Oral Supplementation Based on Low Molecular Weight Bioactive Collagen Peptides Improves Facial Wrinkles and Skin Hydration: A Randomized, Double-blind, Placebo-Controlled Study

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Abstract:

In recent decades, there has been a growing focus on the effects of aging on both the appearance and functionality of the skin, accompanied by 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 in 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, and after 6 weeks of intervention.

In comparison to the placebo group, we observed a significant ($p<0.001$) reduction of biometric skin wrinkle parameters (volume, area and depth) as well as an improvement on skin moisturization in the group taking the supplement. The food supplement did not significantly modify skin firmness or fatigue and had only slight beneficial effects on skin elasticity. The nutraceutical product was well tolerated.

The measured effects were fully in agreement with the subjective assessments of the study participants.

Keywords: low molecular weight collagen peptides, skin health, wrinkles, moisturization, elasticity, nutraceutical, randomized controlled trial.

Conflict of Interest Statement: The authors declare no conflicts of interest. The sponsor had no influence on execution, analysis and interpretation of the data.

Introduction

The skin is the largest organ in the human body and serves several important functions. As well as having a sensory role, it constitutes an active interface between the internal and external environment of the body and allows for the permanent adaptation and acclimatisation of the organism throughout its life.

Collagen, the most abundant component of the extracellular matrix, is accounting for more than 75% of the dry weight of the young and healthy human skin dermis. This protein is crucial to determine skin physiology, maintaining the skin's structure and allowing its many functions to be carried out (Quan & Fisher, 2015). The extracellular matrix retains water, ensuring the maintenance of smooth, firm and resilient skin and its ability to adapt and respond to constantly changing aggressors. The structure of collagen is reminiscent of a rope. Three chains wind around each other to form a collagen triple helix. These building blocks combine to form collagen fibrils of enormous resilience and tensile strength (Quan & Fisher, 2015).

Aging of the skin is a continuous process related to a depletion in the physiological function of this body tissue often accelerated by environmental factors or dermatological disorders (Tobin, 2017). Aging induces a decline in the enzymes involved in the post-translational processing of collagen, reducing the number of fibroblasts that synthesize collagen and vessels that supply the skin. Therefore, collagen formation decreases in mature skin and the skin biomatrix starts to collapse when the collagen scaffold loses its strength and stability (Krutmann et al., 2017; Sato, 2017). Therefore, the decline in skin quality with age is characterized by a reduction in collagen synthesis and a decrease in skin vascularity (Calleja-Agius et al., 2007; Castelo-Branco et al., 1992).

Furthermore, extrinsic factors such as sunlight, smoking, environmental pollution, alcohol abuse and consumption of an unbalanced diet and stress-related micronutrient deficiencies, can accelerate this process leading to an age-dependent loss of collagen in the skin (Krutmann et al., 2017; Lee et al., 2015; Nistico et al., 2021).

As a result of this, the skin undergoes regressive changes with age, losing its integrity, and becoming increasingly thin and dry, unable to retain enough moisture. Moreover, lines and skin wrinkling also progress as dermal thickness and elasticity are reduced over time (Varani et al., 2006).

Therefore, the rejuvenation of the biomatrix can be effectively enhanced only through a sufficient supply of nutrients delivered by the bloodstream. The use of nutraceuticals as supplements has seen an increase in recent years.

Collagen-based products are found in a lot of pharmaceuticals, medicine, food, and cosmetics products for a wide variety of applications (Lupu et al., 2019; Shenoy et al., 2022; Wang, 2021a). Therefore, knowledge and understanding of the effects of collagen-based products on different aspects of health and disease due to their increasing rate of use are urgently requested.

Among these supplements, hydrolyzed collagen appears to be a popular and promising skin anti-aging nutraceutical (Branquinho França et al., 2023; de Miranda et al., 2021a; Schwartz & Park, 2012).

COLLinstant® LMW is a novel cosmeceutical with low molecular weight (≤ 1000 Da) collagen peptides. In contrast to other regular collagen hydrolysates, COLLinstant® LMW contains a high amount of glycine and proline-rich peptides (Gly-X-Y).

The main purpose of this study was to evaluate the anti-wrinkle, moisturizing and skin elasticity efficacy of COLLinstant® LMW administered orally in a single- centre, randomized, double-blind, placebo-controlled clinical trial for six weeks. A secondary objective was to compare skin improvement, satisfaction with the product, and adverse events among the middle-aged female volunteers.

Materials and methods

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).

Participants were individually randomized (1:1 ratio) to a strategy of receiving either COLLinstant® LMW (collagen group) or a placebo regimen and followed up for 6 weeks. Subjects were instructed to dose both product regimens as per the manufacturer`s package instructions or, when appropriate, investigator guidance.

The study was approved by the Clinical Research Ethics Committee of the University Hospital San Pedro de Alcántara (Cáceres, Spain). 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 with national regulations of Spain, and in full compliance with the applicable principles of good clinical practice (GCP) (ICH, 2018). Written informed consent was obtained from all subjects prior to any study procedures being initiated.

Intervention/study products

The test product is classified as a food supplement. 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.

The investigational product and placebo were presented as a powder for oral suspension and sealed in sachets that were identical in appearance and odor, and labelled per the ICH-GCP requirements and applicable local regulations.

Each sachet of the active COLLinstant® LMW (Viscofan DE GmbH, Weinheim, Germany) contained low molecular weight hydrolyzed 2.5 g collagen peptides. Other ingredients, which were also contained in the

placebo, were 467 mg lemon flavour, 150 mg citric acid, 8.5 mg sucralose and 7.1 mg stevia (97%). The placebo did not contain any nutrients.

Study subjects

We recruited a total of 80 women (aged 30-65 years) with phototypes I-IV, who were mentally and physically healthy, had a BMI 20.0-29.9 kg/m^2 and displayed visible signs of natural and photoaging on their face, with crow´s feet, which refers to a wrinkle-score around the eyes greater than grade 3.

The Fitzpatrick scale is a numerical classification for human skin color (in a scale of I-VI), with the amount of melanin in the skin indicating the type of skin, its susceptibility to burns and its ability to tan (Gupta & Sharma, 2019).

During the screening phase, the participants satisfied all inclusion and exclusion criteria and agreed to avoid prolonged exposure to ultraviolet (UV) radiation for the duration of the study.

Subjects were excluded in case of pregnancy, lactation, acute or chronic skin disease or dermatological disorder; use of natural health supplements for improving the skin within 1 month before the start of the study; low protein diet; planned or unavoidable exposure to UV radiation; tattoos on or near the test area; use of systemic corticosteroids or applied topical alpha hydroxyl acids near the test site within 4 weeks of enrolment; use of topical medications near the test area within 6 weeks of enrolment; Botulinum toxin A (Botox) treatment or filler injection (collagen, hyaluronic acid, etc) near the test sites within 2 years of enrolment; subjects cognitively impaired and/or unable to give informed consent; or had any other condition which in the medical investigator's opinion may adversely affect the individual's ability to complete the study or its measures or which may pose significant risk to the individual.

Study schedule and biometric evaluation

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

For all women participating in the study, biometric characteristics were assessed at baseline (T0), and after 6 weeks of treatment with the products (T6).

Measurement of skin wrinkling parameters (volume, area and depth) was evaluated in the crow`s feet area and changes were analyzed and digitally photographed in all patients by VisioFace® 1000D (equipped with a high-resolution reflex camera) (Bazargan et al., 2023).

Afterwards, subjects were acclimatized for at least 30 min in the air-conditioned measurement room at a temperature of 21 ± 1°C and a relative humidity of 50 ± 5 %.

Skin elasticity was measured at the crow's feet region, so a Cutometer® dual MPA 580 (Courage &

Khazaka) was used to assess skin biomechanical properties (Ryu et al., 2008). This non-invasive tool evaluated the skin elasticity by negative force that distorts the skin mechanically. The functional principle is based on suction of the skin using a probe with negative pressure (450 mbar), which causes the test area to be drawn into the aperture of the probe. A non-contact optical measuring system determined the penetration depth of the skin (Ohshima et al., 2013; Stroumza et al., 2015). The parameters evaluated were R0, R2, R5, R7 y R9 and measurements were carried out in triplicate.

R0 represents the final distension of the first curve i.e., the passive behavior of the skin to the suction force and correlates to the skin firmness. This parameter is measured from the highest point of amplitude at the end of the suction phase to the baseline reading (R0 = Uf).

The R2 parameter is related to the gross elasticity/ viscoelasticity, which is the skin's resistance to the mechanical suction force versus its ability to recover (R2 = Ua/Uf).

R5 refers to net elasticity and is represented by the ratio of the elastic portion of the suction stage to fast recovery throughout relaxation stage (R5 = Ur/Ue), meaning the higher the value, the more elastic the skin.

R7 is related to biological elasticity. R7 is the immediate elastic recovery in the first 0.1 s compared with the amplitude (total deformation) after suction (R7 = Ur/Uf) and can be interpreted as another marker of elasticity, with aging causing its reduction; and R9, the residual deformation at the end of the measuring cycle, indicating the tiring effects or fatigue of skin after repeated suction (R9 = R3 - R0) (Ryu et al., 2008; Woo et al., 2014).

In addition, measurement of stratum corneum hydration was performed at each study visit by the electrical capacitance method using a Corneometer® CM 825 (Courage & Khazaka, Cologne, Germany). At least five determinations per measurement area at four different locations (middle forehead, both right and left cheek bone and the chin area) were performed, then the average was used for analysis.

Self-reported measures

After 6 weeks of treatment, filled out questionnaires, to subjectively assess their perception of different parameters such as efficacy and organoleptic properties and satisfaction since the last time they took the product. the Treatment Satisfaction Questionnaire with Medication (TSQM) (Atkinson et al., 2004), in the present study, the Spanish version of the questionnaire was used. It was assessed by a Likert scale with the following items: no satisfaction, slightly satisfied and well satisfied.

Statistical methods

All statistical analyses were performed using IBM® SPSS® Statistics for Windows (version 27.0) and JASP (JASP Team, 2023).

The analysis of the distribution and normality tests of the variables were carried out using the Kolmogorov-Smirnov and Shapiro-Wilk tests.

All the data measured are given as the mean ± SD. For categorical variables, the number and percentage of volunteers included in each category were calculated. The measured skin parameters -wrinkling, elasticity and hydration- were evaluated by descriptive analysis at T0 (baseline) and T6 (after 6 weeks of supplementation). Efficacy was determined by relative changes of these parameters, which were determined by the differences of the means (T6-T0).

The results are presented graphically by means of box-and-whisker plots. The box ranges from the 25th percentile up to the 75th percentile. Whiskers are drawn from the ends of the boxes to the largest and smallest values that did not represent outliers. Outliers are defined as being values more than 1.5 times the interquartile range away from the box. Outliers are represented by symbols beyond the whiskers.

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

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

Results

CONSORT (Consolidated Standards of Reporting Trials) flowchart of the controlled interventional trial

Eighty women aged 30 to 60 years were statistically analysed. The mean age in the placebo group (n= 40) was 47 ± 7.7 years and in the experimental group (n= 40), 45 ± 7.1 years. No subjects had to be excluded during screening or throughout the study. No protocol violations occurred. Compliance during the trial was excellent thus, all volunteers (n= 80) who were screened for eligibility and requested to participate, completed the study protocol, and could therefore be analysed. According to this, the intention-to-treat (ITT) population and the per-protocol (PP) population were identical. The safety population (SP) also included all 80 enrolled subjects. The flow of subjects through the controlled intervention trial is depicted in a diagram according to CONSORT (Schulz et al., 2010) (Figure 1).

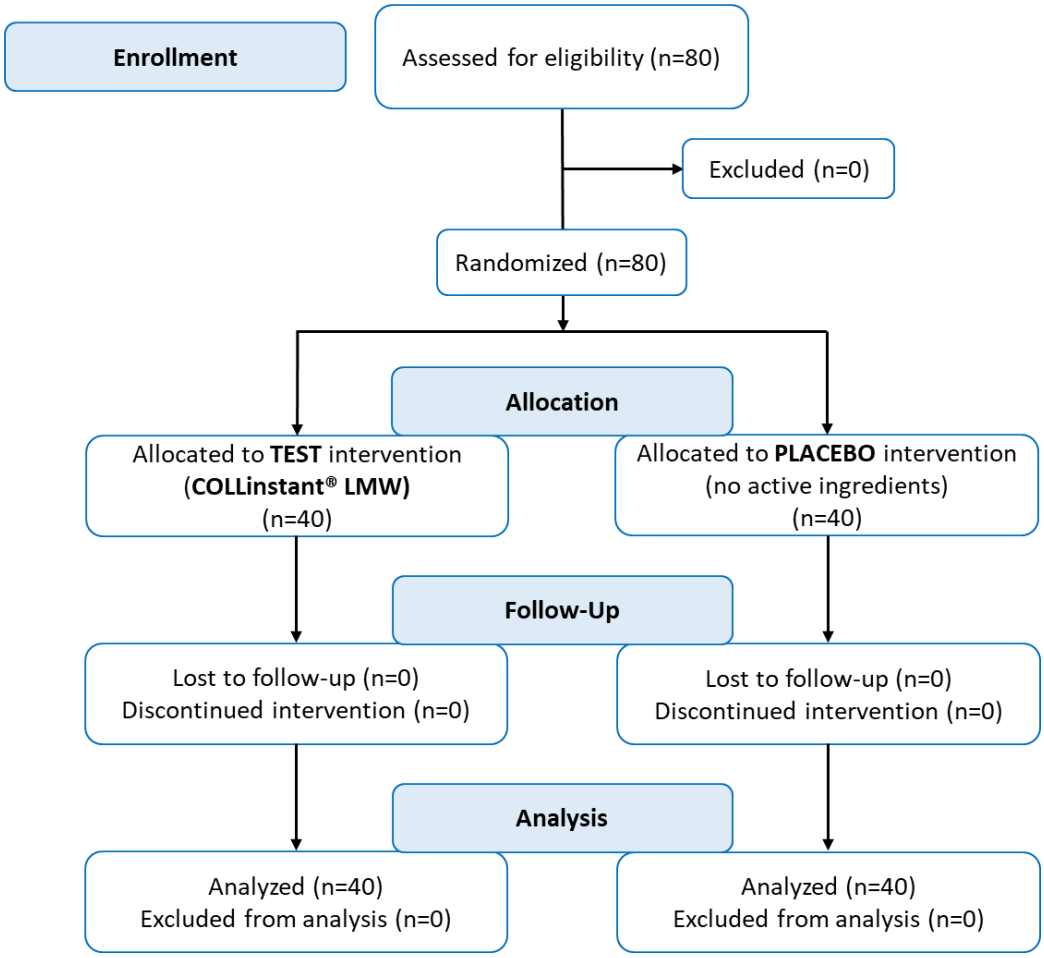


Figure 1. Recruitment of eligible subjects with the intervention protocol and assessment.

Characteristics of the population

Participants (n= 80) were randomized at baseline (T0) and allocated to the test group (n= 40) or the placebo group (n= 40) The active treatment group included 40 women who received the bioactive collagen peptide-based food supplement orally.

Demographic and general features of the volunteers did not show any significant difference between the test product and the placebo group at baseline (Table 1).

The median age in the group receiving food supplements (n=40) was 45 years (age range between 30 and 58 years). In the group of volunteers with placebo (n= 40), the median age was 48 years, with an age range between 32 and 60 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 with regard to this variable ($p = 0.27$).

Among all participants, the most represented Fitzpatrick skin classification was phototype III (slightly brown skin and brown hair) that was predominant in both groups (90% in the placebo group; 85% in the

group with collagen supplementation). 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 2).

Table 1. Demographics and general characteristics of women randomly allocated to the placebo and the test group.

		Placebo (n=40)	Collagen (n=40)	p
Age, years		47 ± 7.7	45 ± 7.1	0.22
Height (m)		1.68 ± 0.06	1.69 ± 0.07	0.68
Weight (kg)		69.09 ± 9.13	68.5± 9.27	0.59
BMI (kg/m²)		24.5 ± 2.7	24.1 ± 2.6	0.71
Skin type, n (%)	Normal	30 (75)	27 (67.5)	0.27
	Sensitive	8 (20)	12 (30)	
	Dry	0 (0)	1 (2.5)	
	Oiled	2 (5)	0 (0)	
Skin phototype, (Fitzpatrick[a]), n (%)	II	0 (0)	3 (7.5)	0.20
	III	36 (90)	34 (85)	
	IV	4 (10)	3 (7.5)	

Data are presented as means ± SD, except where otherwise indicated.

[a]Fitzpatrick scale based on numerical classification for human skin color, with the amount of melanin in the skin indicating the type. Type I always burns, never tans (pale white; blond or red hair; blue eyes; freckles). Type II usually burns, tans minimally (white; fair; blond or red hair; blue, green, or hazel eyes). Type III sometimes burns, tans uniformly (cream white; fair with any hair or eye color). Type IV burns minimally, always tans well (moderate brown). Type V very rarely burns, tans very easily (dark brown). Type VI never burns, never tans (deeply pigmented dark brown to darkest brown).

Table 2. Skin biometric parameters at baseline (T0).

		Placebo (n= 40)	Collagen (n= 40)	
	Parameter	**Mean (SD)**	**Mean (SD)**	**p value***
Skin wrinkling	Volume (px³)	51.2 (45.3)	61.3 (56.5)	0.53
	Area (px²)	3.8 (2.6)	4.6 (3.3)	0.34
	Depth (px)	12.03 (2.9)	11.55 (2.5)	0.46
Elasticity	R0 (mm)	0.40 (0.09)	0.38 (0.10)	0.25

	R2 (%)	53.28 (15.7)	53.14 (11.6)	0.89
	R5 (%)	48.59 (17.4)	48.16 (13.9)	0.96
	R7 (%)	34.03 (14.5)	32.1 (10.2)	0.95
	R9 (mm)	0.07 (0.03)	0.07 (0.02)	0.27
Hydration		55.27 (10.9)	55.5 (8.6)	0.52

*p values for test intragroup comparisons at baseline.

Effect of LMW collagen on 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 3. 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 2).

Regarding the intraindividual comparison from baseline (T0-T6), all biometric parameters (volume, area and depth) considerably improved at T6, after 6 weeks of treatment in the collagen group, whereas they remained unchanged in the placebo group during the same period (Table 3).

Table 3. Skin crow`s feet parameters (volume, area, and depth) assessed by VisioFace® 1000D.

		Placebo (n= 40)		Collagen (n= 40)		
Parameter	**Time-point**	**Mean (SD)**	**p value***	**Mean (SD)**	**p value***	**Test/Placebo p value[ł]**
Volume (px^3)	Baseline	51.2 (45.3)		61.3 (56.5)		
	Week 6	52.6 (48.4)	0.07	35.7 (41.4)	0.001	0.02
Area (px^2)	Baseline	3.8 (2.6)		4.6 (3.3)		
	Week 6	3.8 (2.4)	0.64	2.7 (2.5)	0.001	0.01
Depth (px)	Baseline	12.0 (2.9)		11.5 (2.5)		
	Week 6	12.3 (2.8)	0.10	10.4 (2.3)	0.001	0.001

*p values for intraindividual comparisons with baseline values (T0-T6; Wilcoxon signed-rank test). [ł]p value for intergroup comparisons (experimental vs placebo group) after 6 weeks of treatment (at T6; Mann-Whitney U test).

No significant differences were found for the mean skin wrinkle volume at T0 between the placebo group (51.2 px^3) and the collagen group (61.3 px^3) (Table 2). After 6 weeks of intake of the study products, at T6, the mean volume of wrinkles significantly decreased from baseline (T0-T6) by -44.0% [-90.8% – (-2.9%)] in

the collagen group (61.3 vs. 35.7 px^3; $p < 0.001$) but remained relatively unchanged by -0.05% (-90.4% - 20.9%) in the placebo group (51.2 vs. 52.6 px^3) (Table 3).

With regard to the skin wrinkle volume at the crow`s feet region, the difference between groups at T6 (Table 3 and Figure 2) proves to be significant ($p < 0.02$) in favor of the test product.

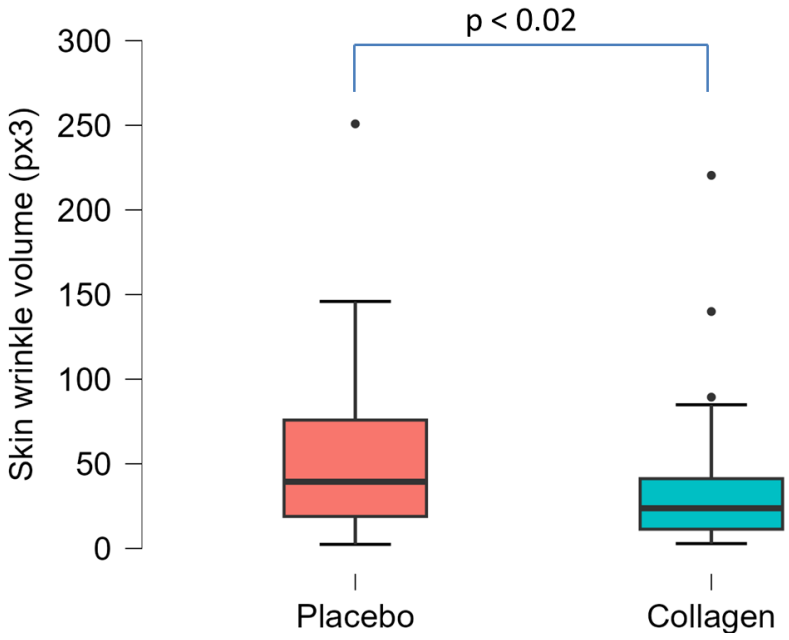
Starting at baseline from similar initial mean wrinkle area of 3.8 px^2 (placebo group) and 4.6 px^2 (collagen group) (Table 2), after an intake of 6 weeks of either placebo or collagen, the area of wrinkle significantly decreased from baseline (T0-T6) by -43.2% [-94.4% – (-2.6%)] (4.6 vs. 2.7 px^2; $p < 0.001$) in the collagen group, but the intraindividual difference was almost negligible by 1.66% (-24.6% – 41.7%), in the placebo group (3.8 vs. 3.8 px^2) (Table 3).

At T6, the intergroup comparison (placebo group vs. collagen group) of the mean wrinkle area (Table 3 and Figure 2) proved to be significant ($p < 0.01$) in favor of the test product.

Table 2 shows that mean wrinkle depth values were similar at baseline of 12.0 px (placebo) and 11.5 px (collagen group). However, in line with improvements in skin wrinkle volume and area, skin wrinkle volume decreased during intake of the active product.

As shown in Table 3, at T6, the depth of wrinkles significantly decreased from baseline (T0-T6) by -9.0% (-40.0% – 0.0%) (11.5 vs. 10.4 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%) (12.0 vs. 12.3 px) in the placebo group.

The differences between the groups at T6 (placebo group vs. collagen group) of the mean wrinkle depth (Table 3 and Figure 2) proved to be highly significant ($p < 0.001$) in favor of the test product.



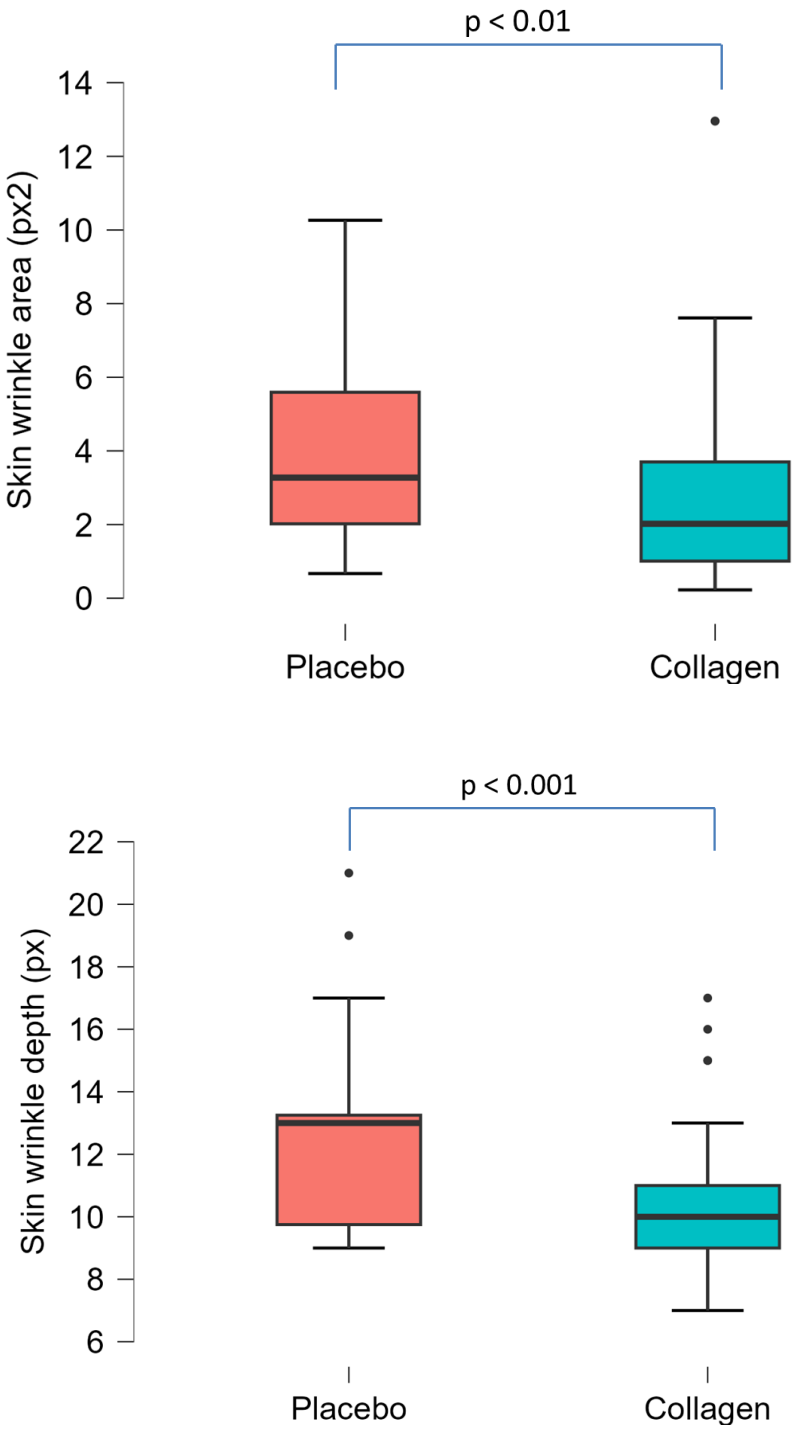
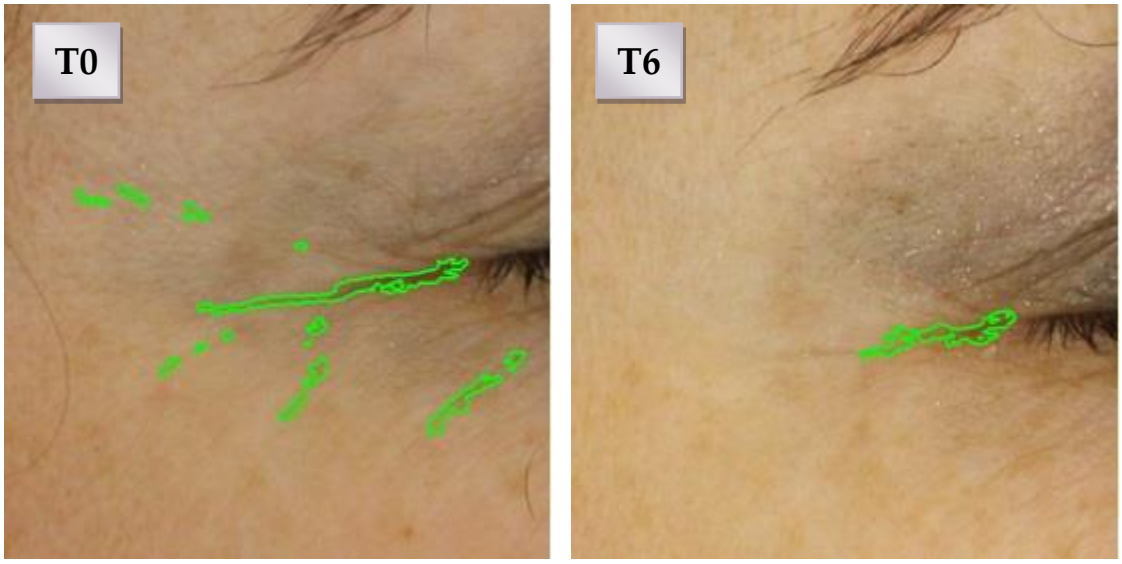


Figure 2. Boxplot representing the mean values of crow`s feet biometric parameters (volume, area and depth), after a 6-week period of treatment (at T6) in the group of women receiving a low molecular weight (LMW) collagen preparation or placebo (n= 40/group). The intergroup comparison shows statistically significant differences for all measured skin wrinkle biometric parameters.

Figure 3 illustrates photographs of three volunteers showcasing the improvement from the initial visit at T0 in facial wrinkling following the consumption of the active product 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.



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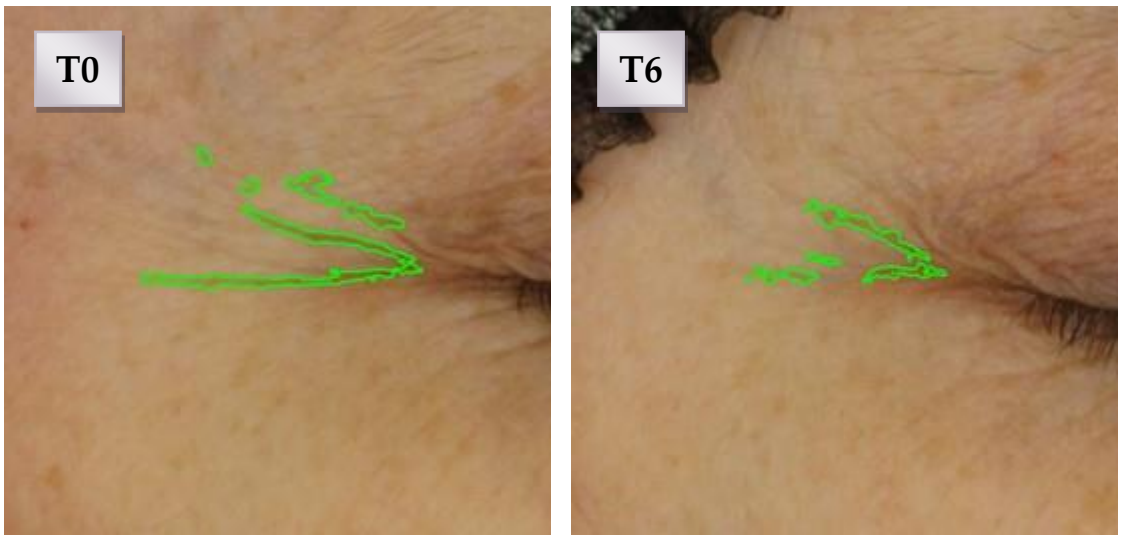

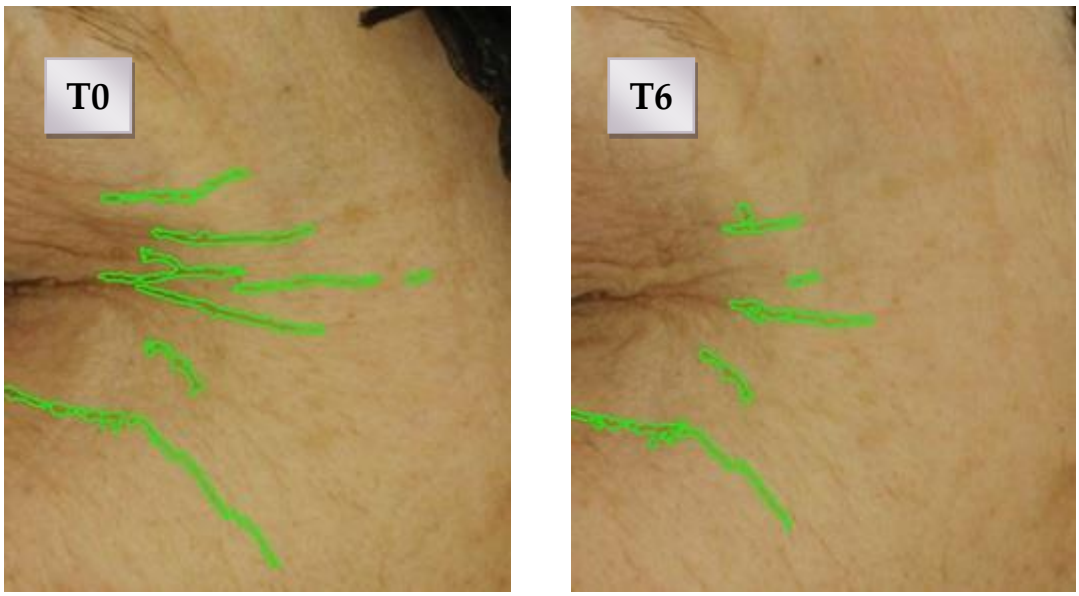

Figure 3. Oral supplementation with the test product for 6 weeks (T6) improved the appearance of wrinkle in the crow`s feet area when compared to baseline (T0). Data from Visioface 1000 D in three volunteers, no. 13 (top); no. 60 (middle) and no. 70 (bottom).

Effect on 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 4. 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 2).

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 group of treatment receiving placebo and the collagen group (table 4). At the end of the study, at T6, we could not demonstrate significant differences in the R0 parameter between both group means (Table 4).

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 4).

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 4) indicating that the food supplement did not show an improvement in most skin elasticity parameters in our study.

Table 4. 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®.

		Placebo (n= 40)		Test group (n= 40)		
Elasticity Parameter	**Time-point**	**Mean (SD)**	**p value***	**Mean (SD)**	**p value***	**Test/Placebo p value[ł]**
R0 (mm)	Baseline	0.402 (0.09)		0.380 (0.09)		
	Week 6	0.362 (0.09)	0.001	0.329 (0.07)	0.001	0.07
R2 (%)	Baseline	53.28 (15.7)		53.14 (11.66)		
	Week 6	51.31 (18.1)	0.31	51.86 (13.7)	0.68	0.53
R5 (%)	Baseline	48.59 (17.5)		48.16 (13.9)		
	Week 6	45.84 (17.6)	0.15	48.54 (15.5)	0.95	0.39
R7 (%)	Baseline	34.03 (14.5)		32.09 (10.2)		
	Week 6	31.65 (13.7)	0.09	32.27 (13.2)	0.65	0.63
R9 (mm)	Baseline	0.073 (0.03)		0.070 (0.02)		
	Week 6	0.066 (0.02)	0.14	0.065 (0.02)	0.13	0.41

*p values for intraindividual comparisons with baseline values. [ł]p value for intergroup comparisons (experimental vs placebo group) after 6 weeks of treatment at T6.

Effect on 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 5. The mean value of five determinations at four different locations (middle forehead, both right and left cheek and the chin) was used for analysis. At baseline (T0) the skin hydration values were similar between both groups of treatment, 55.3 AU (placebo group) vs. 55.5 AU (collagen group) (Table 2).

Corneometric methodology corroborates the improvement in skin hydration in the volunteers who received the test product. Compared to baseline (T0), it has been detected a statistically significant improvement in skin hydration by 34.4% (9.2% - 98.0%) (55.5 AU vs. 74.12 AU, $p < 0.001$) in the volunteers who received the active product for 6 weeks (T0-T6) (table 5). On the other hand, the percentage change in skin hydration was non-significant and limited to 1.0% (-30.3% - 17.6%) (55.3 vs. 55.5 AU) in the placebo group.

The differences between the groups at T6 (placebo group vs. collagen group) of the mean hydration values (Table 5 and (Figure 4) proved to be highly significant ($p < 0.001$) in favor of the test product.

Table 5. Skin hydration values (mean ± SD) before intake of the study products at baseline (T0) and after 6 weeks of treatment (T6).

		Placebo (n= 40)		Collagen (n= 40=		
	Time-point	**Mean (SD)**	**p value***	**Mean (SD)**	**p value***	**Test/Placebo p value¹**
Skin hydration(AU)	Baseline	55.3 (10.9)		55.5 (8.6)		
	Week 6	55.5 (11.2)	NS	74.1 (11.9)	0.001	0.001

*p values for intraindividual comparisons with baseline values. ¹p value for intergroup comparisons (experimental vs placebo group) after 6 weeks of treatment at T6. AU indicates arbitrary units.

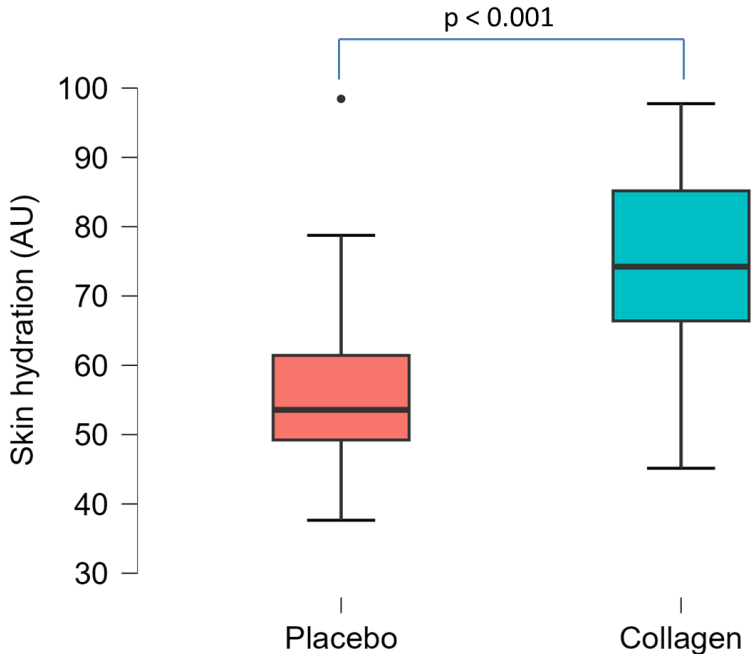

Figure 4. Boxplot representing the mean values in skin hydration at T6, in the group of women receiving a low molecular weight (LMW) collagen preparation and in the placebo group (n= 40/group). The intergroup comparison shows statistically significant differences ($p < 0.001$).

Overall assessment of the efficacy of the food supplement

Compared to placebo, the efficacy of the test product is summarized in Figure 6. The differences between the relative changes (T0-T6) in skin wrinkle parameters (volume, area and dept), 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 collagen group, but the food supplement did not significantly modify

skin firmness or fatigue and had only slight beneficial effects on skin elasticity but we could not demonstrate significance.

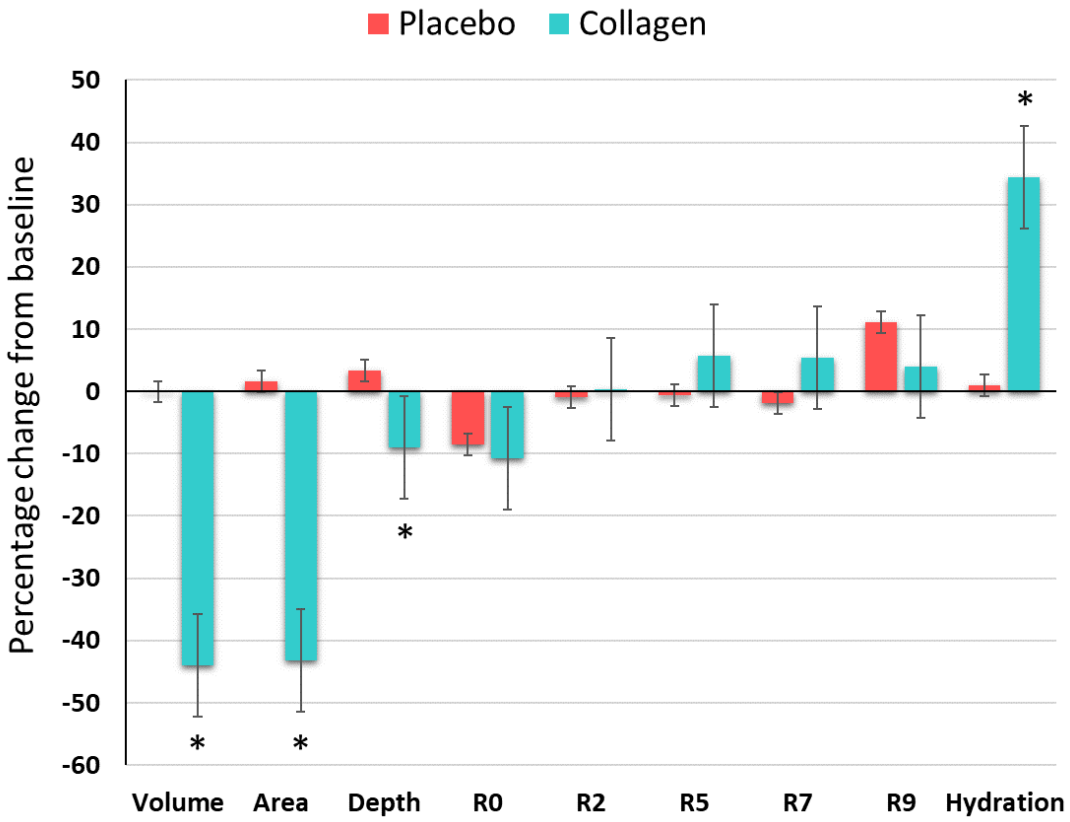

Figure 6. Percentage change of biometric skin parameters from baseline after 6 weeks of the interventional period (T0-T6) in the placebo group (red bars) and in the test group (green bars). Error bars indicate the standard error of the mean.

*indicates $p < 0.001$ for intergroup comparison (placebo group vs. collagen group).

Subjective Rating

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 was highly satisfied with the brief time period it takes for the supplement to start showing its effect, underlining the significance of obtaining quick results with a collagen food supplement.

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.

Table 6. Results of the survey conducted by the principal investigators with the volunteers who received the food supplement.

	Good	Fair	Poor
Effectiveness	76.2	21.3	2.5
Tolerability	88.8	10.0	1.25
Acceptability of the product	88.8	10.0	1.25

Discussion

Skin aging involves an intricate number of biological process characterized by changes that affect several components of the skin over time, influencing its appearance as an individual ages. The natural process of intrinsic skin aging is driven by factors such as genomic instability, cellular senescence, and telomere shortening, leading to a deterioration in both the function and appearance of the skin. Additionally, aging is accelerated by external factors such as UV radiation, pollution, tobacco smoke, and alcohol consumption, hastening the process and resulting in premature skin aging (Liang et al., 2023).

During the aging process, a pivotal molecular event is the dysregulation of extracellular matrix turnover, particularly the degradation of collagen fibers by matrix metalloproteinases (MMPs) and other proteases (Quan, 2023). Consequently, the skin undergoes structural changes, including a thinning and a reduction in epidermal thickness, along with damage to the dermis (Calleja-Agius et al., 2013; Robins, 2007). These structural changes result in regressive changes such as dehydration and loss of elasticity, leading to dry and loose skin with the appearance of furrows or wrinkles (Krutmann et al., 2023; Quan, 2023; Rostkowska et al., 2023).

Therefore, the psychosocial impact of skin aging has spurred the demand for effective interventions, including topical creams, injectable fillers, and collagen supplements.

Nutraceuticals have gained popularity in recent years as a strategy to enhance skin health and maintain a youthful appearance (Honigman & Castle, 2006; Lordan, 2021; Martinez et al., 2023; Schagen et al., 2012). Among the various nutraceutical strategies, collagen supplements, particularly those containing hydrolyzed collagen, have emerged as a safe and cost-effective option, compared to other collagen-based strategies. These products have the advantage of being taken orally, making them easy to incorporate into daily routines (Avila Rodríguez et al., 2018; Choi et al., 2019; Czech & As, 2016; Garcez Duarte, 2017; León-López et al., 2019).

Hydrolyzed collagen has shown promise in improving skin hydration and elasticity (de Miranda et al., 2021b). It has been demonstrated that the administration of collagen peptides can positively impact on various skin conditions and aging (Choi et al., 2019; Pu et al., 2023).

However, it is crucial to note that not all sources of hydrolyzed collagen are equally effective, and further studies are warranted to determine the optimal source and therapeutic duration against skin aging (Wang, 2021b; Pu et al., 2023).

This clinical trial specifically aimed to assess the efficacy, safety, and tolerability of 2.5 g COLLinstant® LMW, a novel cosmeceutical containing low molecular weight collagen peptides, on various skin parameters. COLLinstant® LMW is the first bovine collagen hydrolysate in the market, providing a beneficial amino acid profile that serves as building material and stimulator for the synthesis of new collagen, elastin and hyaluronic acid in the skin. In the context of skincare and nutraceuticals, hydrolyzed bovine collagen has become a popular ingredient. As stated by other authors (Bolke et al., 2019), one reason for the significant improvements of skin parameters shown in the present study might be the high similarity between the collagen peptides provided by the bovine collagen complex and those of human collagen. Hydrolysis of the bovine collagen yields specific bioactive short chain peptides that are characterized by a high coverage of their amino acid profile not only with the amino acid sequence of human collagen I, but also with elastin and hyaluronic acid in the skin.

The results demonstrated a significant reduction in wrinkle volume, area, and depth, increased skin hydration around the eyes, and moderate improvement in skin elasticity compared to the placebo. The effects were exclusive to COLLinstant® LMW, as the subjects had no other form of cosmetic treatment.

Notably, a daily intake of 2.5 g COLLinstant® LMW for 6 weeks yielded beneficial effects on skin health, contrasting with the higher dosages and longer durations recommended for standard molecular weight collagen hydrolysates to achieve clear beauty or health benefits (Asserin et al., 2015; de Miranda et al., 2021a).

The unique composition of COLLinstant® LMW, enriched with bioactive glycine- and proline-rich peptides, enhances gastrointestinal absorption (Sibilla et al., 2015), making it highly bioavailable and detectable in human blood shortly after ingestion (Ohara et al., 2007; Proksch et al., 2013). Topically applied skin care products (creams, lotions and sera) often fail to reach the deeper layers of the skin and given that the tested product is an oral supplement, the improvements observed most likely resulted from changes in protein turnover and restoration of collagen synthesis within the dermal layer of the skin (Oesser et al., 1999).

It was also confirmed by the present study, particularly by the improvement of the volume, area and depth of the skin wrinkle. Moreover, the effects were not only fully confirmed in objective test methods, but also in the subjective assessment of the volunteers. Collagen supplementation was safe and no adverse effects were reported.

In summary, low molecular weight hydrolyzed collagen holds promise as a natural intervention for maintaining skin health and combating signs of aging. Regular supplementation may contribute to smoother, more radiant skin.

Conclusions

This randomized, placebo-controlled clinical trial confirmed that skin aging could be addressed using nutrients that can restore altered skin biometric parameters that were objectively measured.

We have observed a significant reduction of wrinkles, a considerable increase in skin hydration, and modest improvements in skin elasticity after intake of 2.5 g low molecular weight collagen peptides, as a daily oral supplement for six weeks.

The treatment with low molecular weight collagen peptides presents a promising natural intervention for maintaining skin health and combatting signs of aging. Regular supplementation may contribute to smoother, more radiant skin by providing essential building materials by stimulating the synthesis of new collagen, elastin, and hyaluronic acid in the skin. Finally, the collagen supplementation regimen has been found to be devoid of any adverse effects and proved to be safe and well tolerated throughout the entire administration period.

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