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Supplementation with Evelle® improves skin smoothness and elasticity in a double-blind, placebo-controlled study with 62 women

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OBJECTIVE: To investigate whether nutritional intervention with a proprietary formulation and other micronutrients may favourably alter skin roughness and elasticity.
METHODS: Sixty-two women aged 45–73 years participated in a double-blind, placebo-controlled trial testing the efficacy of a proprietary oral supplement for skin nutrition (Evelle®), for improvement of skin elasticity and roughness. The active ingredients were vitamins C and E, carotenoids, selenium, zinc, amino acids and glycosaminoglycans, blueberry extract and Pycnogenol®.
RESULTS: Skin elasticity, measured using an optical cutometer, was found to be statistically significantly increased by 9% after 6 weeks of treatment compared with placebo ($p=0.0351$). Skin roughness, as evaluated by three-dimensional microtopography imaging, was found to be statistically significantly lowered by 6% compared with the control group after 12 weeks treatment ($p=0.0157$).
CONCLUSION: Evelle can potentially improve visible signs of cutaneous ageing. (*J Dermatol Treat* (2004) **15:** 222–226)

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Introduction

Human skin represents the body's barrier to the external environment, protecting it from mechanical damage, noxious substances, invading micro-organisms, and radiation. Moreover, the skin plays an important role in controlling water retention, regulating body temperature and is an essential part of the immune system.

Further to the vital biological functions, the skin plays a pivotal role in the feeling of well-being and in physical attractiveness. Skin appearance is determined by colour, surface texture and physiologic properties such as elasticity, sweat, scent and sebum production.[1] With increasing age, skin appearance gradually declines, ultimately leading to the reduction and coarsening of collagen, elastosis, wrinkling, laxity, atrophy, irregular pigmentation and dryness.[2] Contributory environmental factors are UV radiation, free radicals, toxic and allergic compounds, immune and hormonal status, mechanical strain, cigarette smoking and stress.

Skin functioning and attractiveness rely on specific nutritional needs, as evidenced by the development of skin disorders in response to various nutritional deficiencies.[3] Supplementation with deficient vitamins, minerals and other dietary constituents were shown to improve skin conditions.[4] An approach to retard the progression of skin ageing has been proposed to rest on two premises.[5] The skin requires antioxidant defence and the mechanical properties, elasticity and texture, rely on sufficient density and geometry of collagen and elastic fibres. In consequence, damage and loss of collagen and elastin impair contractile properties, resulting in skin laxity and wrinkling.

Vitamin C is an essential co-factor of prolyl 4-hydroxylase required for the generation of hydroxyproline in collagen and elastin.[6] Dietary supplementation with vitamins C and E as well as carotenoids provide

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significant antioxidant activity in human skin with demonstrated UV-protection and enhancement of cutaneous immune response.[1]

Selenium, a component of antioxidant selenoproteins, was shown to protect skin cells from UV-induced damage, DNA oxidation and lipid peroxidation.[7] Zinc is an essential element of more than 200 metalloenzymes, such as superoxide dismutase. Zinc is a component of enzymes required for DNA replication, gene transcription, and RNA and protein synthesis. Roughened skin and impaired wound healing have been reported in association with a mild zinc deficiency, implicating changes in skin.[8]

The vitamin biotin is an essential cofactor for several carboxylases which catalyse vital steps in intermediary metabolism in the skin. Deficiency of biotin is known to manifest in various skin disorders, including dermatitis, scaling and alopecia.[9]

While the above-mentioned vitamins and minerals fulfil the basic needs for healthy skin tissue, another micronutrient has been suggested to support collagen skin density. An extract of French maritime pine bark (*Pinus pinaster*), Pycnogenol®, a mixture of procyanidins and phenolic acids, displays physical affinity to collagen and elastin and protects it against proteolytic degradation.[10]

The impetus of this study was to investigate whether nutritional intervention with a proprietary formulation comprising vitamins, antioxidants, minerals, Pycnogenol and other micronutrients may favourably alter skin roughness and elasticity in women aged 45 years or older.

Patients and methods

A total of 62 volunteers were recruited for this study who met the following inclusion criteria: age 45–75 years, female, skin phototype I–IV. As an investigation site the inner side of the left forearm (volar forearms) was chosen because it is more homogeneous and covered from environmental factors such as sunlight and the renounce of cosmetic products is more feasible than, for example, the face. The investigation site had to be free of acute skin diseases, warts, scabs, with little or no hair and no tattooing. Exclusion criteria were chronic diseases, pregnancy or breastfeeding, as well as other studies on the forearms during the past 2 weeks. Subjects were advised to refrain from using a sauna and swimming, and to discontinue use of skin care products, shower oils and dermatological therapeutics on the tested area of the arm during the trial period. Written informed consent was obtained from all subjects.

The study design was double-blinded and placebo-controlled. After a run-in period of 7 days for conditioning the subjects, they were assigned to either the verum or placebo group according to their initial skin roughness value to ensure equal distribution. One group of 31 women was advised to take two Evelle® tablets twice a day, a trademarked proprietary blend of ingredients geared to support optimal nutrition of the skin as well as hair and nails. One Evelle tablet provides the following micronutrients: Pycnogenol (10 mg), vitamin C (30 mg), vitamin E (*d*-α-tocopherylacetate) (5 mg), biotin (75 μg), selenium (25 μg), zinc as gluconate (7.5 mg), bio-marine complex (hydrolised collagen and glycosaminoglycans from salmon) (50 mg), horsetail herb extract (natural source of silicate) (40 mg), blueberry extract (15 mg) and tomato extract (dietary carotenoids, β and γ-carotene, lycopene and lutein) (34 mg). The control group received identical-looking tablets containing none of the above ingredients.

Skin elasticity was recorded using the Cutometer SEM 575 (Courage & Khazaka Electronics, Collogne, Germany) 1 day before start of supplementation and then again after 6 weeks. Measurements of visco-elastic properties were performed according to the manufacturer's instructions (available on website: www.courage-khazaka.de) with the following parameter settings: 350 mbar, on-time 5 s, off-time 5 s followed by computer-aided evaluation using parameter R5 (=Ur/Ue), representing the net skin elasticity, as previously described.[11] Measurements were always repeated twice.

Skin roughness was evaluated using a three-dimensional microtopography imaging system, PRIMOS (GF Messtechnik, Teltow, Germany: www.gfmesstechnik.com). The scanned skin area size was $13 \times 18\,mm^2$, with three separate sites of the investigation site being measured. Calculation of skin roughness was carried out using the PRIMOS computer programme using the parameter star roughness (polynom level 5) according to the manufacturer's instructions. Each line of the star had a length of 12.8 mm, with 16 lines being analysed. The result was recorded as skin roughness in μm, as previously described.[12] Evaluation of skin roughness was carried out 1 day before the start of supplementation and a second time after 12 weeks.

Skin parameters were measured in a climate-controlled room at 21.5°C (±1°C) and 50% (±5%) relative humidity. Subjects were conditioned to this indoor climate 20 min before measurements were taken.

Statistical analysis was carried out using computer programmes Excel (Microsoft) and STATISTICA. Excel was used for the calculation of relative data, the means and standard deviation. STATISTICA was applied to test the significance of differences between verum and placebo. The distribution of original and relative data was checked using the Kolmogorov–Smirnov test. In case of normal distribution of data, the test of significant differences between verum and placebo was performed with the *t*-test for independent samples. Differences between treatment situations with a *p*-value $\leqslant 0.05$ were accepted as statistically significant.

Results

A total of 58 women completed the trial, 29 each in placebo and verum group. Two subjects in the placebo group and another two in the verum group discontinued the study. All four subjects described symptoms of gastric discomfort which resolved after termination of tablet intake.

The initial skin elasticity of both groups was comparable and not statistically significantly different. The mean skin elasticity in the verum group was 0.638±0.119 and 0.677±0.104 in the placebo group. After 6 weeks the mean elasticity in the placebo group decreased to 0.675±0.117, while the mean elasticity in the Evelle group rose to 0.681±0.085. The results are presented relative to t_0 in Figure 1, with verum relative elasticity being statistically significantly higher by 9% than placebo.

At trial start, skin roughness of investigated sites in both groups were statistically significantly indistinguishable from each other. The starting level was 211.1±21.6 µm in the verum group and 211.2±29.5 µm in the placebo group. After 12 weeks of oral treatment there was an increase of skin roughness in the placebo group, while the verum group did not encounter an increase of skin roughness. As shown in Figure 2, the mean skin roughness of the placebo-treated women was statistically significantly higher by 6% than in the verum-treated group.

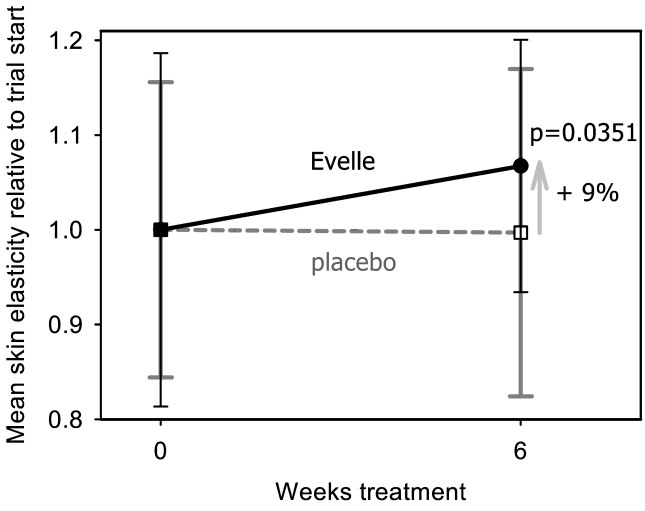

Figure 1
Mean skin elasticities of both treatment groups are presented relative to the initial value (=1) measured at t_0. Skin elasticity was measured prior to the start of supplementation and again after 6 weeks of supplementation with either verum or placebo. Presented are the mean values and SD of 29 subjects in each group. The statistical significance of difference between treatment groups was obtained by t-test after normal distribution of data was found according to the Kolmogorov–Smirnov test.

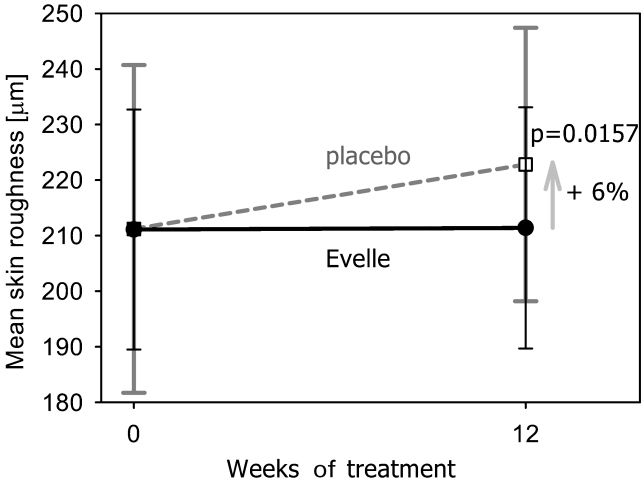

Figure 2
Mean of subjects' skin roughness values in both treatment groups are presented. Skin roughness was measured by three-dimensional microtopography imaging at trial start and after 12 weeks of supplementation with either verum or placebo. Presented are the mean values and SD of 29 subjects in each group. The statistical significance of difference between treatment groups was obtained by t-test after normal distribution of data was found according to the Kolmogorov–Smirnov test.

Discussion

In this study, the objective parameters, skin elasticity and roughness, were favourably altered by oral treatment with a supplement created for improved skin nutrition. Placebo treatment had no measurable effect on skin elasticity. In our study we chose to first study an effect of the nutritional supplement on skin elasticity, as we expected this parameter to be affected first. Only when this showed promising results was the study planned to continue for a further 6 weeks to measure a possible effect on skin roughness. Unexpectedly, the skin roughness in the placebo group rose considerably, while this was not the case in the verum group. We suspect that outdoor climate may have contributed to this finding as the study was carried out in the course from winter to summer. At the trial start the outdoor climate represented mild winter weather conditions with temperatures around 6°C and moderate humidity. After 6 weeks, when the effect on skin elasticity was measured, a moderately warm spring weather prevailed (around 11°C and low relative humidity). At the end of the study, when skin roughness was measured, hot spring weather with around 22°C and low relative humidity predominated.

Despite the unexpected increase of skin roughness in the placebo group, skin roughness in the verum group was statistically significantly lower. This finding, together with the improved elasticity, suggests that Evelle bears nutritional benefits for the skin.

As reviewed by Boelsma et al, vitamins C and E and carotenoids have been extensively researched for skin nutrition, showing protection against photo-ageing, antioxidant protection and improved cutaneous immune responses.[1] Because studies have suggested that broad mixtures of carotenoids are more effective than isolated species, a tomato extract was chosen as the carotenoid source in Evelle as it provides lycopene and lutein and further carotenoid species in addition to β- and γ-carotene.[13] In addition to providing the minerals selenium and zinc, which are essential co-factors of various enzymes, blueberry extract was added because of the ascribed potent antioxidant activity.[14]

In order to provide abundant supply of amino acids necessary for collagen production, a standardised fish extract (bio-marine complex), consisting of hydrolised collagen and glycosaminoglycans, was incorporated into Evelle. Glycosaminoglycans represent modified carbohydrates such as hyaluronic acid, which play an important role in skin hydration. A need for supplementation appears reasonable in light of the documented age-related decreased content of glycosaminoglycans in aged skin.[5]

A pivotal ingredient of Evelle is Pycnogenol, a standardised extract of *Pinus pinaster* bark. Pycnogenol is a powerful antioxidant which can recycle oxidized ascorbate and protect vitamin E from oxidation, thus prolonging the activity of these vitamins.[16] Furthermore, Pycnogenol, as well as its human metabolites, were found to inhibit matrix metalloproteinases (MMPs) from degrading collagen.[17] An imbalance between MMPs and their natural inhibitors, tissue inhibitors of MMPs (TIMPs), occurs in ageing skin – particularly during UV-exposure and in smokers. Indeed, the common perception that smokers and sun-lovers look older is believed to be the consequence of increased dermal collagen degradation.[18] Pharmacological studies have demonstrated that Pycnogenol accelerates wound healing, suggesting increased collagen matrix remodelling.[19]

Oral consumption of Pycnogenol was shown to increase UV-light-induced minimal erythema dosage in humans in a dose-dependent manner.[20] Pycnogenol's antioxidant activity prevents redox-regulated activation of gene transcription factor NF-κB, thus preventing expression of pro-inflammatory adhesion molecules and cytokines. In pharmacological studies, Pycnogenol was shown to reduce the incidence of skin tumours during chronic exposure of skin to UV-light, indicating potent photo-ageing protection. Oral Pycnogenol administration was found to be helpful for women with hyperpigmentation (chloasma). The size of the affected skin area, as well as pigmentation intensity, was significantly reduced, most likely because of a tyrosinase inhibitory effect.[21] Furthermore, in pharmacological and clinical studies, Pycnogenol was found to increase micro-circulation by enhancing production of endothelial nitric oxide.[22] The improved micro-circulation has been proposed to support better oxygen and nutrient supply as well as better waste removal in the skin.[10]

In conclusion, the chosen micronutrients combined in Evelle can potentially alleviate the visible signs of skin ageing.

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