

Original Article

Effects of a nutritional supplement containing collagen peptides on skin elasticity, hydration and wrinkles

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ABSTRACT

Context: Many people around the world strive to achieve younger-looking skin. This is often promised by topical treatments. Oral treatments for skin ageing have been unsuccessful due to their constituents being broken down by acid and enzymes in the gut; however several studies have shown that hydrolyzed collagen is absorbed in the gut and then delivered to skin and joints through the blood stream.

Aims: The aim of this study was to determine whether an oral nutritional supplement drink containing hydrolyzed collagen and other specific ingredients reported to have antiageing properties, would have a positive effect on skin wrinkling, elasticity and hydration.

Materials and Methods: A double-blind, randomized, placebo controlled clinical trial was conducted on healthy subjects to assess whether this oral supplement could improve certain specific skin properties of post-menopausal women, namely depth of facial wrinkles, skin elasticity and hydration.

Results: The evidence provided here proves that the combination of specific ingredients present in this nutritional drink acts to significantly reduce the depth of facial wrinkles and increase skin elasticity and hydration.

Conclusions: This study shows that the oral nutritional supplement consisting of hydrolyzed collagen, hyaluronic acid, and essential vitamins and minerals, leads to a significant improvement in wrinkle depth. It is also able to induce noticeable improvement in elasticity and hydration of the skin.

Key words: Hydrolyzed collagen, nutritional supplement, skin ageing, skin elasticity, skin hydration, wrinkles

INTRODUCTION

Skin is the largest organ in the body and like other organs, it changes with time. The alterations due to the ageing process include elastosis, leathery aspect or roughness, pigmentation, and the appearance of fine lines. As well as changes due to natural ageing process (known as intrinsic ageing), skin is subject to deterioration caused by dermatological disorders or environmental

conditions (wind, air conditioning, heating). The intrinsic ageing process may also be accelerated by exposure to the sun (photo-ageing)^[1,2] or other lifestyle issues (extrinsic ageing: smoking, alcohol, stress, lack of sleep).^[3,4]

There are many fundamental processes that lead to the changes observed in aged skin, such as alterations in the turnover of the extracellular matrix proteins within the dermal layer of the skin, a rise in the inflammatory markers, and reduced blood flow. Fine lines begin to appear when the breakdown of collagen within the dermis exceeds its synthesis. In fact, as the main component of skin, together with elastin and hyaluronic acid, collagen has a key role in providing integrity and elasticity to this organ.

Collagen represents a family of 28 different proteins,^[5] which account for 30% of the total protein mass in the

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human body and play a pivotal role in the structure of several tissues, such as skin and bones, providing rigidity and integrity.^[6] All members of this family share a triple-helical structure composed of α -chains;^[7] multiple triple-helices form a collagen fibril. In general, collagen fibrils are made of different collagen types: collagen I and III in the skin; collagen II and III in cartilage.^[8]

The first evidence that collagen peptides may have a chemotactic activity on fibroblasts was presented by Postlethwaite *et al.*^[9] in 1978. In this study the chemotactic response of human dermal fibroblasts to type I, II, and III human collagens and collagen-derived peptides was observed with an *in vitro* assay. The result showed that all three native human collagens and constituent α -chains could act as chemoattractants for fibroblasts *in vitro*. In addition, di- and tri-peptides containing the hydroxyproline were chemotactic for fibroblasts. The authors suggested that both collagen and collagen-derived peptides might function as chemotactic stimuli for fibroblasts also *in vivo* and attract these cells to repair damaged tissues.

As mentioned previously, the processes that bring about the changes associated with skin ageing take place in the deep dermal layer and therefore they may not be affected by topical products. Influencing the collagen-producing fibroblasts in the dermis through the intake of different nutrients is a more effective method. Interestingly, alterations in diet can change the way skin functions as evidenced by the effects of dietary deprivation on skin health. For example essential fatty acid deficiency^[10] or accumulation of abnormal fatty acids^[11] results in so-called skin scaling and poor skin barrier function. Moreover, a recent publication shows that in a placebo-controlled double-blind study, the addition in the diet of the omega-3 oils from flaxseed and omega-6-rich oils from the borage plant leads to a decrease in skin roughness and scaling.^[12]

Scientific studies provide evidence that nutrients can help lessen the effect of skin ageing and improve skin appearance. Such nutrients may have specific properties, which attract water molecules thus improving hydration in the skin, scavenge free radicals or reduce inflammation. Vitamins such as Vitamin E and Vitamin C form part of the skin's natural defense against reactive oxygen species and their interaction is thought to be particularly important in the protection of skin against photo oxidation.^[13] Vitamin C is also an essential co-factor in the biosynthesis of collagen, an important process in the prevention of skin ageing.^[14]

Consumption of hydrolyzed collagen has also been shown to have beneficial effects in the skin. Three studies from

Japan in particular have demonstrated a clear effect. The benefits of daily ingestion of hydrolyzed collagen (10 g) on skin hydration of 20 healthy Japanese women compared to the placebo group (19 volunteers) were evaluated by Sumida *et al.*^[15] In comparison with the placebo group, gradual improvement of water absorption capacity was observed through 60 days in volunteers who ingested collagen peptides. Matsumoto *et al.*^[16] presented results of a trial also suggesting that a daily ingestion of collagen peptides improve skin hydration. The authors reported subjective improvement of the skin condition of woman's volunteers after ingestion of fish collagen peptides for 6 weeks. The percentage of positive response between the subjects was very high. This study was followed by a double-blind placebo-controlled study by the same research group^[17] on healthy women volunteers aged 25-45. In this study 2.5, 5 and 10 g of fish collagen peptide were administered and compared to the placebo. The hydration of the stratum corneum was measured at baseline and after 4 weeks. A significant difference was observed in subjects older than 30 years between the treated group (5 g and 10 g) and placebo.

When administered orally, hydrolyzed collagen reaches the small intestine where it is absorbed into the blood stream. Several scientific studies have described the bioavailability of hydrolyzed collagen after oral administration in animals and humans. ¹⁴C-labeled hydrolyzed collagen was used in a study by Oesser *et al.*,^[18] who investigated the time course of hydrolyzed collagen absorption and its subsequent distribution in various tissues in male mice. The test group received 10 mg of ¹⁴C-labeled gelatin hydrolyzate/g body weight, while the control group ¹⁴C-labeled proline, along with unlabeled gelatin hydrolyzate (10 mg/g body weight). The time course of radioactivity within the mice subsequent to absorption of orally administered ¹⁴C-labeled hydrolyzed collagen was measured. The results showed that about 90% of orally administered hydrolyzed collagen was absorbed within the first 12 h from the intake. Radioactivity in skin attained its peak values 12 h after the administration of ¹⁴C-labeled hydrolyzed collagen and in contrast to plasma, ¹⁴C-activity remained relatively high up to 96 h.

Through the network of blood vessels, collagen peptides are distributed in the human body, in particular to the dermis, where it has been proven they can remain up to 14 days.^[19] The function of collagen peptides on the migration and growth of mouse skin fibroblasts was investigated by Shigemura *et al.* in Japan.^[20] They reported that the number of cells migrating from the explanted skin increased significantly after treatment with

proline-hydroxyproline (Pro-Hyp) peptide. They also showed that Pro-Hyp increases significantly fibroblasts growth.

The aim of the present study is to evaluate the effect of hydrolyzed collagen together with a combination of other ingredients reported to influence key factors involved in skin ageing: namely collagen synthesis. A supplemented drink containing hydrolyzed collagen, hyaluronic acid, vitamins and minerals was given to a group of women. Females aged 45 years and above were considered as a useful cohort to study, with respect to skin ageing, as the quality of skin declines dramatically with age, especially following the menopause. Any intervention aimed at correcting the symptoms of skin ageing is therefore likely to have a greater effect in this target population.

MATERIALS AND METHODS

Study design

A double-blind, placebo-controlled study was conducted by an independent Clinical Research Organization (Wales, UK) between April and July 2012. Eligible subjects were Caucasian, healthy, nonsmoking females aged 45-64 years, who were not using other nutritional supplements. 18 females were enrolled into this study. All subjects completed the study and no adverse events associated with the product were recorded.

Subjects were allocated to two different groups according to a preprepared randomization code. Study sponsor, investigators and statisticians remained blind to the allocation in order to avoid bias. One group was given the test product containing all the active ingredients. Test product was a fruit-based drink containing Vitamin C, Vitamin E, hydrolyzed collagen, among other ingredients, outlined in Table 1. The other group was given placebo drink containing no active ingredients. Subjects were asked to consume one dose of their allocated drink per day before breakfast for 12 weeks and to complete a diary card recording this. Subjects were instructed to keep the product out of reach of children and store it in a cool and dry place, away from direct sunlight and heat. Subjects were informed that once a bottle was opened, it had to be consumed within 24 h.

The properties of the skin on the inner aspect of the left forearm were assessed using a noninvasive instrumentation. All assessments were carried out at weeks 0, 3, 6, 9 and 12 [± 2 days; Table 2]. The assessments were for skin hydration, skin elasticity and skin surface micro-topography. In order to standardize the area for measurements, a template was constructed that allowed the marking of three adjacent test areas on the forearm corresponding to the three measurement techniques

Table 1: Study products and active ingredients

Ingredients in test product	Ingredients in placebo
Water	Water
Hydrolyzed collagen	Glucose fructose syrup
Glucose fructose syrup	Citric acid anhydrous
Citric acid	Stabilizer (soybean polysaccharide)
Soybean polysaccharide (stabilizer)	Peach flavor
Malic acid	DL-malic acid
Ascorbic acid (Vitamin C)	Sucralose
Flavoring substances (peach flavor)	
Hyaluronic acid	
Borage oil emulsion (borage oil 20%)	
d-alpha-tocopherol (Vitamin E)	
N-acetylglucosamine	
Sucralose	
Zinc gluconate	
Pyridoxine hydrochloride (Vitamin B6)	
Piper nigrum (black pepper extract)	
Copper (cupric gluconate)	
D-biotin	
Vitamin D3	

Table 2: Study design

Week	Activity
-1	Administration and recruitment. Consent procedures. Evaluation of inclusion/exclusion criteria. Relevant medical history
0	Subjects enrolled - baseline measurements using Corneometer [®] , Cutometer [®] and Visioline [®] . Dispensal of investigation product and patient diary
3,6,9	Measurements using Corneometer [®] , Cutometer [®] and Visioline [®] . Compliance check. New products dispensed. Gather adverse event reports
12	Measurements using Corneometer [®] , Cutometer [®] and Visioline [®] . Gather adverse event reports. Complete a short questionnaire. Compliance check. End of study

used. The position of this template on the forearm was by means of reference to the center of the elbow crease and the wrist.

The Cardiff Independent Research Ethics Review Committee approved the study, and all subjects gave written informed consent. All the subjects were monitored for the occurrence of serious adverse events during the study.

Study outcomes

The primary endpoint of the study was the measurement of wrinkle depth. This was assessed using a skin surface micro-topography system (Visioline[®] VL 650, Courage and Khazaka, GmbH) Cutometer: (Cutometer[®] dual MPA 580, Courage and Khazaka, GmbH) Corneometer: (Corneometer[®] CM 825, Courage and Khazaka, GmbH) were also measured. All instruments were calibrated according to the manufacturer's instruction before the start of the analysis.

Study products

Study drinks were prepared on behalf of MINERVA Research Labs Ltd., in Japan. Drinks (50 ml units) were supplemented

with the required level of individual active ingredients, except those used for the placebo group. To preserve study blinding, all test and placebo products were of similar appearance and were packaged and labeled in an identical way.

Statistical analyses

Study data were analyzed using Wilcoxon signed rank test with the change from week 0 after 3, 6, 9 and 12 weeks. Results were considered significant if $P < 0.05$ (95% confidence interval) as calculated using R (www.r-project.com). P values for the differences between test group and placebo were calculated using Mann-Whitney U-test. The two nonparametric tests were used as the sample size was small and we could not assume that our population was normally distributed.

RESULTS

Wrinkle replica analysis

Skin roughness is considered one of the main indicators of wrinkle depth.^[21] To assess wrinkle depth, silicone rubber replicas were taken from the inner part of the left forearm at the beginning of the study and at 3, 6, 9, 12 weeks, and analyzed at each time point using the Visioline[®] equipment. The values were automatically calculated for each replica by the image analysis software.

The mean starting values in both groups were very similar. As the study progressed, the mean values showed little variation. However by week 9, the group taking the test product experienced statistically significant reduction in average wrinkle depth, compared to week 0 [Figure 1]. This effect was not observed in the placebo group.

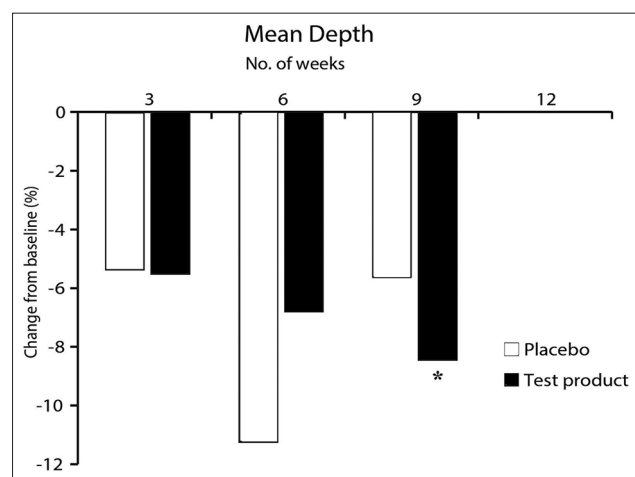


Figure 1: Mean wrinkle depth values, expressed as percentage change from week 0. At week 9 a significant decrease was observed in people taking the test product. Mean \pm standard deviation was calculated for the wrinkle depth values both for test product and placebo groups. The mean was then used to calculate percentage change from week 0. Significance (*) is displayed compared with week 0 using Wilcoxon

A placebo effect was noted, and there was some reduction in the wrinkle depth in the placebo group, mainly after 6 weeks. However this was not significant when compared to week 0. Furthermore, with the test product, there was a gradual reduction in the depth of wrinkles from 5% to 8% by week 9, whereas with placebo there was no visible trend. By week 12 the values for placebo had increased and were close to those for the group taking the active supplement, which also returned to baseline values.

Interestingly, a clear correlation between the average values of wrinkle depth at week 9 and the percentage of reduction is shown: in fact, the greatest reductions in wrinkles depth were reported for deeper wrinkles [Figure 2].

Firmness and elasticity

Skin elasticity was measured using Courage and Khazaka Cutometer[®] (probe aperture size 8 mm) at week 0 and week 3, 6, 9, and 12. Three measurements were taken from the site on the inner part of the left forearm. The data were sorted and the values for the Cutometer[®] parameters R5 (net elasticity) and R7 (ratio of immediate retraction to total deformation-Biological elasticity) obtained. The average value for R5 and R7 was calculated for each subject at each time point. This value was used in subsequent analysis.

Statistically, significant difference from week 0 was observed for the treated group after 6 weeks. The data continued to be significant at week 9 and week 12 [Figure 3]. This was seen with both net elasticity [Figure 3a] and biological elasticity [Figure 3b]. However, no significant difference was observed between placebo at week 6 and week 0. No statistical significance was observed between test product and placebo. Two illustrative examples of treatment over time are shown in Figure 4, both for test

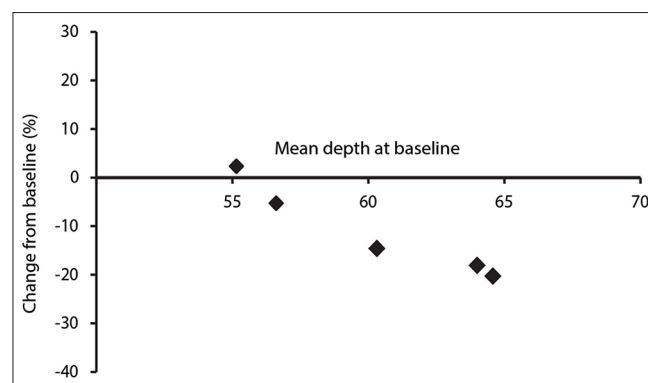


Figure 2: A comparison of percentage change in wrinkle depth against baseline values. A correlation between the values of wrinkle depth at baseline and the percentage of change from baseline at week 9 is shown: the deeper the wrinkle the greater the reduction in depth

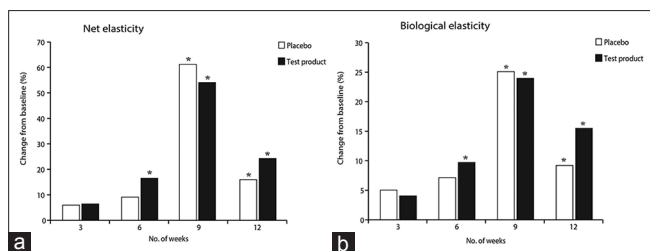


Figure 3: Measurements of elasticity (Cutometer®), expressed as percentage change from week 0. A significant increase both in net elasticity (a) and biological elasticity (b) was observed in people taking the test product after 9 weeks. Results are displayed as percentage change from week 0 calculated by using the mean of the readings for each group taken at each time point (a) net elasticity, (b) biological elasticity. Significance (*) is displayed compared to week 0 using Wilcoxon

product and placebo. In the case of the subject who was taking the test product a constant increase in elasticity was observed at 6, 9 and 12 weeks [Figure 4a]. On the contrary, this trend was not present in the subject treated with placebo [Figure 4b].

Skin hydration

Skin hydration was also measured using Courage and Khazaka Corneometer® at week 0 and after 3, 6, 9 and 12 weeks of intervention. Five measurements were taken from the site on the inner part of the left forearm and the average value calculated for each subject at each time point. This value was used in subsequent analysis. After 6 weeks, there was a significant increase in hydration from week 0 in people taking the test product [Figure 5]. However, no statistical difference was measured between placebo and test product at any time point (data not shown).

DISCUSSION

The aim of this double-blind, randomized controlled study was to discover whether an oral supplement containing a select blend of ingredients, put together as a pleasant tasting drink, would be effective in improving skin properties. The investigation was carried out according to the principles of International Good Clinical Practice. The products were coded and provided to participating subjects at random. All subjects, investigators and statistician remained “blind” to the coding until initial data analysis was complete.

A group of 18 postmenopausal women were asked to test the product, and the results were compared to a similar group of women taking placebo.

The results show that a combination of hydrolyzed collagen and hyaluronic acid, together with other ingredients, when consumed orally for 9 weeks can significantly reduce

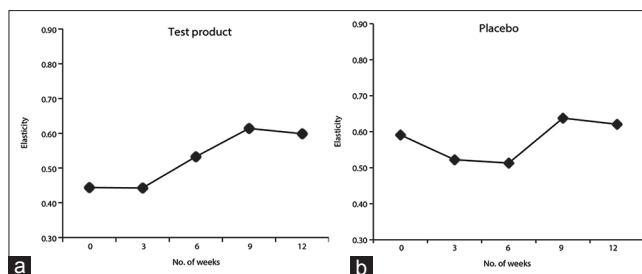


Figure 4: Example of treatment over time on elasticity. (a) Female aged 64 taking test product. (b) Female aged 63 taking placebo

the depth of wrinkles, whereas there was no significant reduction with placebo. In fact, there was 8% reduction in wrinkle depth in the group taking the test product, which was found to be significant ($P = 0.037$).

By comparing baseline values at week 0 with wrinkle depth after 9 weeks treatment, we demonstrated a correlation between the two that is, the deeper the wrinkle, the greater the reduction in depth. This finding provides an indication to the possible mechanism of action of the product. Fine lines probably result from alterations in the surface of the skin or the epidermis, whereas deep wrinkles are formed by changes in the dermis. Thus, nutritional supplements that have an effect on the dermis will most likely reduce the deeper wrinkles to a greater extent compared with products that act on the surface of the skin.

We also found that the specific formulation of nutritional ingredients within the test product, proven to have benefits on the skin, enhance skin elasticity. Even though, there was the lack of significant overall difference between elasticity values of subjects taking test product compared to the placebo group, a significant increase in elasticity was observed at week 6 with the test product. This suggests the product may have some beneficial effect on the elastin network.

We also demonstrated significant benefit of the test product on skin hydration of individuals consuming it on a daily basis. The water content of the dermis increased by 14% at week 6 from the baseline value. Even though, some increase in hydration was also observed with placebo, this was not significant compared to week 0, confirming that the retention of water molecules in skin tissues is primarily mediated by the active ingredients present in the test product. Thus, this study supports previous finding that the intake of hydrolyzed collagen protected mice against dermal dehydration.^[22]

Recently, there has been a growing interest in the role of nutrients in promoting skin health, with much research carried out in this area. There is clinical evidence that

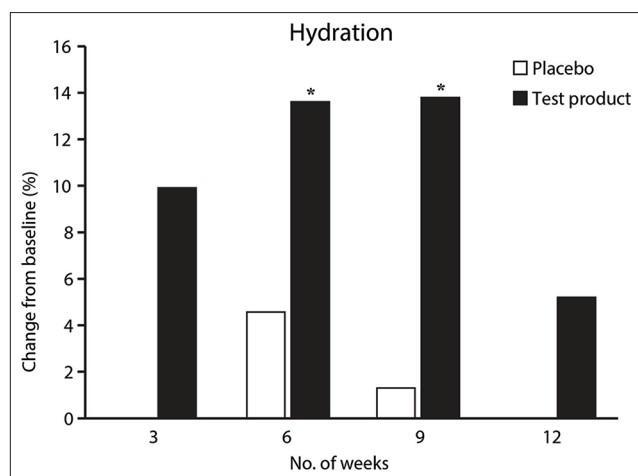


Figure 5: Measurements of hydration (Corneometer®), expressed as percentage change from week 0. A significant increase in skin hydration was observed in people taking the test product after 6 weeks. This increase was still significant after 9 weeks. The mean values were calculated and the results are displayed as percentage change from week 0. Significance (*) is displayed compared with week 0 using Wilcoxon

shows the positive effects of Vitamins E and C in case of photodamage.^[23,24] In addition, we have demonstrated the benefits of taking oral hydrolyzed collagen in combination with hyaluronic acid and vitamins. We have shown that this oral supplement would be particularly beneficial for postmenopausal women as abrupt decline in estrogen levels leads to less collagen production and the onset of visible skin ageing.^[25-27]

The decline in the production and quality of collagen with age is thought to be a key factor in the appearance of wrinkles and reduced elasticity.^[28] It means any product that aims to counteract the ageing process would need to replenish collagen levels within the dermis. The reduction in wrinkle depth observed suggests the test product used in this study acts by increasing collagen in the skin. The efficacy of the test product described here is supported by other clinical trials. In a pilot open-label study, Schwartz and Park investigated the effect of a dietary supplement, containing a hydrolyzed collagen type II, hyaluronic acid and chondroitin sulfate in 26 healthy females who displayed visible signs of natural and photo-ageing in the face. Daily supplementation with 1 g of hydrolyzed collagen for 12 weeks led to a significant reduction of skin dryness/scaling and global lines/wrinkles. In addition, a significant increase in the content of hemoglobin and collagen in the skin dermis was observed after 6 weeks of supplementation. At the end of the study, an increase in hemoglobin remained significant, while the increase in collagen content was maintained, but the difference from baseline was not significant. The authors suggested that dietary supplementation with hydrolyzed collagen can physiologically counteract natural and photo-ageing processes to reduce visible aging signs in

the human face. A placebo-controlled study is necessary to verify these observations.^[29]

In 2005 Béguin tested the efficacy and safety in skin ageing of a micronutrient supplement, containing marine proteins, with 4 months randomized double-blind controlled study. The trial included 40 subjects. The supplement was tested against placebo for 3 months followed by a 1-month supplement-free period to assess lasting effects. Efficacy measurements included skin surface evaluation, ultrasound measurement of sun-exposed and protected areas of the skin and photographic assessment. All investigated parameters showed a continuous and significant improvement in the active group during the 3 months of supplementation as compared to placebo. Photographs showed visible improvement of the overall skin appearance and reduction of fine lines. Ultrasound measurements showed an increase in dermis density of up to 78% in the active group. The final assessment after 1-month without supplementation showed no further improvements, but a slight decrease was observed in most improved parameters. No treatment-related side-effects were reported. The study demonstrated that the supplement appears to be effective and safe as an oral supplement to protect the skin and support its repair process. Further evaluations are needed.^[30]

In another study, Choi *et al.* evaluated the effect of daily collagen peptide supplementation on skin properties. 32 healthy volunteers were randomized to receive for 12 weeks either:

- No supplement
- Collagen peptide 3 g
- Collagen peptide 3 g and Vitamin C 500 mg
- Vitamin C 500 mg.

Skin properties such as hydration, transepidermal water loss and elasticity were evaluated. The data showed that daily collagen peptide supplementation improved skin hydration and elasticity, but concomitant intake of low-dose Vitamin C did not enhance the effect of collagen peptide on skin properties.^[31]

In conclusion, this study demonstrates that daily consumption of the test product in its current formulation is able to induce a clinically measurable improvement in the depth of facial wrinkles, skin elasticity and hydration.

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