The effect of SOLUGEL® collagen peptides intake on skin conditions: a randomized double-blind clinical trial

[1] Telma Garcia, MSc; [2] Stephan Dierckx, PhD; [1] Yingying Wu, MSc; [1] Milagros Patrizi, BSc; [1]Erwin Quanten, PhD; [1] Reyhan Nergiz Unal, PhD

[1] PB Leiner, part of Tessenderlo Group, [2] Tessenderlo Innovation Center

© 2022 – PB Leiner proprietary information. All rights reserved.

Abstract

Background: Collagens are particularly important for the strength and resilience of the skin. Changes in the amount and structure are thought to be responsible for the deterioration of the skin. Therefore, the aim of the study was the in vivo assessment of the effect of the daily intake of hydrolyzed collagen composed of collagen peptides alone as a bioactive ingredient, on skin hydration, elasticity, and firmness of the skin in a clinical study design.

Methods: A randomized double-blind clinical trial study on women aged between 35 and 55 years was undertaken in order to investigate the effect of the oral intake of collagen peptides (10g/day of SOLUGEL®) for 8 weeks on skin properties. A total of 54 women selected randomly according to strict participation criteria were included in the study. Skin hydration was measured by the Corneometer®; firmness and elasticity were measured by the Cutometer®; the clinical scoring of skin condition was observed by an expert dermatologist working in the field; additionally, self-assessment of the skin improvement perceived by the subjects was also observed.

Results: In a relatively short period, the oral intake of 10g collagen peptides has a significant increase in the moisturizing effect of 14% on the skin after 8 weeks. Furthermore, collagen peptide intake led to improvements in the biomechanical properties of the skin, such as firmness (17.4%) and elasticity (10.6%) compared to baseline. Clinical score data showed a significant improvement of skin elasticity of 17.1% and skin moisture with an increase of 20.6%. Supplementation with SOLUGEL® collagen peptides was found to be well-tolerated.

Conclusion: The benefits of taking a daily dosage of collagen peptides for 56 days are seen in the significant improvement of the skin condition and aspects such as hydration, firmness and elasticity. Collagen peptide intake for a longer period would offer the natural solution to wrinkle formation and skin aging.

Keywords: Collagen peptides, skin anti-aging, hydration, firmness, elasticity

Introduction

Collagen is the most abundant protein in the human body and a major protein in the skin's structure [1]. Collagen represents up to 35% of the total proteins present in the body and ensures the cohesion, elasticity, and regeneration of the tissue [2]. Collagen is synthesized by specific cells called fibroblasts and is assembled in connective tissues. Skin properties are conferred by its particular architecture, resulting in the interactions between the extracellular matrix and the fibroblast cells, allowing both the synthesis and the degradation of the matrix components such as collagen for hydration, elasticity and firmness of the skin [3].

The structure, integrity and appearance of the skin are affected by various environmental factors (ultraviolet radiation, nutrition, etc.) and especially chronological aging [4, 5]. During chronological aging, all the layers of the skin (epidermis, dermis and hypodermis) undergo modifications [5, 6]. Within the dermis, the fibroblast density declines, which leads to reduced synthesis of matrix elements [7]. Moreover, fibers are damaged by oxidant stress, they lose their elasticity due to glycosylation and their features due to decreased synthesis of anabolic enzymes. With aging, decreases in the hydration of the outermost layer of the skin epidermis stratum corneum, impairment of sebaceous and sweat glands, and increases in transepidermal water loss occur [6, 7]. All these processes contribute to the loss of skin hydration, structure and firmness observed during aging [7]. On the other hand, importantly, in the aging process, the production of collagen by fibroblasts slows down and from the age range of 25-30 its level diminishes within the body, leaving the first aging signs to appear: for example, the appearance of wrinkles and loss of skin elasticity and firmness [8].

Oral collagen supplements have been proposed to modulate skin functions and can, therefore, be useful in the treatment of skin aging [9]. A small amount of accumulating clinical studies have shown that, in addition to a healthy and balanced life, oral hydrolyzed collagen can improve skin aging. This supplementation stimulates the growth of fibroblasts, which are responsible for the collagen synthesis, and, in addition, the elastin and hyaluronic acid synthesis that would allow for improving skin moisture and less wrinkle formation [10-13].

Collagens are particularly important for the strength and resilience of the skin [3, 8]. Collagen was shown in preclinical studies to improve skin barrier function, which induces the synthesis of collagen and hyaluronic acid and promotes the growth and migration of fibroblasts [14-18]. Notwithstanding this, changes in the amount and structure of collagen in the skin may be responsible for wrinkle formation, loss of skin elasticity, and hydration [19, 20]. Various supplements containing collagen peptides are claimed to have skin anti-aging properties; however, there are a limited number of human studies supporting each of these claims for collagen hydrolysate alone [13, 21-23]. There is still a gap in research to fill the effectiveness of supplemented hydrolyzed collagen alone on skin biophysical parameters related to cutaneous aging. Therefore, the aim of the study was the in vivo assessment of the effect of the daily intake of hydrolyzed collagen composed of collagen peptides (SOLUGEL®) alone as a bioactive ingredient, on skin moisture-related skin properties (hydration), and on biomechanical properties (elasticity and firmness) of the skin in a clinical study design.

Materials and Methods

Subject inclusion and exclusion criteria

This study was a blinded randomized and mono-centered clinical trial. The sample size was

estimated by power analysis of means. 29 healthy Caucasian women for each group aged 35–55 years (mean age: 45.34 years) with average body weight (mean weight: 58.9kg) were recruited based on the inclusion/exclusion criteria. A total of 58 participants were enrolled and 54 concluded the study. This study resulted in the following number of valid cases: 26 for the 10g SOLUGEL® group and 28 for the placebo group.

Inclusion criteria were Fitzpatrick skin phototypes II and III, body mass index from 18 to 25kg/m^2, the presence of wrinkles on the face, crow's feet with a grade ranging between ≥ 2 and < 5 (Bazin R. and Doublet E. Skin Aging Atlas, Vol. 1, p. 41), the presence of dry skin on the face (corneometric index range between 30UA and 50UA at the level of one cheekbone) and no applied body care products. Exclusion criteria were pregnancy, smokers, diabetics, those addicted to alcohol or other narcotic drugs, history of adverse reaction to cosmetics, systemic diseases, auto-immune disease, skin disease and the use of any cosmetics or supplementation during the study.

Study manipulation period

The study was approved by the independent French Ethics Committee, *Les Comités de protection des personnes* (CPP) and authorized by the French competent authority, *l'Agence nationale de sécurité du médicament et des produits de santé* (ANSM). The recruitment of the subjects was performed through the COSderma laboratory's database and declared to the CNIL under number 1015566. Each subject participating in the test was fully informed and gave informed consent in accordance with the last revision of the Declaration of Helsinki . The study was carried out in 2017 by the contract research organization Les Laboratoires COSderma (Bordeaux, France), in cooperation with the Dermatology Unit at Hôpital Saint-André (Bordeaux, France) and in accordance with the regulatory requirements (International Conference on Harmonization [ICH] Good Clinical Practices [GCP] (ICH Topic E6 ref: CPMP/ICH/135/95).

The participants were instructed not to use cosmetic products two weeks before and during the study period. All participants agreed to avoid being exposed to the sun during the period of the study. All the measurements were performed before the oral collagen peptide intake (baseline) and after 8 weeks of daily intake. The effects were eventually perceived by the subjects who received the tested product and determined by the dermatologist at the hospital.

This study was performed for a duration of 10 weeks: a pre-participation period (wash-out period) of 2 weeks during which subjects were allowed to use neutral cream, followed by 8 weeks of collagen peptide supplementation period. One sachet of instant powder beverage containing 10g/day of SOLUGEL® collagen peptides or 10g/day maltodextrin was taken once in the evening before bedtime. SOLUGEL® pure collagen peptides is an instant powdered product that is fit for human consumption, manufactured by PB Leiner (Part of Tessenderlo Group, Belgium) according to a production process and in respect of specific hygiene rules that are compliant with the European Regulation (EC) n° 853/2004. The proposed placebo for the study was corn maltodextrin (dehydrated based corn glucose syrup) of which the appearance, the organoleptic quality and the caloric value were similar to those of the collagen peptides. The content of the sachet (sugars, acidifier, flavoring and natural colorings) was diluted in a glass filled with water (150mL).

The assessment of the hydration of the upper layers of the epidermis by capacitance measurements

The hydration level of the upper layers of the epidermis on one cheekbone was assessed with the Corneometer® CM 825, which ran at frequencies between 0.9 and 1.2MHz. The surface of measurement for the probe was 49mm². The capacitance of the system (electrodes and upper skin layers) was influenced by changes in the dielectric constant of the skin surface. The dielectric properties of the medium and, consequently, the capacitance of the system, were modified when the hydration level of the stratum corneum varied. The capacitance values (corneometric indexes) were collected and expressed in arbitrary units (AU) ranging from 0 to 120 [24, 25].

The instrumental measurements were performed in triplicate according to a consecutive, shift method. The measurements of a randomly chosen cheekbone (on the bone, specifically the higher part of the cheekbone) were performed at baseline and after 8 weeks of collagen peptide intake. The corneometric value given as a result is the mean of the three measurements. Very dry skin was characterized with Corneometer® units below 30; dry skin was characterized between 30 and 50; and normal skin was characterized as higher than 50 arbitrary units.

The assessment of the hydration of the cutaneous surface by illustrative acquisitions

The hydration level of the upper layers of the epidermis was assessed using the Moisture Map. In addition to the Corneometer® measurements of hydration, quantitative Moisture Map analysis was performed. With this analysis, the distribution of water could be shown [11, 12]. The principle of measurement was based on a capacitance measurement of the dielectric environment. Any change in the level of hydration of the skin's surface engendered a modification of the dielectric constants and the capacitance measurement. It was performed on 2 balanced groups, each comprising 5 subjects. The 10 subjects were selected randomly amongst all subjects already included for the other techniques of this study. The measurements were performed by the responsible technician on one randomly chosen forearm at baseline and after 8 weeks of collagen peptide intake. Illustrative pictures were taken to show the hydration of the skin.

The capacitance measurements reflect the level of hydration of the skin that is expressed in the software of acquisition in the form of a picture of gray levels of the studied area. The scale of gray level was expressed as follows: (i) gray level: « 0 » = black picture (value of calibration with an aqueous gel), (ii) gray level: « 255 » = white picture (measurement in the air).

The assessment of the biomechanical properties of the skin

The biomechanical properties of the skin were measured by cutometric measurements (Cutometer®) on one temple. Briefly, the principle of measurement was based on the suction and the strain. The device generates a negative pressure (between 20 up to 500mbar) that causes, the zone of skin to be measured to be sucked up in the opening of the probe by this depression. When the depression stops, the skin returns to its initial state (phase of relaxation). The depth of penetration of the skin inside the opening of the probe was determined without contact by an optical measurement system [26, 27]. This method allows the firmness, elasticity and tonicity of the skin to be measured.

The instrumental measurements were performed in triplicate according to a consecutive, shift method. All the measurements were performed before the oral collagen peptide intake (at baseline) and after 8 weeks of daily intake by

applying the probe at the level of a delimited area with a template on a randomly chosen temple (the opposite side to the corneometric measurements).

The assessment of the cutaneous state by clinical scoring

The clinical scoring consisted of a visual and tactile assessment of the cutaneous state at the level of the face, before and after the intake of collagen peptides, to determine its cosmetic efficacy. In order to be as accurate as possible and always under the same intensity of light, some landmarks were set in the examination room so that the subject would be installed in the same position as at baseline [24].

The clinical scoring was performed by the co-investigator dermatologist on the face at baseline and after 8 weeks using a scale scored in 10 points (from 0 to 9) according to the following parameters related to hydration and elasticity of the skin: (i) elasticity of the skin (0 = not elastic, 9 = very elastic), tactile, right-hand side cheek; (ii) smooth aspect of the skin (0 = not smooth, 9 = very smooth), visual, whole face; and (iii) moisturized aspect of the skin (0 = not moisturized, 9 = very moisturized), visual, whole face.

The self-assessment of the efficacy perceived by the subjects

The study also consisted of a self-assessment questionnaire that aimed at measuring the skin improvement as perceived by the subjects after 8 weeks provided conditions of normal use. Questions were asked relating to the overall improvement of the state and the aspect of the skin, firmness, general appearance, smoothness, hydration, visible wrinkles and self-reported satisfaction related to the efficacy of used collagen peptides. The answering modalities were as follows: “very good”, “good”, “neutral”, “bad” and “very bad”, “yes” or “no”. For each item, the percentage of satisfaction (= percentage of “very good” + percentage of “good”, or percentage of “yes”) was calculated [28].

Safety of the collagen peptides

Hydrolyzed collagen is deemed to be safe. As non-gelling gelatin, the collagen hydrolysate can be considered GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA) (Ref: GRAS SCOGS report # 58 (SCOGS 58 Gelatin)). In addition, the FDA has a GRAS notification for hydrolyzed animal proteins that includes hydrolyzed gelatin/polypeptides (REF: 21 CFR 184.1553 Peptones). By these references, the GRAS status of hydrolyzed collagen is affirmed.

Recording procedure and notification of adverse events

At each visit or if the subject called in, the subject was interviewed in order to obtain impartial answers regarding the occurrence of adverse events, serious or not. All modifications of the subject’s condition from the inclusion visits related or not to the tested products were considered an adverse event. There were no severe adverse effects during the study. The test product was found to be safe and very well tolerated.

Statistical analysis

Statistical analysis was performed with SPSS version 17.0 (Statistical Analysis Software Inc., Chicago, IL, USA) by the COSderma laboratory. Homogeneity between groups at baseline was tested by ANOVA, followed by a Tukey or Dunnett’s post hoc test for multiple comparisons. Intragroup differences over time and between

treatment groups were calculated by ANOVA/ANCOVA with a post hoc analysis using the Tukey or Dunnett's post hoc test for multiple comparisons. Statistical significance was considered when $p<0.05$.

Results

Subjects

In total, 54 subjects completed the study. 4 subjects were withdrawn from the study: 1 subject voluntarily withdrew from the study, 2 subjects did not respect the study's constraints and 1 subject withdrew from the study for other medical reasons. This study resulted in the following number of valid cases: 26 of the group getting 10g/day of SOLUGEL® collagen peptides; 28 in the placebo group.

The effect on the hydration of the upper layers of the epidermis by capacitance measurements

The hydration effect was assessed by capacitance measurements, using a Corneometer®. After 8 weeks of oral intake of SOLUGEL®, there was a statistically significant increased moisturizing effect of 14% (58.4 AU) compared to baseline (51.3 AU) (**Figure 1A**). A scan was taken from a fixed area of the skin and processed to illustrate the moisture level, which was expressed in a moisture map. The effect on the skin hydration of the daily intake of 10g of SOLUGEL® and its moisturizing effect could also clearly be seen as illustrated in **figure 1B** for one particular subject that showed an increase in the corneometric measurements.

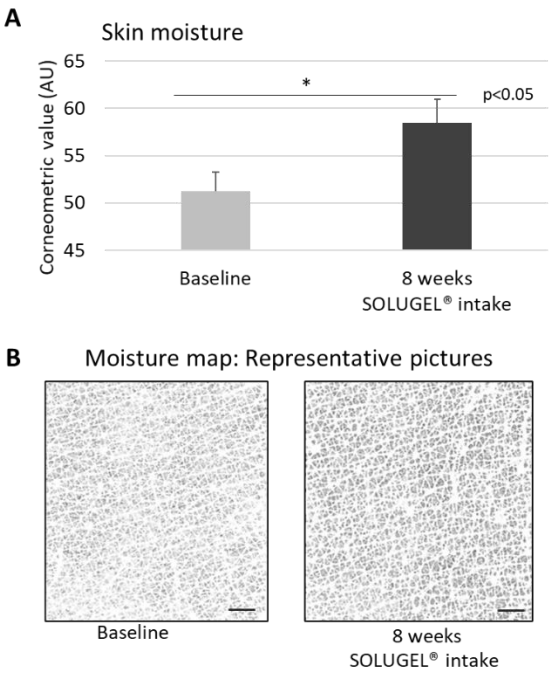

Figure 1. Effect of oral intake of collagen peptides on the hydration of the upper layers of the epidermis. *(A) The moisture level of the upper layers of the epidermis at baseline and after 8 weeks of supplementation were assessed using the Corneometer® (AU: arbitrary units); (B) Representative pictures of the upper layers of the epidermis were assessed using the moisture map technique. The data was presented as mean ± standard deviation ($\bar{X}$ ± SD). * Refers to statistically significant difference ($p<0.05$). NS: not significant ($p>0.05$).*

The effect on the biomechanical properties of the skin

The biomechanical properties of the skin, such as elasticity and firmness, were assessed by Cutometer® measurements. The situation at baseline and after 8 weeks of intake of 10g of SOLUGEL® were compared to clarify the collagen peptide supplementation effect. The calculation of the percentage change from baseline after 8 weeks of supplementation is shown in **figure 2**. The skin firmness (17.4%) and skin elasticity (10.6%) were significantly improved after 8 weeks of collagen peptide supplementation compared to baseline (**Figure 2**).

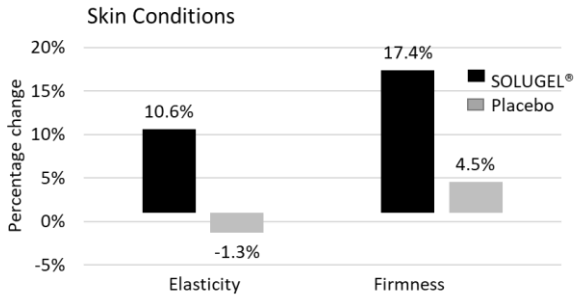

Figure 2. Effect of oral intake of SOLUGEL® collagen peptides on biomechanical properties. *The properties such as elasticity and firmness of the skin at baseline and after 8 weeks of supplementation were measured using a Cutometer®. The calculation of the percentage of change from baseline after 8 weeks of supplementation is shown in the figure. The data was presented as mean ± standard deviation ($\bar{X}$ ± SD) or percentage (%). * Refers to statistically significant difference (p<0.05). NS: not significant (p>0.05).*

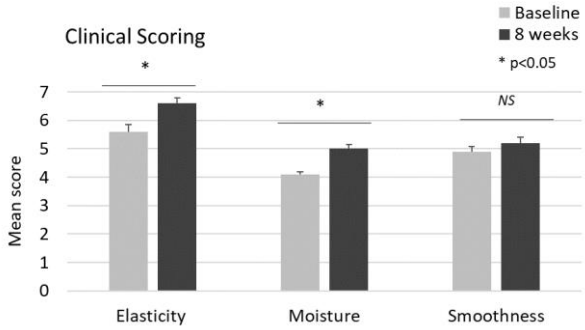

Figure 3. Clinical scoring of the effect of oral intake of SOLUGEL® collagen peptides. *Clinical scoring of the effect on elasticity, moisture and smoothness of the skin. The clinical scoring was performed by the expert dermatologist on the face using a scale scored in 10 points (from 0 to 9). The data was presented as mean ± standard deviation ($\bar{X}$ ± SD). * Refers to statistically significant difference (p<0.05). NS: not significant (p>0.05).*

The effect on the cutaneous state by clinical scoring

The result for the clinical scoring of elasticity and moisturized aspect of the skin shows a clear effect of collagen peptides for 8 weeks. The mean score for skin elasticity ($p<0.001$), moisture ($p<0.001$) and smoothness ($p>0.05$) were 5.6, 4.1 and 4.9 at baseline and 6.6, 5.0 and 5.2 after 8 weeks supplementation respectively. Data showed a significant improvement of skin elasticity by more than 17.1%. A similar observation can be made for the moisturized aspect of the skin with an increase of nearly 20.6% (**Figure 3**).

The self-assessment of the efficacy perceived by the subjects and product evaluation

At the end of the study (which lasted 8 weeks), the subjects completed a self-assessment questionnaire to measure the skin improvement as felt by the subjects. Overall, all of the products were well accepted with no differences in appreciation of the drinks with either maltodextrin as a placebo or 10g of SOLUGEL®. More than half of the volunteers taking SOLUGEL® demonstrated a better perception of the product efficacy in terms of skin hydration, firmness of the skin, the appearance of the skin, as well as the overall improvement of the state and aspect of the skin. This further confirms the results of the measurements as described above.

Discussion

Collagen is the main component in human skin structure. Changes in the amount and structure are proposed to be responsible for skin aging. The increase of the collagen in the skin layers would enable a better construction of the extracellular matrix to ultimately allow biomechanical

improvements of the skin [6-8]. The intake of collagen peptides, which has been claimed to have effects on wrinkle formation and skin aging, requires more attention in science [4, 8, 29].

Collagen, which is the extracellular connective tissue matrix component, is particularly important for the strength and resilience of the skin [4]. With age, there is a loss of collagen fibers implicated in diminished moisture, resulting in vulnerable skin for the dynamics of the overall skin structure. In this context, in our study, a higher level of the hydrating effect of collagen peptides (10g/day of SOLUGEL®) after 8 weeks of intake was determined by capacitance measurements and scans from the skin to illustrate the moisture level ($p<0.05$). Objective clinical evaluation by the dermatologist showed comparable hydration affected by collagen peptides ($p<0.05$). Our results are in line with two recent studies that have directly shown an induction of skin moisture improvement in response to oral collagen peptide administration (10g/day for 8 weeks) [21, 23]. However, these studies showed the effect of collagen ingestion on a single parameter, moisture content. For hydration, there may be a kind of dynamic equilibrium at a certain plateau value as the skin may show a kind of maximum moisture retention capacity and this may be a rather quick effect. On the other hand, in another current study, moisture content was not changed after CP ingestion (5g/day) for 8 weeks [22]. In addition, as was the case in our study, there is a study that determines the skin condition in terms of aspects other than hydration. However, this showed the overall skin firmness and elasticity effect of 10g/day CP intake when used for a period of 4 weeks longer than our study (the other study lasted a total of 12 weeks) [30]. Regarding the human skin's physiology, cutaneous water content (moisture) is known to play an important role in skin barrier/envelope function, and a lack of hydration of the skin may be associated with several dermatological dysfunctions besides wrinkles.

Aging damages the elastic capacity of the skin, and thus, aging skin is marked by a lack of elasticity, fragmentation, and collagen bundle fragility [5]. In order to look further into the impact of this collagen peptide ingestion on the skin's physiology, we also assessed firmness and elasticity to detect improvements in the plasticity of the skin. The skin firmness and elasticity were improved after 8 weeks of intake of collagen peptides (10g/day of SOLUGEL®) compared to baseline ($p<0.05$). An objective clinical evaluation by the dermatologist showed that the elasticity of the skin was affected by collagen peptides for 8 weeks ($p<0.05$). Our results are in line with one recent study that measured skin elasticity in sun-exposed areas of only post-menopausal women after 4 weeks of collagen ingestion [13]. In parallel, another current study determined that skin elasticity was improved after CP ingestion for 8 weeks [22]. Based on the accumulated data, it can be assumed that the most prominent physiological changes in aging are localized in the dermis and result from the metabolism of collagen fibers.

The increase of the collagen in the dermis would enable a better construction of the extracellular (cutaneous thickening) and therefore an improvement of the firmness as well as epidermal functions such as skin moisture. CP supplementation reduced skin wrinkles and had positive effects on dermal matrix synthesis, i.e. the content of procollagen type I and elastin after 8 weeks [10]. So far, only one randomized clinical trial has been conducted in a comparable study group to investigate the effect of orally administered CP on various skin parameters such as hydration, elasticity, and firmness. [30]. The underlying mechanisms for the build-up or restoration of the collagen network in the skin, related to hydration, elasticity and firmness are different. Hydration as a biomechanical skin property is quite different from elasticity and firmness. Higher dosages of collagen may be required, or the effect is only clear after a longer

period at lower dosages. Studies over a longer period of time, including different dosages, are required to confirm these hypotheses. The tested dosages within those several studies vary between 5 and 10g of daily intake for 4-12 weeks. Comparable human studies based on chronological aging [13, 31, 32] and other experimental studies related to ultraviolet-induced skin damage [33, 34] have demonstrated that collagen peptides, administered orally at a dose of 5-10g per day for approximately 8 weeks, provide better skin conditions and fewer wrinkles. In some articles, this effect appeared significantly at a later stage, such as at 12 weeks [21, 28]. This duration and dosage difference may be due to the differences in the collagen peptides or the design of the study.

Collagen peptide supplementation has been proposed to be effective in a better construction of the extracellular matrix [17], cutaneous thickening [23], and an improvement of biomechanical properties such as firmness [14], elasticity [30] as well as skin moisture [11] in a couple of studies. However, there are studies in which some of these parameters were not affected statistically by collagen intake [21, 22, 30]. These conflicting data may be due to characteristics of study design, duration, and collagen dosage, proving the need for more human studies.

The effects of a multi-nutrient supplement blend containing collagen peptides on skin health is another matter. Daily oral supplementation with a relatively lower dosage of collagen peptides (2.5-5g/day) combined with vitamins/minerals (vitamin C, E, zinc, etc.) [11, 12, 35, 36] and other bioactive compounds (glucosamines, antioxidants, plant extracts, silicone, etc.) for 8-12 weeks [28, 32, 36-40] have shown to improve skin health. However, it has not been proven whether there is any synergistic effect of any compound in these studies. All the ingredients demonstrated here can have a combined effect with collagen. It is not known whether it is a synergistic or an additive effect. The mechanism by which each ingredient acts needs an explanation for the human health and safety of these mixtures. However, in our study, we show the pure effect of hydrolyzed collagen.

There is a limited amount of clinical research on the mechanism of collagen peptides on skin improvements [10, 35, 38]. Some preclinical studies have demonstrated the effect of collagen peptides on fibroblasts [17, 19]. One hypothesis of mechanical action in collagen peptides may be recognized by the cells as a marker of the degradation of endogenous collagen. In response to this "degradation" signal, cells such as fibroblasts produce collagen [16]. These cells originate from the same stem cells and share certain characteristics, such as the capacity to produce endogenous collagen [16, 17, 19]. Therefore, it has been demonstrated through in vitro studies that collagen peptides can stimulate collagen production by such cells. This needs to be confirmed by the human study design to extrapolate this data on skin health benefits.

Conclusion

Collagen peptides have been known for offering various health benefits since the 1980s. Numerous scientific studies have introduced the beneficial effects of collagen peptides on weight management, joint health, bone health, physical activity and exercise recovery, wound healing, as well as skin health. This is a clinical study suggesting the beneficial effect of pure SOLUGEL® collagen peptides on skin condition. The benefits of taking a daily dosage of 10g collagen peptides for 56 days are seen in the significant improvement of skin condition aspects such as hydration, firmness, and elasticity. Collagen peptides may improve skin health and offer a natural solution to skin aging.

Authorship

All the authors were involved in the design of the study, collection and analyzing the data, and finalizing of the manuscript. All authors read and approved the final manuscript.

Acknowledgment

We would like to thank Marion Potier, who worked at PB Leiner from 2013-2017 for leading the study. We would also like to thank Professor Alain Taieb and Dr. Emilia Bucur from Hôpital Saint-André for the data collection; Jérôme Asserin and the COSderma Team Manager from the COSderma Laboratory for the study design and analysis; Dr. Carlos Castells for leading the study.

References

1. Solano, F., *Metabolism and Functions of Amino Acids in the Skin.* Adv Exp Med Biol, 2020. 1265: p. 187-199.
2. Sato, K., *The presence of food-derived collagen peptides in human body-structure and biological activity.* Food Funct, 2017. 8(12): p. 4325-4330.
3. Yousef, H., M. Alhajj, and S. Sharma, *Anatomy, Skin (Integument), Epidermis*, in *StatPearls*. 2021: Treasure Island (FL).
4. Kathryn J. Martires, P.F., A.M.P.K.D. Cooper, and E.D. Baron, *Factors That Affect Skin Aging.* Arch Dermatol, 2009. 145(12): p. 1375-1379.
5. Fisher, G.J., et al., *Mechanisms of photoaging and chronological skin aging.* Arch Dermatol, 2002. 138(11): p. 1462-70.
6. Tobin, D.J., *Introduction to skin aging.* J Tissue Viability, 2017. 26(1): p. 37-46.
7. Puizina-Ivi, N., *Skin aging.* Acta Dermatoven APA, 2008. 17(2): p. 47-54.
8. Lupu, M.A., et al., *Beneficial effects of food supplements based on hydrolyzed collagen for skin care (Review).* Exp Ther Med, 2020. 20(1): p. 12-17.
9. Jhawar, N., J.V. Wang, and N. Saedi, *Oral collagen supplementation for skin aging: A fad or the future?* J Cosmet Dermatol, 2020. 19(4): p. 910-912.
10. Proksch, E., et al., *Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis.* Skin Pharmacol Physiol, 2013. 27(3): p. 113-9.
11. Nomoto, T. and S. Iizaka, *Effect of an Oral Nutrition Supplement Containing Collagen Peptides on Stratum Corneum Hydration and Skin Elasticity in Hospitalized Older Adults: A Multicenter Open-label Randomized Controlled Study.* Adv Skin Wound Care, 2020. 33(4): p. 186-191.
12. Bolke, L., et al., *A Collagen Supplement Improves Skin Hydration, Elasticity, Roughness, and Density: Results of a Randomized, Placebo-Controlled, Blind Study.* Nutrients, 2019. 11(10).
13. Sangsuwan, W. and P. Asawanonda, *Four-weeks daily intake of oral collagen hydrolysate results in improved skin elasticity, especially in sun-exposed areas: a randomized, double-blind, placebo-controlled trial.* J Dermatolog Treat, 2020: p. 1-6.
14. Zhang, L., et al., *Ingestion of collagen hydrolysates alleviates skin chronological aging in an aged mouse model by increasing collagen synthesis.* Food Funct, 2020. 11(6): p. 5573-5580.
15. Matsuda, N., et al., *Effects of ingestion of collagen peptide on collagen fibrils and glycosaminoglycans in the dermis.* J Nutr Sci Vitaminol (Tokyo), 2006. 52(3): p. 211-5.
16. Yoshihiro Tokudome, et al., *Effects of soybean peptide and collagen peptide on collagen synthesis in normal human dermal fibroblasts.* International Journal of Food Sciences and Nutrition, 2012. 63(6): p. 689-695.
17. Zague, V., et al., *Collagen peptides modulate the metabolism of extracellular matrix by human dermal fibroblasts derived from sun-protected and sun-exposed body sites.* Cell Biol Int, 2018. 42(1): p. 95-104.
18. Oba, C., et al., *Collagen hydrolysate intake improves the loss of epidermal barrier function and skin elasticity induced by UVB irradiation in hairless mice.* Photodermatol Photoimmunol Photomed, 2013. 29(4): p. 204-11.
19. Edgar, S., et al., *Effects of collagen-derived bioactive peptides and natural antioxidant compounds on proliferation and matrix protein synthesis by cultured normal human dermal fibroblasts.* Sci Rep, 2018. 8(1): p. 10474.
20. Song, H., et al., *Effect of Orally Administered Collagen Peptides from Bovine Bone on Skin Aging in Chronologically Aged Mice.* Nutrients, 2017. 9(11).
21. Hiroki Oharą, et al., *Improvement in the Moisture Content of the Stratum Corneum Following 4 Weeks of Collagen Hydrolysate Ingestion.* Nippon Shokuhin Kagaku Kogaku Kaishi 2009. 56(3): p. 137-145.
22. Proksch, E., et al., *Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: a double-blind, placebo-controlled study.* Skin Pharmacol Physiol, 2014. 27(1): p. 47-55.
23. Asserin, J., et al., *The effect of oral collagen peptide supplementation on skin moisture and the*

dermal collagen network: evidence from an ex vivo model and randomized, placebo-controlled clinical trials. Journal of Cosmetic Dermatology, 2015. 14(4): p. 291-301.
24. Qassem, M. and P. Kyriacou, *Review of Modern Techniques for the Assessment of Skin Hydration.* Cosmetics, 2019. 6(1).
25. *Measurement of hydration in the stratum corneum with the MoistureMeter and comparison with the Corneometer.* 2004.
26. Ryu, H.S., et al., *Influence of age and regional differences on skin elasticity as measured by the Cutometer.* Skin Res Technol, 2008. 14(3): p. 354-8.
27. Ahn, S., et al., *Correlation between a Cutometer and quantitative evaluation using Moire topography in age-related skin elasticity.* Skin Res Technol, 2007. 13(3): p. 280-4.
28. Genovese, L., A. Corbo, and S. Sibilla, *An Insight into the Changes in Skin Texture and Properties following Dietary Intervention with a Nutricosmeceutical Containing a Blend of Collagen Bioactive Peptides and Antioxidants.* Skin Pharmacol Physiol, 2017. 30(3): p. 146-158.
29. de Miranda, R.B., P. Weimer, and R.C. Rossi, *Effects of hydrolyzed collagen supplementation on skin aging: a systematic review and meta-analysis.* Int J Dermatol, 2021.
30. Evans, M., et al., *A randomized, triple-blind, placebo-controlled, parallel study to evaluate the efficacy of a freshwater marine collagen on skin wrinkles and elasticity.* J Cosmet Dermatol, 2021. 20(3): p. 825-834.
31. Proksch, E., M. Schunck, and S. Oesser, *Positive effect of fish-derived Bioactive Collagen Peptides on skin health.* Nutrafoods, 2020. 1: p. 127-133.
32. Irwin Kantor, et al., *Results of a Study Evaluating the Use of a Dietary Supplement Formula in the Management of Age-Related Skin Changes in Women with Moderate to Severe Wrinkling of the Periorbital Area.* JANA, 2002. 5(2): p. 10-19.
33. Suhigara, F. and N. Inoue, *Clinical effects of collagen hydrolysates ingestion on UV-induced pigmented spots of human skin: A preliminary study.* Health Sciences, 2012. 28(2).
34. Koyama Y I, et al., *Supplemental ingestion of collagen peptide suppresses ultraviolet-induced erythema - A randomized double-blind placebo-controlled study.* Japanese Pharmacology and Therapeutics 2014. 42(10): p. 781-790.
35. Laing, S., et al., *A Dermonutrient Containing Special Collagen Peptides Improves Skin Structure and Function: A Randomized, Placebo-Controlled, Triple-Blind Trial Using Confocal Laser Scanning Microscopy on the Cosmetic Effects and Tolerance of a Drinkable Collagen Supplement.* J Med Food, 2020. 23(2): p. 147-152.
36. Czajka, A., et al., *Daily oral supplementation with collagen peptides combined with vitamins and other bioactive compounds improves skin elasticity and has a beneficial effect on joint and general wellbeing.* Nutr Res, 2018. 57: p. 97-108.
37. Borumand, M. and S. Sibilla, *Effects of a nutritional supplement containing collagen peptides on skin elasticity, hydration and wrinkles.* Journal of Medical Nutrition and Nutraceuticals, 2015. 4(1).
38. Yoon, H.S., et al., *Supplementing with dietary astaxanthin combined with collagen hydrolysate improves facial elasticity and decreases matrix metalloproteinase-1 and -12 expression: a comparative study with placebo.* J Med Food, 2014. 17(7): p. 810-6.
39. Duteil, L., et al., *Effect of Low Dose Type I Fish Collagen Peptides Combined or not with Silicon on Skin Aging Signs in Mature Women.* Juniper Online Journal of Case Studies, 2018. 6(4).
40. Motwani, M.S., et al., *Efficacy of a collagen hydrolysate and antioxidants-containing nutraceutical on metrics of skin health in Indian women.* J Cosmet Dermatol, 2020.