Anti-Aging Effects of Low-Molecular-Weight Collagen Peptide Supplementation on Facial Wrinkles and Skin Hydration: Outcomes from a Six-Week Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract: In recent decades, there has been a rising demand for anti-aging interventions aimed at postponing or potentially reversing indicators of skin aging. The use of collagen-based nutraceutical supplements has gained popularity as they have shown promise in enhancing skin health and reducing signs of aging. The aim of this randomized, placebo-controlled, blinded study was to investigate the effects of 2.5 g COLLinstant® LMW, a novel cosmeceutical containing low-molecular-weight (≤1000 Da) collagen peptides, on skin aging and health. The trial was conducted with 80 healthy women aged 30 years and older. They received a daily oral dose of either the food supplement (n = 40) or placebo (n = 40) for six weeks. Skin assessment was performed based on validated objective methods, such as Visioface 1000D (skin wrinkling), cutometry (elasticity and fatigue), and corneometry (skin hydration) at baseline (T0) and at week 6 (T6). After 6 weeks, participants that received collagen had significant improvements in biometric skin wrinkle parameters from baseline, with a reduction in volume by 46%, in area by 44%, and in depth by 9%, along with a greater increase in skin moisturization (by 34%) than those in the placebo group (p < 0.001). The food supplement did not significantly modify skin firmness or fatigue and had only slight beneficial effects on skin elasticity. The investigational product was well tolerated. The observed effects aligned closely with the subjective assessments reported by study participants. The study provides substantiated evidence supporting the efficacy of low-molecular-weight collagen peptides in restoring altered skin biometric parameters, as objectively assessed. Thus, regular supplementation with this nutraceutical may contribute to achieving smoother and more radiant skin.

Keywords: low-molecular-weight collagen; anti-aging; skin health; facial wrinkles; moisturization; nutraceutical; randomized controlled trial

1. Introduction

The skin, the body's largest organ, plays a crucial role in numerous functions. Beyond its sensory capacity, it acts as a dynamic interface between the internal and external environments, enabling the continuous adaptation and acclimatization of an organism throughout its lifespan [1,2].

Collagen, a protein primarily synthesized by fibroblasts in the connective tissues, constitutes the most abundant component of the extracellular matrix of the skin, representing over 75% of the dry weight of a young and healthy human dermis [3,4]. This protein is...
characterized by a triple-helix structure formed by the repetition of glycine every third residue, with proline and hydroxyproline occupying the remaining positions [5,6]. These amino acids are essential in the structure of collagen. Fibroblasts, as connective tissue cells within the dermis, play a pivotal role in the synthesis and organization of the collagen matrix. These cells exhibit sensitivity to both physical and chemical stimuli, which can trigger fibroblast activation and proliferation. Chemical stimuli operate via a key–lock mechanism, wherein small ligands bind to receptors located on the fibroblast extracellular membrane, leading to their activation. On the other hand, physical stimuli directly influence the interactions between collagen and fibroblasts [3,4].

Thus, collagen is a cornerstone in the skin’s extracellular matrix, essential for maintaining structural integrity and physiological functions. It retains water and plays a pivotal role in maintaining the skin’s smoothness, firmness, and resilience [4]. This enables the skin to effectively respond to the ever-changing onslaught of environmental stressors [7].

The aging process of the skin is an ongoing phenomenon, marked by a gradual decline in structural and physiological functions, often exacerbated by environmental factors and dermatological disorders [8,9].

Aging precipitates a decline in collagen synthesis within mature skin, attributed to diminished activity of enzymes involved in collagen post-translational processing, a reduction in the population of collagen-synthesizing fibroblasts, and a decrease in skin vasculature [10–12]. Consequently, the skin’s biomatrix begins to deteriorate as the collagen scaffold loses its strength and stability [4,13,14].

Extrinsic factors such as sunlight exposure, smoking, pollution, alcohol consumption, an unbalanced diet, and stress-related micronutrient deficiencies expedite this process, contributing to the process of collagen loss associated with aging skin [4,14–16]. The consequences of aging on the skin are manifold. It undergoes regressive changes, losing its integrity and becoming progressively thin and dry, leading to a compromised ability to retain enough moisture. Additionally, the reduction in dermal thickness and elasticity over time manifests as lines and wrinkles, further contributing to the visible signs of aging [17].

The psychosocial impact of skin aging has spurred the demand for effective interventions, including topical creams, injectable fillers, and collagen supplements [2,18].

While topically applied collagen from skincare products such as creams, lotions, and serums often fails to penetrate the deeper layers of the skin [2,19], injectable fillers, such as hyaluronic-acid-based products, can be costly and may cause adverse effects, including bruising, swelling, and infection, among others [20].

On the other hand, orally administered hydrolyzed collagen has gained significant popularity in recent years as a safe and cost-effective option to enhance skin health and maintain a youthful appearance [4,21–24]. It is available in various formulations, including gels, liquids, capsules, and powder, making them easy to incorporate into daily routines.

The use of low-molecular-weight collagen hydrolysate has been shown to be a promising and effective strategy to improve skin hydration and elasticity, thereby counteracting the changes associated with aging [2,4,25]. However, a comprehensive understanding and further research are paramount to substantiate these claims and optimize collagen use in dermatological applications.

Therefore, COLLinstant® LMW is a novel cosmeceutical that distinguishes itself by containing a high proportion of low-molecular-weight (≤1000 Da) glycine- and proline-rich bioactive peptides.

This product is the result of a tailored enzymatic digestion of native bovine collagen (type I and III) that preserves the collagen-specific sequence Gly-Pro-Hyp (GPH), yielding a low-molecular-weight (LMW) hydrolysate enriched with this active tripeptide, which appears to exert beneficial effects in various tissues, including skin, muscles, joints, and bones [26–28].

Unlike standard-weight collagen, which is degraded in the gastrointestinal tract, low-molecular-weight collagen (LMW) peptides, such as GPH and PH, easily cross the intestinal barrier via the PEPT1 transporter and remain intact throughout the gastrointestinal tract.
nal pathway [26,29,30]. These bioactive components are highly stable and facilitate rapid and efficient uptake, enhancing bioavailability and overall efficacy by providing essential building blocks and stimulating the synthesis of new collagen, elastin, and hyaluronic acid [27,28].

The objective of the present study is to assess the effectiveness of daily supplementation with COLLinstant® LMW over a period of six weeks in ameliorating visible signs of aging. This includes assessing its impact on skin wrinkle reduction, as well as its potential to enhance skin elasticity and moisturization. COLLinstant® LMW was administered orally in a single-center, randomized, double-blind, placebo-controlled clinical trial. A secondary objective involves comparing skin improvement, assessing product satisfaction, and monitoring adverse events among middle-aged female volunteers.

2. Materials and Methods

2.1. Study Design and Ethical Aspects

This was a 6-week, prospective, randomized, placebo-controlled, double-blind, monocentric study performed at GALA Laboratories in Don Benito–Villanueva (Badajoz, Spain). This study is listed on the ClinicalTrials.gov registry (NCT06321770).

Participants were individually randomized (1:1 ratio) to a strategy of receiving either COLLinstant® LMW (collagen group) or a placebo regimen and were followed up with for 6 weeks. Subjects were instructed to follow the administration guidelines provided in the manufacturer’s package for both product regimens and, if necessary, investigator guidance.

The investigation was performed according to the ethical guidelines detailed in the Declaration of Helsinki (amendment of the 64th General Assembly, Fortaleza, Brazil, October 2013) and the national regulations of Spain, and in full compliance with the applicable principles of good clinical practice (GCP) and International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use [31]. The trial protocol was approved (code 075/2022) by the Clinical Research Ethics Committee at the University Hospital of Cáceres (Cáceres, Spain) and written informed consent was obtained from all subjects prior to any study procedures being initiated.

2.2. Investigational Product

The preparation under study was COLLinstant® LMW (Viscofan DE GmbH, Weinheim, Germany), an oral food supplement based on bovine bioactive hydrolyzed (type I and III) collagen peptides.

Following ICH-GCP requirements and applicable local regulations [31], the investigational product and placebo were formulated as powder-containing sachets for oral suspension that were identical in appearance and odor.

Each sachet of the investigational product (COLL instant® LMW) contained 2.5 g low-molecular-weight hydrolyzed collagen peptides. Detailed information of each ingredient is shown in Table 1. The placebo did not contain any nutrients.

Table 1. Ingredient composition of the investigational product and the placebo.

<table>
<thead>
<tr>
<th>Supplement Facts</th>
<th>Sachet Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investigational Product (COLL instant® LMW)</strong></td>
<td><strong>Placebo</strong></td>
</tr>
<tr>
<td>Amount</td>
<td>%DV</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>14 sachets per container</td>
</tr>
<tr>
<td>Serving size</td>
<td>1 sachet (3.13 g)</td>
</tr>
<tr>
<td>Calories</td>
<td>11.30</td>
</tr>
<tr>
<td>Fat</td>
<td>0.004 g</td>
</tr>
<tr>
<td>Protein</td>
<td>2.7 g</td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Supplement Facts</th>
<th>Sachet Type</th>
<th>Investigational Product (COLL_instant® LMW)</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>%DV</td>
<td>Amount</td>
</tr>
<tr>
<td>Sodium</td>
<td>37.40 mg</td>
<td>1.62%</td>
<td>37.40 mg</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>0.126 g</td>
<td>0.046%</td>
<td>2.7 g</td>
</tr>
<tr>
<td>Low MW collagen peptides</td>
<td>2.5 g</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lemon flavor</td>
<td>467 mg</td>
<td>467 mg</td>
</tr>
<tr>
<td>Anhydrous citric acid</td>
<td>150 mg</td>
<td>+</td>
<td>150 mg</td>
</tr>
<tr>
<td>Sucralose</td>
<td>8.5 mg</td>
<td>+</td>
<td>8.5 mg</td>
</tr>
<tr>
<td>Stevia (97%)</td>
<td>7.1 mg</td>
<td>+</td>
<td>7.1 mg</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>2.5 g</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

% Daily values (DV) are based on a 2000 Calorie diet, + daily values (DV) not established for the ingredient.

2.3. Study Subjects

We recruited a total of 80 women (aged 30–65 years) with phototypes I–IV (Fitzpatrick scale) [32] who were mentally and physically healthy, had a BMI 20.0–29.9 kg/m², and displayed visible signs of natural and photoaging on their face (crow’s feet) rated from moderate to severe [33].

The Fitzpatrick scale is a numerical classification system for human skin color, ranging from type I to type VI, based on the amount of melanin in the skin. This classification informs the skin’s susceptibility to burns and its ability to tan [32,34].

During the screening phase, participants met all inclusion and exclusion criteria. Subjects were excluded if they were pregnant or lactating, had acute or chronic skin diseases or dermatological disorders, used natural health supplements for skin improvement within one month prior to the study commencement, followed a low-protein diet, had planned or unavoidable UV radiation exposure, had tattoos on or near the test area, used systemic corticosteroids or applied topical alpha hydroxy acids near the test site within four weeks of enrollment, used topical medications near the test area within six weeks of enrollment, underwent Botulinum toxin A (Botox) treatment or filler injection (collagen, hyaluronic acid, etc.) near the test sites within two years of enrolment, were cognitively impaired and/or unable to provide informed consent, or had any other condition that, in the medical investigator’s opinion, might adversely affect the individual’s ability to complete the study or its measures or pose significant risk to the individual.

2.4. Study Schedule and Biometric Evaluation

All participants (test and placebo groups) were instructed to consume the content of one sachet daily, in the morning, on an empty stomach for 6 weeks. The product was required to be dissolved in at least 100 mL of water, juice or other liquid.

Participants agreed to refrain from prolonged exposure to ultraviolet (UV) radiation, consuming any similar dietary supplements, and using any skincare treatments such as face masks, packs, and massages. They were also not permitted to apply topical cosmetics except those provided during the study. This restriction was enforced for a 2-week washout period before the study began and continued throughout the 6-week study period to maintain consistent skin conditions. Each participant visited the research center twice for assessment: once prior to intake of the study formulation at baseline (T0), and again 6 weeks after intake of the study formulation (T6), for efficacy measurements and safety evaluations.
For all women participating in the study, skin parameters were assessed at baseline (T0), and biometric changes were also evaluated after 6 weeks of treatment with the products (T6). Measurement of skin wrinkling parameters (volume, area, and depth) was evaluated at the crow’s feet region and changes were analyzed and digitally photographed in all patients by a VisioFace® 1000D (equipped with a high-resolution reflex camera) [35].

According to Mödinger et al. [36], subjects underwent acclimatization for a minimum of 30 min in the air-conditioned measurement room set at a temperature of 21 ± 1 °C and a relative humidity of 50 ± 5%.

Skin elasticity at the crow’s feet region was quantified using a Cutometer® dual MPA 580 (Courage + Khazaka), a non-invasive instrument designed to assess skin biomechanical properties [37]. This device evaluates skin elasticity by applying negative force that mechanically deforms the skin. The operational principle involves using a probe with negative pressure (450 mbar) to suction the skin, drawing the test area into the probe’s aperture. A non-contact optical measurement system then determines the depth of skin penetration. The evaluated parameters (R0, R2, R5, R7, and R9) are key indicators used to characterize skin biomechanical properties following suction force application [38–44].

R0 represents the final distension of the initial curve, reflecting the passive response of the skin to suction force and correlating with skin firmness. It is calculated as the difference between the highest point of amplitude at the end of the suction phase and the baseline reading (R0 = Uf). The R2 parameter is related to the gross elasticity/viscoelasticity, representing the skin’s resistance to mechanical suction force relative to its ability to recover (R2 = Ua/Uf). R5, referred to as net elasticity, signifies the ratio of elastic deformation during suction to rapid recovery during relaxation (R5 = Ur/Ue), indicating higher skin elasticity with increasing values. R7 is related to biological elasticity, quantifying the immediate elastic recovery within the first 0.1 s compared to the total deformation after suction (R7 = Ur/Uf) and can be interpreted as another marker of elasticity, with aging causing its reduction. Lastly, R9 denotes residual deformation at the conclusion of the measurement cycle, reflecting skin fatigue following repeated suction (R9 = R3 − R0) [37,42,43,45,46]. The measurements were carried out in triplicate.

Furthermore, stratum corneum hydration was assessed at each study visit using the electrical capacitance method with a Corneometer® CM 825 (Courage + Khazaka, Cologne, Germany). A minimum of five measurements were taken per measurement area at four distinct locations (middle forehead, both cheekbones, and the chin area), and the average value was used for subsequent analysis [36,44,46].

2.5. Safety and Self-Reported Measures

Safety was evaluated based on adverse reactions reported by the patients during the treatment period. Adverse events were documented at the final visit following six weeks of treatment.

At the end of the treatment, the volunteers filled out questionnaires to subjectively assess their perception of different parameters such as efficacy, organoleptic properties, tolerability, and satisfaction since the last time they took the product. The Spanish version of the Treatment Satisfaction Questionnaire with Medication (TSQM) [47] and a 3-point Likert scale with the items dissatisfied, slightly satisfied, and very satisfied were used.

2.6. Statistical Methods

Statistical analyses were performed according to the principles of ICH (International Council for Harmonization) guideline E9(R1) on “Statistical Principles for Clinical Trials” [48] using IBM® SPSS® Statistics for Windows (version 27.0) and Jeffreys’s Amazing Statistics Program (JASP) (version 0.17.1) computer software. The analysis of the distribution and normality tests of the variables were carried out using the Shapiro–Wilk tests.

All measured data are presented as mean ± SD (standard deviation). Categorical variables were summarized using the number and percentages of patients in each category.
Skin parameters (wrinkling, elasticity, and hydration) were evaluated descriptively at baseline (T0) and after 6 weeks of collagen supplementation (T6). The efficacy was assessed based on relative changes in these parameters, calculated as the differences between means at T0 and T6.

Graphical representations of the results were generated using box-and-whisker plots. The box spans from the 25th to the 75th percentile of the data. Whiskers extend from the edges of the box to the minimum and maximum values that are not considered outliers. Outliers, defined as values more than 1.5 times the interquartile range from the box, are depicted as symbols beyond the whiskers.

The between-group comparison (placebo group vs. verum group) was carried out to determine population homogeneity at baseline (T0) and treatment-related differences after 6 weeks (at T6) were analyzed by means of the Mann–Whitney U test, a non-parametric test applied to two independent samples. Comparisons between categorical variables were performed using the Chi-square test.

The within-group mean changes of skin parameters between the initial (T0) and final (T6) visits were evaluated by the non-parametric Wilcoxon signed-rank test for paired data. The threshold of statistical significance was set, in all cases, at a value of $p < 0.05$.

### 3. Results

#### 3.1. CONSORT (Consolidated Standards of Reporting Trials) Flowchart of the Controlled Intervventional Trial

Eighty women aged 30 to 60 years were included in the statistical analysis. No subjects were excluded during screening or throughout the study, and no protocol violations occurred. Compliance during the trial was excellent; therefore, all volunteers ($n = 80$) who were screened for eligibility and requested to participate completed the study protocol, and could therefore be analyzed. The flow of subjects through the controlled intervention trial is depicted in a diagram according to CONSORT guidelines (Figure 1) [49].

![Flow chart of subjects' recruitment, randomization, and follow-up.](image)
3.2. Characteristics of the Population

Participants \((n = 80)\) were randomized at baseline (T0) and allocated to the verum group \((n = 40)\) or the placebo group \((n = 40)\). Demographic and general features of the volunteers did not show any significant difference between the test product and the placebo group at baseline (Table 2).

Table 2. Demographics and general characteristics of women randomly allocated to the placebo and the test group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo ((n = 40))</th>
<th>Collagen ((n = 40))</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47 ± 7.7</td>
<td>45 ± 7.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.06</td>
<td>1.69 ± 0.07</td>
<td>0.68</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.1 ± 9.13</td>
<td>68.5 ± 9.27</td>
<td>0.59</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.5 ± 2.7</td>
<td>24.1 ± 2.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Skin type, (n) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>30 (75)</td>
<td>27 (67.5)</td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>8 (20)</td>
<td>12 (30)</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Oiled</td>
<td>2 (5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Skin phototype, (n) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0 (0)</td>
<td>3 (7.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>III</td>
<td>36 (90)</td>
<td>34 (85)</td>
<td>0.20</td>
</tr>
<tr>
<td>IV</td>
<td>4 (10)</td>
<td>3 (7.5)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SD, except where otherwise indicated. *Fitzpatrick scale [32].

The investigational treatment group included 40 women who received the bioactive collagen peptide-based food supplement orally.

The mean age in the placebo group \((n = 40)\) was 47 ± 7.7 years (age range between 32 and 60 years) and in the experimental group \((n = 40)\) 45 ± 7.1 years (age range between 30 and 58 years), with no significant differences between the two groups \((p = 0.22)\).

The predominant skin type in both groups (placebo and experimental) was “normal skin” (67.5% in the experimental group vs. 75% in the placebo group), followed by “sensitive skin” (30% in the experimental group vs. 20% in the placebo group), with no significant differences between the two groups \((p = 0.27)\).

Among all participants, the most represented Fitzpatrick skin classification was phototype III (slightly brown skin and brown hair), which was predominant in both groups (90% in the placebo group; 85% in the verum group). No significant differences were detected between the two groups for this dermatological parameter \((p = 0.20)\).

Homogeneity tests between groups revealed no significant differences for the mean initial skin parameters at baseline (T0) between the placebo group and the collagen group (Table 3).

Table 3. Skin biometric parameters at baseline (T0).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo ((n = 40))</th>
<th>Collagen ((n = 40))</th>
<th>(p) Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin wrinkling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (px(^3))</td>
<td>48.50 (35.2)</td>
<td>58.77 (47.7)</td>
<td>0.52</td>
</tr>
<tr>
<td>Area (px(^2))</td>
<td>4.13 (2.6)</td>
<td>4.53 (3.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Depth (px)</td>
<td>11.95 (2.7)</td>
<td>11.50 (2.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>Elasticity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0 (mm)</td>
<td>0.40 (0.09)</td>
<td>0.38 (0.10)</td>
<td>0.25</td>
</tr>
<tr>
<td>R2 (%)</td>
<td>53.28 (15.7)</td>
<td>53.14 (11.6)</td>
<td>0.89</td>
</tr>
<tr>
<td>R5 (%)</td>
<td>48.59 (17.4)</td>
<td>48.16 (13.9)</td>
<td>0.96</td>
</tr>
<tr>
<td>R7 (%)</td>
<td>34.03 (14.5)</td>
<td>32.1 (10.2)</td>
<td>0.95</td>
</tr>
<tr>
<td>R9 (mm)</td>
<td>0.07 (0.03)</td>
<td>0.07 (0.02)</td>
<td>0.27</td>
</tr>
<tr>
<td>Hydration (AU)</td>
<td>54.77 (9.4)</td>
<td>55.5 (8.6)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* \(p\) values for intergroup comparison were determined by Mann–Whitney U test.
3.3. Skin Wrinkling

The descriptive analysis of skin wrinkling biometric parameters (volume, area, and depth) of the facial crow’s feet region before intake of the product (at T0) and after 6 weeks of intake (at T6) is summarized in Table 4. The mean value of three determinations was used for analysis. At baseline, the mean skin wrinkling biometric parameters were similar between both groups of treatment (Table 3). Regarding the intraindividual comparison from baseline (T0–T6), all biometric parameters (volume, area, and depth) considerably decreased at T6 after 6 weeks of treatment in the collagen group, whereas they remained unchanged in the placebo group during the same period (Table 4).

Table 4. Skin crow’s feet parameters (volume, area, and depth) assessed by VisioFace® 1000D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time-Point</th>
<th>Placebo (n = 40)</th>
<th>Collagen (n = 40)</th>
<th>Test/Placebo p Value *</th>
<th>Test/Placebo p Value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (px³)</td>
<td>Baseline</td>
<td>48.50 (35.2)</td>
<td>58.77 (47.7)</td>
<td>0.13</td>
<td>30.24 (24.4)</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>50.30 (40.4)</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Area (px²)</td>
<td>Baseline</td>
<td>4.13 (2.6)</td>
<td>4.53 (3.1)</td>
<td>0.92</td>
<td>2.60 (2.1)</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>4.14 (2.5)</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Depth (px)</td>
<td>Baseline</td>
<td>11.95 (2.7)</td>
<td>11.50 (2.4)</td>
<td>0.23</td>
<td>10.35 (2.1)</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>12.25 (2.6)</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

* p value for within-group comparison (pre-treatment vs. post-treatment; T0–T6) (Wilcoxon signed-rank test).
† p value for intergroup comparison (experimental vs. placebo group) at T6, after 6 weeks of treatment (Mann–Whitney U test).

At baseline (T0), no significant differences were found for the mean skin wrinkle volume at the crow’s feet region between the placebo group (48.50 px³) and the collagen group (58.77 px³) (Table 3). After 6 weeks of intake of the study products, at T6, the mean volume of wrinkles significantly decreased from baseline (T0–T6) by −45.9% [−90.8%–(−2.9%)] in the collagen group (58.77 vs. 30.24 px³; p < 0.001) but remained relatively unchanged by 0.32% (−90.4%–20.9%) in the placebo group (48.50 vs. 50.30 px³) (Table 4 and Figure 2). The difference between groups (placebo vs. collagen) at T6 proves to be significant (p < 0.01) in favor of the test product (Table 4 and Figure 2).

![Figure 2](image-url)
collagen, the area of skin wrinkles at the crow’s feet region significantly decreased from baseline (T0–T6) by −43.8% [−94.4%–(−2.6%)] (4.53 vs. 2.60 px²; \( p < 0.001 \)) in the collagen group, but the intradividual difference was almost negligible at 1.66% (−24.6%–41.7%) in the placebo group (4.13 vs. 4.14 px²) (Table 4 and Figure 3). At T6, the intergroup comparison (placebo group vs. collagen group) of the mean wrinkle area proved to be significant (\( p < 0.001 \)) in favor of the test product (Table 4 and Figure 3).

![Figure 3](image1.png)

**Figure 3.** Box plot representing skin wrinkle area at crow’s feet region before (T0) and after intake of the product for 6 weeks (T6) in the group of women receiving a low-molecular-weight (LMW) collagen preparation or placebo (\( n = 40 \)/group). The mean value (\( x \)) is also represented. The asterisk (*) indicates statistically significant differences in the intergroup comparison (\( * p < 0.001 \)).

Table 3 shows that mean wrinkle depth values were similar at baseline of 11.95 px (placebo) and 11.50 px (collagen group). However, in line with improvements in volume and area, skin wrinkle depth decreased during intake of the investigational product. As shown in Table 4 and Figure 4, at T6, the depth of wrinkles significantly decreased from baseline (T0–T6) by −9.0% (−40.0%–0.0%) (11.50 vs. 10.35 px; \( p < 0.001 \)) in the collagen group. On the other hand, the intraindividual variation was non-significant and limited to 3.3% (−33.3%–40.0%) (11.95 vs. 12.25 px) in the placebo group. The differences between the groups at T6 (placebo group vs. collagen group) of the mean wrinkle depth proved also to be highly significant (\( p < 0.001 \)) in favor of the test product (Table 4 and Figure 4).

![Figure 4](image2.png)

**Figure 4.** Box plot representing skin wrinkle depth at crow’s feet region before (T0) and after intake of the product for a 6-week period (T6) in the group of women receiving a low-molecular-weight (LMW) collagen preparation or placebo (\( n = 40 \)/group). The mean value (\( x \)) is also represented. The asterisk (*) indicates statistically significant differences in the intergroup comparison (\( * p < 0.001 \)). ° represent outliers.
Figure 5 illustrates photographs of three volunteers showcasing the improvement in the appearance of skin wrinkles in the crow’s feet areas from the initial visit at T0, following the consumption of the investigational product COLLinstant® LMW for six weeks. The skin assessment was conducted using objective and validated methods (Visioface 1000D). In volunteer no. 13, there was a percentage change in volume, area, and depth of −59.18%, −51.91%, and −15.4%, respectively. For volunteer no. 60, the corresponding changes were −64.8%, −46.2%, and −40.0%, and for volunteer no. 70, the changes were −37.1%, −31.4%, and −7.2%, respectively.

![Figure 5](image-url)

**Figure 5.** Appearance of wrinkles at the crow’s feet region during the treatment period with the investigational product. Photographs of the frown lines in three volunteers, no. 13 (top), no. 60 (middle), and no. 70 (bottom), at baseline (images (a,c,e)) and at T6 after 6 weeks of oral supplementation with the test product (images (b,d,f)). The green lines provide a visual guide to researchers to precisely observe and analyze skin features. Data obtained from Visioface® 1000D.
3.4. Skin Elasticity

The descriptive analysis of skin elasticity obtained from the Cutometer® device before intake of the product (at T0) and after 6 weeks of intake (at T6) is summarized in Table 5. The mean value of three determinations at the crow’s feet region was used for analysis. At baseline (T0), the skin elasticity parameters were similar between both groups of treatment (Table 3).

Table 5. Mechanical characteristics of the skin through the analysis of elasticity parameters (mean ± SD) at the crow’s feet region, before intake of the study products at baseline (T0) and after 6 weeks of treatment (T6) as assessed by Cutometer®.

<table>
<thead>
<tr>
<th>Elasticity Parameter</th>
<th>Time-Point</th>
<th>Placebo (n = 40)</th>
<th>Test Group (n = 40)</th>
<th>Test/Placebo p Value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0 (mm)</td>
<td>Baseline</td>
<td>0.402 (0.09)</td>
<td>0.380 (0.09)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.362 (0.09)</td>
<td>0.329 (0.07)</td>
<td></td>
</tr>
<tr>
<td>R2 (%)</td>
<td>Baseline</td>
<td>53.28 (15.7)</td>
<td>53.14 (11.66)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>51.31 (18.1)</td>
<td>51.86 (13.7)</td>
<td></td>
</tr>
<tr>
<td>R5 (%)</td>
<td>Baseline</td>
<td>48.59 (17.5)</td>
<td>48.16 (13.9)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>45.84 (17.6)</td>
<td>48.54 (15.5)</td>
<td></td>
</tr>
<tr>
<td>R7 (%)</td>
<td>Baseline</td>
<td>34.03 (14.5)</td>
<td>32.09 (10.2)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>31.65 (13.7)</td>
<td>32.27 (13.2)</td>
<td></td>
</tr>
<tr>
<td>R9 (mm)</td>
<td>Baseline</td>
<td>0.073 (0.03)</td>
<td>0.070 (0.02)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.066 (0.02)</td>
<td>0.065 (0.02)</td>
<td></td>
</tr>
</tbody>
</table>

* p value for within-group comparison (pre-treatment vs. post-treatment; T0–T6). † p value for intergroup comparison (experimental vs. placebo group) after 6 weeks of treatment at T6.

Results showed that skin firmness (R0) significantly increased in both groups of treatment. Compared to baseline (T0), the assessment of total elongation and skin firmness (R0) showed statistically significant lower values (p < 0.001) after 6 weeks in both the treatment group receiving placebo and the collagen group (Table 5). At the end of the study, at T6, we could not demonstrate significant differences in the R0 parameter between both group means (Table 5).

The percentage of variation in mean skin firmness (R0) from baseline was −8.6% (−50.8–24.1%) in the placebo and −10.7% (−61.3–29.0%) in the group that received the food supplement.

The assessment of the remaining skin elasticity parameters (R2, R5, R7, and R9) showed a moderate improvement, but the differences were not statistically significant (Table 5). At T6, the assessment of the rest of skin elasticity parameters (R2, R5, R7, and R9) obtained with the Cutometer® device did not show any statistical significance between groups (Table 5), indicating that the food supplement did not show an improvement in most skin elasticity parameters in our study.

3.5. Skin Hydration

The descriptive analysis of skin hydration before intake of the product (at T0) and after 6 weeks of intake (at T6) is summarized in Table 6. The mean value of five determinations at four different locations (middle forehead, right and left cheek, and chin) was used for analysis. At baseline (T0), the skin hydration values were similar between both groups of treatment, 54.77 AU (placebo group) vs. 55.50 AU (collagen group) (Table 3).
Table 6. Skin hydration values (mean ± SD) before intake of the study products at baseline (T0) and after 6 weeks of treatment (T6).

<table>
<thead>
<tr>
<th>Time-Point</th>
<th>Placebo (n = 40)</th>
<th>Collagen (n = 40)</th>
<th>Test/Placebo p Value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin hydration (AU)</td>
<td>Mean (SD)</td>
<td>p Value *</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Baseline</td>
<td>54.77 (9.4)</td>
<td>0.79</td>
<td>55.50 (8.6)</td>
</tr>
<tr>
<td>Week 6</td>
<td>55.03 (9.5)</td>
<td>74.13 (11.9)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* p value for within-group comparison (pre-treatment vs. post-treatment; T0–T6). † p value for intergroup comparisons (experimental vs. placebo group) at T6, after 6 weeks of treatment. AU indicates arbitrary units.

A corneometric methodology corroborated the improvement in skin hydration in the volunteers who received the test product. Compared to baseline (T0), a statistically significant increase in skin hydration by +34.4% (9.2–98.0%) (55.50 AU vs. 74.13 AU, p < 0.001) was detected in the volunteers who received the investigational product for 6 weeks (T0–T6) (Table 6). On the other hand, the percentage change in skin hydration was non-significant and limited to +1.0% (−30.3–17.6%) (54.77 vs. 55.03 AU) in the placebo group (Table 6 and Figure 6). The differences between the groups at T6 (placebo group vs. collagen group) of the mean hydration values proved to be highly significant (p < 0.001) in favor of the test product (Table 6 and Figure 6).

Figure 6. Boxplot representing skin hydration before (T0) and after intake of the product for a 6-week period (T6) in the group of women receiving a low-molecular-weight (LMW) collagen preparation or placebo (n = 40/group). The mean value (x) is also represented. The asterisk (*) indicates statistically significant differences in the intergroup comparison (p < 0.001).

3.6. Overall Assessment of the Efficacy

Compared with placebo, the efficacy of the test product is summarized in Figure 7. The differences between the relative changes (T0–T6) in skin wrinkle parameters (volume, area, and depth), skin elasticity, and skin hydration are illustrated. There was a statistically significant improvement (p < 0.001) in the skin wrinkle parameters and skin hydration in the verum group. Regarding skin elasticity, the food supplement had slight beneficial effects on skin elasticity parameters but we could not demonstrate statistical significance.
Figure 7. Percentage change in biometric skin parameters between the baseline visit and the end of the 6 weeks of the interventional period (T0–T6) in the placebo group (red bars) and in the group taking the investigational product (green bars). Error bars indicate the standard error of the mean. The asterisk (*) indicates $p < 0.001$ for intergroup comparison (placebo group vs. collagen group).

3.7. Safety and Subjective Rating

During the study intervention, the collagen supplement did not cause any side effects and proved to be safe and well tolerated during the entire period of application.

Over two thirds of the volunteers rated the overall effectiveness of COLLinstant® LMW as good, a survey result that was matched by the ratings of the treating physicians. Over 90% of the volunteers in the experimental group indicated a high degree of satisfaction with the ability of the supplement to improve skin hydration or wrinkles, confirming the robust effect of the treatment. In addition, 87.5% of the volunteers in this group were highly satisfied with the brief time period it takes for the supplement to start showing its effects, underlining the significance of obtaining quick results with a collagen food supplement (Table 7).

Table 7. Results of the survey conducted by the principal investigators with the volunteers who received the food supplement.

<table>
<thead>
<tr>
<th></th>
<th>Very Satisfied</th>
<th>Slightly Satisfied</th>
<th>Dissatisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectiveness (%)</td>
<td>76.2</td>
<td>21.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Tolerability (%)</td>
<td>88.8</td>
<td>10.0</td>
<td>1.25</td>
</tr>
<tr>
<td>Acceptability of the product (%)</td>
<td>88.8</td>
<td>10.0</td>
<td>1.25</td>
</tr>
</tbody>
</table>

4. Discussion

The current body of evidence indicates that the administration of hydrolyzed collagen can significantly improve skin conditions and mitigate signs of aging, thereby establishing it as a popular and promising nutraceutical for skin rejuvenation and anti-aging interventions [4,50,51].

Upon oral intake of collagen hydrolysates, small bioactive peptides, such as the tripeptide GPH, are readily absorbed intact across the intestinal barrier [3]. These peptides exhibit...
high bioavailability and are detectable in human blood shortly after consumption [52,53]. Subsequently, they are distributed to various tissues, with notable accumulation in the skin, where they remain at elevated levels and persist longer (up to 14 days) compared to other tissue [30]. Within the skin, GPH is further hydrolyzed into the bioactive dipeptide PH [27].

In light of these considerations, COLLinstant® LMW, a novel cosmeceutical containing a unique composition of bioactive glycine- and proline-rich peptides, was selected for the clinical trial due to its potential to enhance the product’s efficacy (Table 1). The study specifically aimed to assess the efficacy, safety, and tolerability of 2.5 g COLLinstant® LMW over a 6-week period by evaluating various skin parameters.

Our observations suggest that the low-molecular-weight peptides in the investigational product are efficiently absorbed and biologically active, leading to a significant reduction in several skin wrinkle biomarkers such as volume, area, and depth in the crow’s feet region. Additionally, compared to the placebo, increased skin hydration and a moderate improvement in skin elasticity were observed. However, we were unable to demonstrate differences in skin elasticity parameters between the control group and the investigational group. The effects were exclusively attributed to COLLinstant® LMW, as the subjects did not receive any other form of cosmetic treatment.

The importance of dosage and duration of the treatment appear as crucial factors to be considered. In contrast to the higher dosages and longer durations recommended for standard-molecular-weight collagen hydrolysates to achieve clear beauty or health benefits [25,54] notably, a daily intake of 2.5 g COLLinstant® LMW for 6 weeks was sufficient to yield beneficial effects on some biometric parameters of skin health in this study.

Regarding skin elasticity, our findings indicated a positive trend (as measured by the Cutometer) in the group treated with the investigational product. However, the observed differences did not reach statistical significance when compared with the placebo group.

Kim et al. [55] reported significant improvement in skin hydration from baseline as early as 6 weeks after intake of 1 g of low-molecular-weight collagen peptides in the verum group. However, benefits in skin wrinkling and elasticity were observed later, after 12 weeks of treatment. Similarly, in another study, the intake of 1.65 g of low-molecular-weight bioactive peptides led to a significant increase in skin hydration and a reduction in skin desquamation after 4 weeks of administration, as well as reduced wrinkling after 12 weeks and increased elasticity after 8 weeks [56].

In light of previous studies on orally based collagen intake from various sources, significant improvements in skin hydration, elasticity, and wrinkling were generally observed following 12 weeks of collagen supplementation [2,57,58]. Thus, to achieve efficacy in enhancing skin elasticity through hydrolyzed collagen supplementation, a treatment duration exceeding 8 weeks appears to be necessary [51]. However, beneficial effects on skin hydration might be obtained earlier.

COLLinstant® LMW is orally administered, making it easy to incorporate into daily routines. Nevertheless, not all sources of hydrolyzed collagen are equally effective, and further studies are needed to determine the optimal source and therapeutic duration for combating skin aging. Different types of collagen used in these supplements, such as fish, porcine, chicken, and bovine collagen, may exhibit varying effects based on their source [2,4,51,59].

Within the scope of skincare and nutraceuticals, hydrolyzed bovine collagen has become a popular ingredient. COLLinstant® LMW is the first bovine collagen hydrolysate on the market, providing a beneficial amino acid profile that acts as both a building material and a stimulator for the synthesis of new collagen, elastin, and hyaluronic acid in the skin as corroborated by various studies [51]. The remarkable improvements in skin parameters observed in this investigation may be attributed to the substantial similarity between the collagen peptides derived from the bovine collagen complex and those naturally present in human collagen. Specifically, the hydrolysis of bovine collagen produces bioactive short-chain peptides that closely match the amino acid profile of human collagen type I, as well
as elastin and hyaluronic acid in the skin [4,50,60–63]. This chemical resemblance likely contributes to the efficacy of the supplementation in improving skin health and appearance.

During the aging process, the dysregulation of extracellular matrix turnover, particularly the degradation of collagen fibers by matrix metalloproteinases (MMPs) and other proteases, is often a pivotal molecular event [64]. Consequently, the skin undergoes structural and degenerative changes such as dehydration and loss of elasticity, resulting in dry, loose skin with the appearance of furrows or wrinkles [64–66]. According to Oesser et al. [67], the skin health benefits observed in our study are likely attributable to changes in protein turnover and the restoration of collagen synthesis within the dermal stratum of the skin. This was notably evidenced by decreases in the volume, area, and depth of skin wrinkles following oral supplementation with low-molecular-weight collagen peptides.

The efficacy of bioactive collagen di- and tripeptides in enhancing skin health appears to be attributed to several key mechanisms. First, these compounds have the ability to induce collagen expression through the mitogen-activated protein kinase 38 (p38 MAPK) pathway [68]. Second, they increase the availability of essential free amino acids, which promotes the synthesis of collagen and elastin fibers. Third, they have the potential to stimulate fibroblasts to produce collagen and hyaluronic acid [3,4,69–72].

The beneficial effects of collagen supplementation were validated not only through objective testing methods but also by the subjective assessments provided by the volunteers. Additionally, consistent with findings from other studies [55,57], the tested product was demonstrated to be safe, with no adverse reactions reported.

5. Conclusions

Exploring its potential as a natural intervention to uphold skin health and combatting signs of aging, low-molecular-weight collagen peptides (COLLinstant® LMW) were investigated in a randomized, placebo-controlled clinical trial, following a daily oral supplementation of 2.5 g of these peptides.

LMW collagen hydrolysate, which contains a high concentration of these characteristic peptides, offers several advantages over standard-molecular-weight products, including quicker and more efficient peptide uptake, higher bioavailability, and enhanced stability and efficacy, particularly in the skin.

The study confirmed the efficacy of these nutrients in restoring altered skin biometric parameters, as objectively assessed. We observed a significant reduction in wrinkles, a considerable increase in skin hydration, and a modest increase in skin elasticity. Regular supplementation may contribute to achieving smoother and more radiant skin.

Ongoing supplementation has the potential to enhance skin texture by providing essential building materials in this tissue, stimulating the synthesis of new collagen, elastin, and hyaluronic acid. Importantly, the collagen supplementation regimen was found to be devoid of any adverse effects, proving to be safe and well-tolerated throughout the entire administration period.


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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee at the Cáceres University Hospital, Cáceres, Spain (no. 075/2022).
**Informed Consent Statement:** Written informed consent has been obtained from the patients to publish this paper. Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

**Data Availability Statement:** The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

**Conflicts of Interest:** L.Q. and B.B. are full-time employees of Viscofan BioEngineering (Viscofan DE GmbH, Weinheim, Germany). The remaining authors (J.A.C.-N., B.G.-M., and R.G.-B.) declare no conflicts of interest. The sponsors had no influence in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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