energy: +50%; $P = 0.00007$; Contrast: −36%; $P = 0.0004$). Therefore, the skin condition has improved significantly only after the active treatment. A significant decrease in the depth of furrows was recorded (volume: −24%, $P = 0.01$; R_1 : −23%, $P = 0.0009$; R_2 : −25%, $P = 0.0005$; R_3 : −27%, $P = 0.0001$; R_4 : −28%, $P = 0.001$; R_5 : −27%, $P = 0.0008$) (Figs 6–11). SC dryness also has decreased significantly (−58%; $P = 0.02$) (Fig. 12).

Clinical assessment

The self-assessment test has shown evident perception's differences of all the estimated parameters

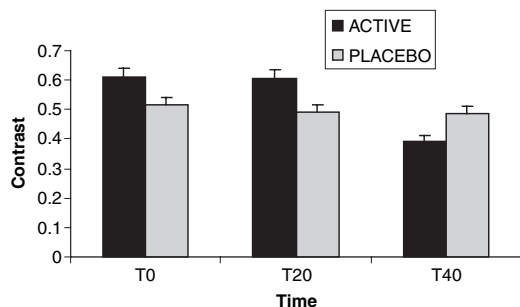


Figure 5 The Contrast parameter, measured with Visioscan VC98, is indicator for the skin homogeneity. It indicates a general overview over the state of the skin. Young, highly hydrated, elastic skin has low contrast value compared with older skin with many wrinkles. In this study, the contrast has significantly decreased only with the active treatment. Therefore, the skin condition has improved significantly only after the active treatment.

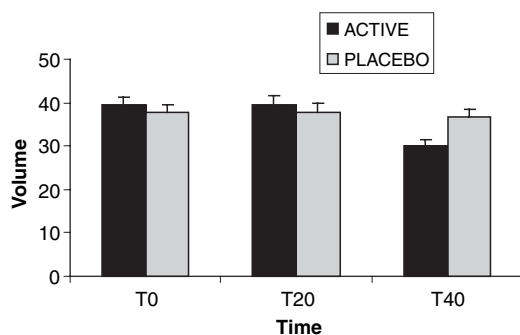


Figure 6 The Volume parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the Volume has significantly decreased only with the active treatment.

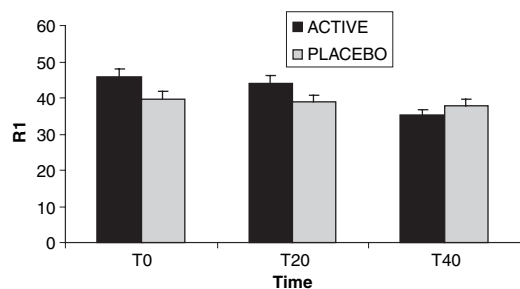


Figure 7 The R_1 parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the R_1 has significantly decreased only with the active treatment.

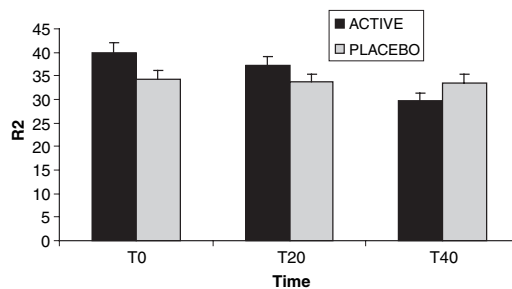


Figure 8 The R_2 parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the R_2 has significantly decreased only with the active treatment.

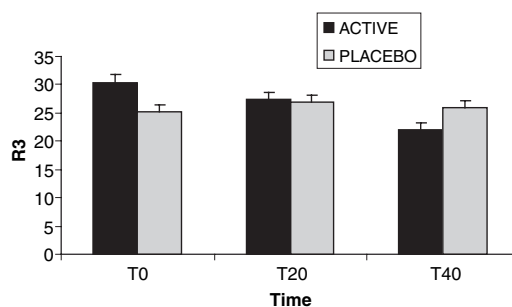


Figure 9 The R_3 parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the R_3 has significantly decreased only with the active treatment.

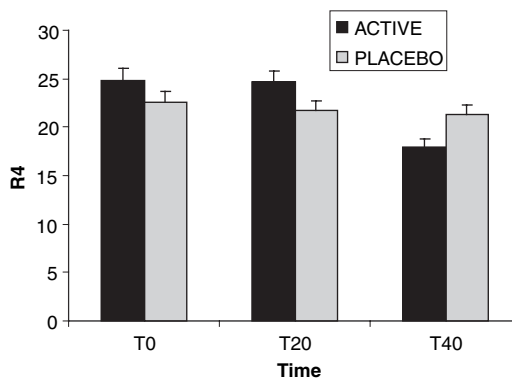


Figure 10 The R_4 parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the R_4 has significantly decreased only with the active treatment.

that are correlated to the product's efficacy. The active treatment has proved much better than placebo. The differences have always turned out statistically significant with the t -test ($P < 0.005$) (Fig. 13). The clinical assessment confirms the results from the biophysical measurements.

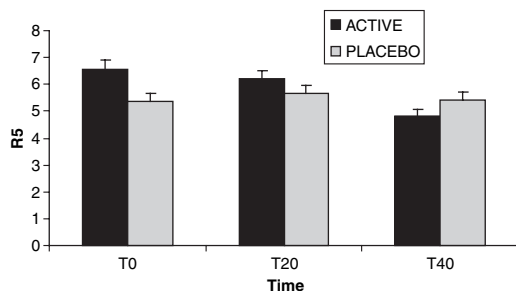


Figure 11 The R_5 parameter, measured with Visioscan VC98, is indicator for the depth of furrows. In this study, the R_5 has significantly decreased only with the active treatment.

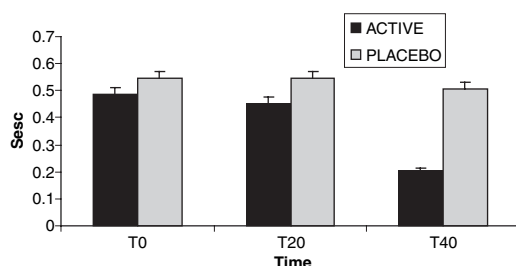


Figure 12 The stratum corneum dryness, measured with Visioscan VC 98, has significantly decreased only with the active treatment.

Discussion

To maintain the proper level of moisturization, the skin epidermis has evolved a finely tuned differentiation programme, which generates and maintains a SC composed of cellular and macromolecular components that provide the required structure, humectancy and barrier to water loss. This structure has been compared with a brick wall, with the 'bricks' representing the terminally differentiated, keratin-filled corneocytes of polyhedral shape, arranged as interdigitating vertical columns, and the 'cement' representing the highly specialized and uniquely organized intercellular lipids. These lipids are primarily ceramides, cholesterol and fatty acid, together with smaller amounts of phospholipids, glucosylceramides, free sphingoid bases and cholesterol sulphate [6].

A soft and flexible skin has an intact and optimally hydrated SC with approximate water content of approximately 10–30% [6]. In the SC water exists in two states: water firmly incorporated into the protein structure termed 'bound

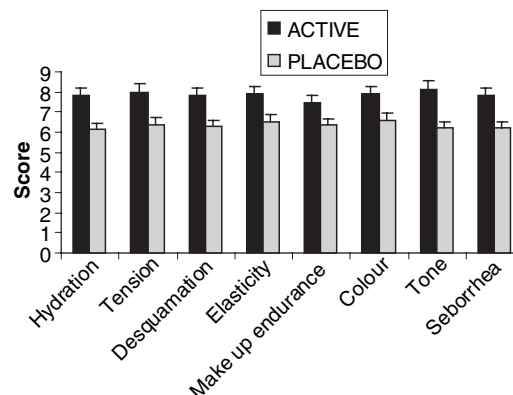


Figure 13 The self-assessment test shows evident perception's differences of all the estimated parameters, that are correlated to the product's efficacy. The subjects assuming the active treatment have always expressed a higher score compared with the one's assuming the placebo.

water', thus the amount held is determined by the hygroscopicity of the SC. The water present in excess of the bound amount is more freely present in other compartments of the SC and is termed 'free water' [7]. The factors which influence the water-holding capacity (WHC) of the SC include: the depth of the survey level carried out on the SC, the thickness of the SC, the survey area, the lipid component and the presence of pathological conditions. Several epidermal components modulate WHC. In the corneocyte, filaggrin rapidly disintegrates, releasing water and low molecular weight substances, which contribute to the so-called natural moisturizing factors (NMF) [8]. These substances are formed during epidermal differentiation and may represent up to 10% of the corneocyte mass. They are principally amino acids, carboxylic pyrrolidone acid, lactic acid, urea, glucose and mineral ions. Keratinization plays an important part in the formation of NMF that exhibit strong osmotic potential attracting the water molecules. The binding of water to NMF is the static aspect of cutaneous hydration [9]. The second, dynamic, aspect is related to the selective permeability of the SC and to its lipid barrier properties, the permeability of which depends on the integrity and nature of the inter-corneocyte lipids and their lamellar organization between the cells. [9, 10].

A dietetic supplement can improve the SC water holding capacity if it contains:

- Pre-formed free amino acids which, together with those in the skin, improve the SC hygroscopicity and help the corneocyte water binding [11];

- Ceramides which improve the skin barrier, decreasing the transepidermal water loss and increasing the corneocyte water binding. In fact these lipids play a key part in both the processes and they are able to firmly bind the water. Therefore, the SC would lose less water from the deep cutaneous compartments [10];
- Antioxidants which improve the SC redox balance, in particular the lipidic peroxidation of the intercellular lipids [12, 13];
- Essential fatty acids which act like anti-inflammatory coadjutants and like epidermal barrier restructuring [14];
- Fish cartilage polysaccharides which are effective in the treatment of photoaging. The active principle in extracts of sea fish connective tissue is chondroitin sulphate, a mixture of acid mucopolysaccharides based on a repeating disaccharide unit esterified by sulphate groups [3].

In fact, in our study, the food supplement containing vegetable ceramides, amino acids, sea fish cartilage, antioxidants and essential fatty acids, has been effective in improving skin hydration and in reducing the cutaneous smoothness and roughness and the depth of furrows. We observed an improvement of almost all the parameters evaluated only in the subjects belonging to the active group, while we did not observe any significant difference in the placebo group. Moreover, concerning several important parameters like SC hydration and skin roughness, the improvement measured exceed the 25%.

The efficacy of the product was further confirmed by a positive subjective perception by the subjects in relation to the parameters concerning the overall cutaneous condition. In fact, in the self-assessment evaluation, the active has always obtained a significant higher score compared with the placebo. No side effects were reported during the study. At the moment no data on the duration of the clinical and instrumental improvements recorded in the present work are available. Further studies to elucidate the dynamics in relation to length of treatment are needed. In conclusion, our study confirms the importance of specific oral supplement in the treatment of skin dehydration. In fact it can be a helpful tool in combination with topically applied active principles exerting a synergistic and simultaneous effect on the epidermis and SC.

References

1. Warner, R.R., Stone, K.J. and Boissy, Y.L. Hydration disrupts human stratum corneum ultrastructure. *J. Invest. Dermatol.* **120**, 275–284 (2003).
2. Kieffer, M.E. and Elsen, J. Imeeden in the treatment of photoaged skin: an efficacy and safety trial over 12 months. *J. Eur. Acad. Dermatol.* **11**, 129–136 (1998).
3. Berardesca, E. EEMCO guidance for the assessment of the stratum corneum hydration: electrical methods. *Skin Res. Technol.* **3**, 126–132 (1997).
4. Tronnier, H., Wiebush, M., Heinrich, U. and Stute, R. Surface evaluation of living skin. *Adv. Exp. Med. Biol.* **455**, 507–516 (1999).
5. Berardesca, E. Disorders of skin barriers: clinical implications. *JEADV* **16**, 559–561 (2002).
6. Visscher, M.O., Tolia, G.T., Wicket, R.R. and Hoath, S.B. Effect of soaking and natural moisturizing factor on stratum corneum water-handling properties. *J. Cosmet. Sci.* **54**, 289–300 (2003).
7. Kennedy, A.H., Golden, G.M., Gay, C.L. et al. Stratum corneum lipids of human epidermal keratinocyte air-liquid cultures: implications for barrier function. *Pharm. Res.* **13**, 1162–1167 (1996).
8. Schurer, N.Y. and Elias, P.M. The biochemistry and function of stratum corneum lipids. *Adv. Lipid. Res.* **24**, 27–56 (1991).
9. Coderch, L., Lopez, O., de la Maza, A. and Parra, J.L. Ceramides and skin function. *Am. J. Clin. Dermatol.* **4**, 107–129 (2003).
10. Sato, J., Katagiri, C., Nomura, J. and Denda, M. Drastic decrease in environmental humidity decreases water-holding capacity and free amino acid content of the stratum corneum. *Arch. Dermatol. Res.* **293**, 477–480 (2001).
11. Meves, A., Stock, S.N., Beyerle, A., Pittelkow, M.R. and Peus, D. Vitamin C derivative ascorbyl palmitate promotes ultraviolet-B-induced lipid peroxidation and cytotoxicity in keratinocytes. *J. Invest. Dermatol.* **119**, 1103–1108 (2002).
12. Moison, R.M., Doerga, R., Beijersbergen, M.J. and van Henegowen, G. Increased antioxidant potential of combined topical vitamin E and C against lipid peroxidation of eicosapentaenoic acid in pig skin induced by simulated solar radiation. *Int. J. Radiat. Biol.* **78**, 1185–1193 (2002).
13. Schurer, N.Y. Implementation of fatty acid carriers to skin irritation and the epidermal barrier. *Contact Dermat.* **47**, 199–205 (2002).
14. Distant, F., Scalise, F., Rona, A., Fluhr, J.W. and Berardesca, E. Oral fish cartilage polysaccharides in the treatment of photoaging: biophysical finding. *Int. J. Cosmet. Sci.* **24**, 81–87 (2002).