

Evaluation of a Food Supplement with Collagen Hydrolysate and Micronutrients on Skin Appearance and Beauty Effects: A Randomized, Double-Blind, Placebo-Controlled Clinical Study with Healthy Subjects

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Abstract

Objective: Skin health, skin appearance and skin beauty are influenced by collagen composition of the dermis. Natural aging affects the structural integrity of the collagen network, resulting in drier skin, wrinkle formation and reduced skin elasticity. Orally ingested hydrolyzed collagen reaches the skin tissue and exerts beneficial effects on human skin from within. However, not only collagen peptides, but also micronutrients can beneficially affect skin appearance. Thus, dietary supplements for cosmetic and beauty effects containing combinations of collagen peptides and selected micronutrients are in demand. Aim of this study was to investigate the effect of a food supplement with collagen hydrolysate and micronutrients on skin beauty. The test product was assessed for its capability to reduce wrinkles and to improve skin hydration and collagen structure.

Methods: Healthy women (n=72) aged 40-65 years with a light to moderate wrinkle depth were enrolled. Over the 12-week intake period, the test product Doppelherz system KOLLAGEN BEAUTY (2.5 g collagen peptides and selected micronutrients) or placebo were daily ingested. At baseline, after 4 and 12 weeks, skin roughness, skin hydration and changes in collagen structure were measured.

Results: Intake of collagen peptides resulted in a reduction of wrinkle depth in the facial area. These effects were evident already after 4 weeks and the impact was especially pronounced in elderly women. Moreover, intake of collagen peptides slightly improved skin hydration in mid-aged females.

Conclusion: Results demonstrate a significant mild positive beauty effect after a 12-week application of the collagen supplement and promotes its concept to support skin beauty from within. The supplement was well tolerated.

Keywords: Collagen; Micronutrients; Skin beauty; Wrinkles; Skin roughness; Skin hydration

Introduction

Collagen is a moisture-retaining fiber protein that plays an essential role in the structure of the skin. About 70-80% of connective tissue consists of collagen, providing firmness and elasticity for the dermis, the middle layer of the skin [1]. Thus, the amount, structure and composition of collagen in the skin influence its external appearance. The moisture content of the skin, the texture of its surface and skin elasticity all contributes to skin beauty and are influenced by collagen characteristics.

Natural aging affects the structural integrity of the dermis, and our skin tends to become drier and loses tension and elasticity, which results in thinner appearance and wrinkle formation. The main underlying reason is the diminishing collagen content, both due to reduced endogenous collagen production, and due to collagen breakdown, accelerated by external factors, such as UV radiation and certain lifestyle factors (i.e. smoking) [2-4].

Hydrolyzed collagen is generated from native collagen and is composed of collagen peptides with low molecular weight. These collagen peptides are easily digestible and are well absorbed and distributed in the human body [5]. Importantly, the collagen peptides reach the skin tissue and are able to remain there for several days [6]. Indeed, a number of clinical studies confirmed beneficial effects of hydrolyzed collagen on human skin [5], for instance by increasing skin elasticity and hydration [7-12].

Skin ageing and the reduction of collagen content may be further exacerbated by stress- or diet-related deficiencies in micronutrients, as micronutrients are known to beneficially affect skin appearance [13-17].

The objective of this randomized, placebo-controlled double-blind clinical study was to investigate the cosmetic effect of a food supplement with collagen peptides and micronutrients on the quality of the skin in healthy women, after an intake period of 12 weeks in

comparison to placebo. In detail, we assessed the capability of the collagen supplement to reduce wrinkles and skin roughness and to improve skin hydration and collagen structure.

Materials and Methods

Study subjects

Healthy female subjects (n=72) at the age of 40-65 years, with a BMI of 19-30 kg/m² and a wrinkle-score of 3-6 were enrolled in the study after having given written informed consent (Figure 1). The suitability of each subject was evaluated according to the inclusion and exclusion criteria. Subjects were excluded in case of pregnancy, drug abuse, infectious diseases, insulin-dependent diabetes, cancers or rheumatic disease. Further, subjects with active or past skin disease, moles, tattoos or scars at the measurement area were not included in the study, and participants were advised to refrain from oral or topical anti-aging products and from the intensive use of skin care products at the measurement area within one month, as well as to abstain from immune-suppressive drugs and antihistamines within 7 days prior to study start. During the entire study duration, subjects were asked to maintain their usual dietary habits.

Confounding effects were controlled, since detailed specifications were defined and followed by the subjects. These included, for instance, to refrain from smoking or water contact in the measurement area within 2 hours prior to the tests, not to apply any leave on cosmetics on the measurement area since the evening before, to avoid intensive exposure of the measurement area to UV light and not to change regularly used skin care or cleaning products throughout the whole study period.

The study was approved by the local ethical committee (Ethikkommission Schleswig-Holstein, approval code: 122/18 II). The study was conducted taking the guidelines for Good Clinical Practice (GCP) set forth by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) as a guide of reference, and in accordance with the Declaration of Helsinki regarding the treatment of human subjects in a study.

Intervention/ study products

The liquid study products (25 mL, Queisser Pharma, Flensburg, Germany) were randomly assigned to the study participants in a 2:1 allocation scheme (verum:placebo). The study products were based on water and pear juice and contained either bovine collagen peptides, micronutrients (Doppelherz system KOLLAGEN BEAUTY; n=48), or no bioactive ingredients (placebo; n=24), and were orally ingested on a daily basis. Doppelherz system KOLLAGEN BEAUTY contained 2.5 g collagen peptides, 100 mg açai berry extract, 80 mg vitamin C, 5 mg zinc, 3 mg vitamin E, 0.96 mg beta-carotin, 150 µg D-biotin and 150 µg copper. Both products were of identical appearance and odor.

Study design and measurements

During the 12-week randomized, double-blind, placebo-controlled clinical study, parameters were assessed at baseline, after 4 and 12 weeks of intervention. The test areas on face (cheek bone, crow's feet region) and thigh (back region) were randomly and balanced assigned to the left and right side. Before any measurement, and after a resting period of about 5-10 min, standardized macro photographs of the face were done by USR-CLiP (Unit for Standardized and Reproducible Clinical Photography) with a high-resolution Hasselblad camera H5D-50c at day 1 and day 85. Afterwards, subjects were acclimatized for at least 30 min in the air-conditioned measurement room at a temperature of 21 ± 1°C and a relative humidity of 50 ± 5 %.

The DermaTOP blue 3D scanner (EoTech SA, Marcoussis, France) was used to capture the 3D surface structure of the facial crow's feet area by means of fringe projection at each study visit. From the obtained 3D structure, the roughness parameters Rz (rough skin structure) and Ra (fine skin structure) were calculated. Measurement of stratum corneum hydration on the cheek bone area was performed at each study visit by the electrical capacitance method using a Corneometer® CM 825 (Courage & Khazaka, Cologne, Germany). Five measurements per measurement area were performed and the mean value was used for analysis. The confocal microscope VivaScope® 1500 (Lucid Inc., Rochester, USA) was used for *in vivo* mapping of the skin to capture different skin microstructures based on particular refraction indexes. The optical section resolution was 5 µm and 3 stacks (40 repetitions) were imaged, starting within the stratum corneum and going down into the upper dermis (200 µm below the skin surface). The collagen structure in the upper dermis was assessed for its appearance by an expert grader at day 1 and day 85 in a blinded manner based on a visual analogue scale.

Methods for safety

During the study intervention, any adverse events and any used concomitant medication were documented.

Data analysis and statistics

Comparison of treatments was performed on differences to baseline for each post-application assessment time separately, using a t-Test for independent samples. Further, pair wise comparisons of assessment times by treatments were done with paired t-Tests on raw data. No adjustments for multiplicity were done as data were interpreted in the context of an explorative analysis. A significance level of α=0.05 was chosen for the supportive data analysis.

For grading of the collagen structure, images were rated in a blind manner. Direct comparison of the two time points (day 1 and day 85) were displayed in pairs and the quality of the collagen structure was rated from -50 (day 1 has the better collagen structure) to +50 (better collagen structure on day 85 after treatment). For each treatment a statistical comparison to benchmark = '0' (no difference between day 1 and day 85) was done on raw data by one sample t-Test with a significance level of α=0.05 for both treatments. The computation of the statistical data was carried out using SAS statistics software (SAS Institute, Cary, USA).

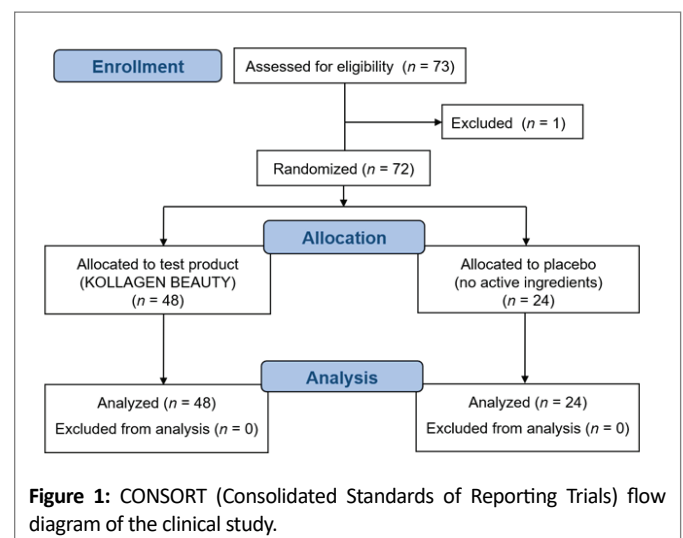


Figure 1: CONSORT (Consolidated Standards of Reporting Trials) flow diagram of the clinical study.

Results and Discussion

Subject characteristics

Seventy-two subjects, aged 54.6 ± 6.1 years, with a mean BMI of $24.45 \pm 2.95 \text{ kg/m}^2$ and a baseline wrinkle score of 4.1 ± 1.0 (light to moderate wrinkle depth) were included in the study.

Twenty-five subjects (34.7 %) used concomitant therapies, but none of these were in conflict with study conduct or with the study objectives. Subjects presented with a baseline capacitance value of 61.7 ± 11.7 , which corresponds to a normal to dry skin. Baseline average skin wrinkle depth Ra (23.9 ± 6) and Rz (95.8 ± 24.5) demonstrated a light to moderate skin wrinkle depth of the study population (Table 1).

Collagen-mediated decrease in wrinkle depth

Skin wrinkles in the crow's feet area were determined by measuring the fine (Ra) and rough (Rz) skin structure, whereby a decrease in the skin roughness parameters implies a reduction of skin wrinkles.

Results showed a significantly improved skin surface profile after intake of the collagen supplement already after 4 weeks. Compared to baseline, Ra decreased by 4.7 % (day 29) and 2.9 % (day 85) in the collagen supplement group, while Ra increased by 5.1 % (day 29) and 7.7 % (day 85) in the placebo group (difference Ra day 29: $p=0.033$; day 85: $p=0.011$). Taking the collagen supplement improved Rz values by -5.2% (day 29) and -4.4% (day 85), while Rz increased by 5.1% (day 29) and 6.0% (day 85) in the placebo group (difference Rz day 29: $p=0.043$; day 85: $p=0.021$) (Figure 2).

Effects on wrinkle depth were particularly pronounced in older subjects aged 51-65 years ($n=59$) (day 85: Ra: collagen supplement -4.6 % vs. placebo: +9.9 %; $p=0.006$; Rz: collagen supplement -6.5 % vs. placebo +7.6 %; $p=0.012$). Effects were even more pronounced in women aged 56-65 years ($n=30$) after 12 weeks of application. Here roughness parameters improved (Ra: -8.8 %; Rz: -10.8 %) under supplementation with collagen peptides, while parameters worsened (Ra: +14.2 %; Rz: + 12.2 %) in the placebo group (difference Ra and Rz $p<0.001$) (Figure 3).

Results showed that especially women from the age of 51 benefited from the application of the collagen supplement. The intake resulted in a decrease of both skin roughness parameters, indicating an improvement of the coarse structure as well as a reduction of fine wrinkles. Importantly, a significantly decreased skin roughness was already measured after 4 weeks of intervention. Exemplarily, a macro photograph highlights the results. Due to the frontal view, the periorbital region is not fully displayed. However, the photograph shows a clear reduction of wrinkles in the forehead area (Figure 4).

The results presented in this study are in accordance with a previously published clinical trial in women, where the intake of 2.5 g collagen peptides resulted in a 7% and 20 % reduced wrinkle depth

Table 1: Demographic and baseline data of the study cohort.

Variable	Mean	SD
Age [years]	54.6	6.1
Height [m]	1.68	0.06
Weight [kg]	69.06	9.15
BMI [kg/m^2]	24.45	2.95
Wrinkle Score [a.u.]	4.1	1.0
Capacitance [a.u.]	61.69	11.74
Skin roughness Ra [μm]	23.90	6.02
Skin roughness Rz [μm]	95.82	24.47

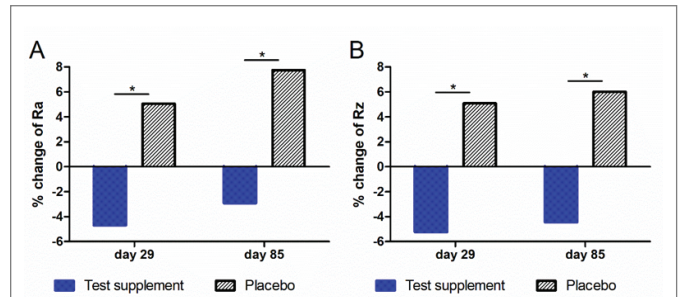


Figure 2: Change of periorbital wrinkles: roughness parameters Ra (fine structure) and Rz (rough structure), shown as percent change to baseline after intake of the test supplement (blue) and placebo (grey). Study population: all subjects.

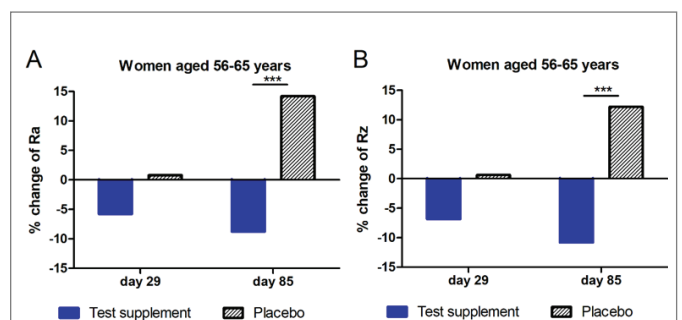
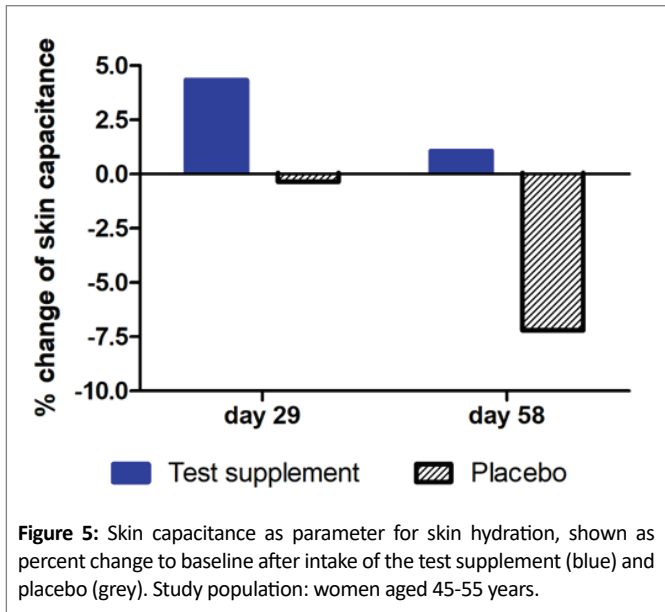


Figure 3: Change of periorbital wrinkles: roughness parameters Ra (fine structure) and Rz (rough structure) shown as percent change to baseline after intake of the test supplement (blue) and placebo (grey). Study population: women aged 56-65 years ($n=30$).



Figure 4: Exemplary macro photographs of the face before (day 1) and after intake of the test supplement (day 85) showing reduced wrinkles in the forehead area.

at 4 weeks and 8 weeks, respectively [18]. Furthermore, previous investigations demonstrated that the daily intake of 0.3 g collagen peptides, together with hyaluronic acid and chondroitin sulfate for 12 weeks significantly reduced facial wrinkles [19], and that an intake of 2.5 g collagen peptides and additional micronutrients over a course of 12 weeks significantly reduced skin roughness [20].



Collagen-mediated improvement of skin hydration in mid-aged females

In this study skin hydration was measured with the electrical capacitance method using the Corneometer. No significant differences over the 12-week intervention period were observed between the study groups, when referring to the whole study population. However, when focusing on mid-aged females (45-55 years, n=36), skin hydration improved in the collagen supplement group (+4.3 %; day 29; +1.5 %; day 85), with the skin being slightly moister compared to the placebo group after 12 weeks (p=0.091) (Figure 5).

Similar to the herein presented results, skin moisture was positively influenced in women aged 35-55 years by a 8-week treatment with collagen hydrolysate (2.5 g or 5 g) and results did also not reach statistical significance [21]. Likewise, in the study by Schwartz and Park, water content was higher (+ 8 %) after 12 week-intake of 0.3 g collagen peptides, hyaluronic acid and chondroitin sulfate, however the increase was not significant [19].

Another clinical trial, including women with normal to dry skin, showed that the 8-week-intake of 10 g fish or porcine collagen resulted in a significant increase in skin hydration (+ 12 % or + 28 %, respectively) [22]. Of note, the dose of collagen peptides used in the study by Asserin J, et al. [22] was higher compared to the one present in the collagen study product. A further study describes a significant increase in skin hydration in males and females after daily intake of 3 g collagen peptides, however after a longer supplementation period (12 weeks), compared to the herein performed study [23].

The influence of the collagen supplement on skin hydration in this study might have been confounded by seasonal changes. The study started in January and was finished in April, and dryness in the ambient air might have changed within this time period.

Collagen microstructure was not affected by intake of the collagen supplement

Collagen microstructure, as assessed by confocal microscopy, appeared indifferent between placebo and the collagen product in this study. No significant differences were found between treatments or assessment times (data not shown). Presumably, the selected

measuring technique was not sensitive enough to detect subtle changes, for instance regarding the different collagen types. Confocal microscopy records variations in the refractive index of different microstructures, and these refractive indices are similar between all collagen types. Moreover, albeit confocal microscopy is able to detect differences in the degree of collagen diffuseness, changes towards more diffuse collagen structures, present in younger and more subtle skin, might not get evident over a time period of 3 months. In contrast, in the study by Asserin J, et al. including 106 healthy women, a reduced collagen fragmentation after the intake of 10 g fish peptides (4 times the dose of the study product) could be observed by using confocal microscopy [22]. These results support, that intake of external collagen peptides impacts the degree of fragmentation in the human dermis.

Product safety

Overall, both study products were well tolerated. In total, twelve adverse events were documented by 9 subjects during study conduct in the collagen group, where of one was classified as being serious (lumbago with short inpatient hospitalization), but none was related to the test product. Equal amounts of adverse events (eleven), albeit in study population half as large, were reported by 7 subjects in the placebo group. None of these adverse events was classified as serious, but for two adverse events a relation to the placebo product could not be excluded (Exacerbation of neurodermatitis with eczema on hand and on skin under left eye). Overall, the study confirmed the good safety profile for the collagen supplement. In both study groups, subjects confirmed the easy and uncomplicated intake of study products (100% of agreement), which were well tolerated.

Conclusion

Concluding, in this study we documented a significant mild positive cosmetic effect on an important anti-aging parameter, which is reduction of wrinkle depth in the facial area after a 12-week lasting application of the collagen test product compared to placebo. Of note, this positive effect was already evident after 4 weeks. The appearance of the skin was positively altered by a significant improvement in fine and coarse structures. The data show that especially older women with already more pronounced wrinkle relief may benefit from the application of the collagen supplement.

Overall, the study results are in line with other clinical studies investigating effects of collagen hydrolysate or of combination products with collagen hydrolysates as main ingredient [5,10]. However, the extent and characteristic of the impact differs between investigations. External factors might impact study results and hamper direct comparisons. Factors contributing to these differences are, for instance, differing study collectives and designs, seasonal factors, differences in applied methodologies and dosages of nutrients, as well as normal physiological variations.

Aside from collagen peptides, the test product contains different micronutrients, including vitamin C, zinc, vitamin E, β -carotin, biotin and copper. These micronutrients can contribute to the maintenance of normal skin and connective tissue, as these vitamins and minerals function as cofactors in various metabolic processes related to skin physiology and collagen metabolism [13-15]. Thus, the herein tested collagen supplement benefits from the interplay of the contained collagen peptides with selected essential nutrients.

In summary, the obtained results support the product concept of Doppelherz system KOLLAGEN BEAUTY to support skin beauty from within.

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