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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

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ABSTRACT

Aging is a multifactorial and natural process that causes physiological changes in organs, tissues and cells over time. In the skin and cartilage, aging leads to a decrease in the synthesis and changes in the arrangement of proteoglycans and collagen, in addition to the loss of glycosaminoglycans, which are responsible for the integrity and health of these tissues. We hypothesized that daily oral supplementation with a liquid nutraceutical containing hydrolyzed fish collagen, vitamins, antioxidants and other active ingredients could improve skin texture and elasticity, and in addition have a protective effect on joint health. A double-blind, randomized, placebo-controlled clinical trial was conducted on 120 subjects who consumed either the test product or placebo on a daily basis for 90 days. Subjects consuming the test product had an overall significant increase in skin elasticity (+40%; $P < .0001$) when compared to placebo. Histological analysis of skin biopsies revealed positive changes in the skin architecture, with a reduction in solar elastosis and improvement in collagen fiber organization in the test product group. As reported in the self-perception questionnaires, these results were confirmed by the subjects' own perceptions in that participants agreed their skin was more hydrated and more elastic. In addition, the consumption of the test product reduced joint pain by –43% and improved joint mobility by +39%. Oral supplementation with collagen bioactive peptides combined with chondroitin sulphate, glucosamine, L-carnitine, vitamins, and minerals significantly improved the clinical parameters related to skin aging and joint health, and therefore, might be an effective solution to slow down the hallmarks of aging.

Abbreviations: SPQ, self-perception questionnaire; GAGs, glycosaminoglycans; ECM, extracellular matrix; OA, osteoarthritis; Pro-Hyp, proline-hydroxyproline; dGEMRIC, delayed gadolinium enhanced magnetic resonance imaging of cartilage; SYSADOA, Symptomatic Slow Acting Drugs for Osteoarthritis.

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1. Introduction

Aging is a natural process which involves changes in the connective tissue and its proteins: collagen and elastin [1]. Aging negatively affects the connective tissues in both the skin and the cartilage. In the skin, aging results in the reduction of collagen and elastin fibers, which eventually leads to skin sagging and the formation of fine lines and deep wrinkles [2,3]. The amount of glycosaminoglycans (GAGs), such as hyaluronic acid, in the epidermis and dermis also decreases with age, thus leading to an increase in skin dryness and a reduced capacity to retain moisture within the skin [4,5]. Cartilage aging can be linked directly to the softening of the articular surface, the decrease in proteoglycan content and the loss of matrix biomechanical properties [6]. Some evidence also suggests that the number of the chondrocytes in the extracellular matrix decreases with age [7]. Aged cartilage is compromised, exposed to deformation during joint movements, and more likely to develop osteoarthritic changes [8]. Osteoarthritis (OA) is a multifactorial disease that develops due to the cumulative effect of several biological and mechanical factors. Development and quicker progression of OA can be associated with knee injuries, structural abnormalities of the joint, repetitive use of the knee joint, or muscle weakness [9-11].

Similarities between dermal fibroblasts and chondrocytes have been shown in *in vitro* studies [12,13]. Dermal fibroblasts can be triggered to differentiate into chondrocytes and express cartilage specific collagen type II [13]. Similarly, under culture conditions, primary chondrocytes are able to switch from the production of cartilage-specific collagen type II to the production of fibroblast-specific collagen type I [12].

Nutraceuticals are orally administrated, biologically active compounds that have been shown to slow down the progression of the signs of aging [14]. Hydrolyzed collagen, as a nutraceutical supplement, has been extensively shown to benefit human skin and cartilage connective tissues [15-20]. Several studies described the mechanism of absorption and distribution of collagen peptides in the body. It has been demonstrated that C^{14}-labeled collagen peptides can reach the skin, cartilage, bones, and muscles and remain in these tissues up to 14 days after a single ingestion [21,22]. Iwai and colleagues showed that hydrolyzed collagen from porcine skin, chicken cartilage, and chicken feet that was ingested by healthy subjects after 12 h of fasting was absorbed and detected in the plasma as small peptides. Hydroxyproline-containing peptides detected in the plasma peaked 2 h after oral ingestion and decreased to half of the maximum after 4 h.

Additionally, ingestion of 9.4-23 g of gelatin hydrolysates from porcine skin, chicken feet, and chicken cartilage resulted in the presence of 25–60 nmol/ml of proline-hydroxyproline (Pro-Hyp) in the plasma [23], suggesting that Pro-Hyp can be considered a digestible peptide. Several *in vivo* studies have demonstrated the efficacy of collagen peptides on skin and cartilage health and aging. Matsumoto and colleagues showed in a preclinical study that ingested fish collagen peptides improved skin hydration in female subjects after 6 months of supplementation [24]. The same group confirmed these results with another double-blind placebo-controlled study carried out on 214 healthy female subjects aged 25–45, who ingested 2.5 g, 5 g, or 10 g of fish hydrolyzed collagen [25]. Hydration of the stratum corneum was measured at baseline and after 4 weeks, with the analysis of the results performed on all subjects showing no differences in skin hydration. Interestingly, stratification according to the different age groups revealed that the hydration of the skin was significantly improved in subjects over 30 years of age. Improvement in skin hydration was observed only in the 5 g and 10 g treated groups in comparison to placebo. In another double-blind, placebo-controlled clinical trial, 69 healthy female subjects, between 35 and 55 years of age, were randomized to 2.5 g, 5 g, or placebo groups and ingested either the hydrolyzed porcine collagen or placebo for 8 weeks. At the end of the study, skin elasticity was statistically improved in both 2.5 g and 5 g groups in comparison to control group [26]. Genovese et al. (2016) reported a double-blind, placebo-controlled study where 120 healthy volunteers were randomly allocated to a treated group (nutraceutical supplement with 5 g of hydrolyzed fish collagen) and a placebo group. The subjects took either the test product or placebo for 90 days. Results showed that the subjects taking the supplement had a significant increase in skin elasticity and an improvement in the skin texture in comparison to the placebo group [27].

Oral supplementation with collagen peptides has also been shown to improve the quality of life of subjects suffering from OA. McAlidon et al. (2011) used an MRI technique of delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) to visualize and assess changes of the GAGs density in the cartilage [19,28]. A prospective, randomized, placebo-controlled, double-blind pilot trial was conducted on 30 participants with mild OA who were supplemented with 10 g of hydrolyzed collagen daily for 24 weeks and 48 weeks [19]. dGEMRIC imaging confirmed improvement in the structure of the human cartilage due to collagen peptides supplementation. Additionally, this study also suggested that orally administrated collagen peptides have a potential protective function and might delay OA progression [19]. In another study, 250 subjects with primary OA were supplemented with 10 g of hydrolyzed collagen for 6 months. The authors reported a reduction of pain and an improvement in the quality of life [20].

Aging is also associated with a decreased intake of some essential nutrients including calcium, zinc, iron, vitamin E and B vitamins [29]. Oral supplementation with vitamins and minerals has been shown to improve mental health [30] and vigor [31], to reduce the levels of oxidative stress [32], to improve general wellbeing [33] and nutritional status [34] in healthy subjects, and to help with recovery following intensive exercise [35]. It has been demonstrated that among people who are physically active, supplementation with vitamins and antioxidants may be beneficial to maintain the integrity of the cellular antioxidant defenses, which is reduced after intensive training [32].

We hypothesize that oral supplementation with a nutraceutical comprised of hydrolyzed collagen, vitamins, antioxidants, and other active ingredients (such as L-carnitine, glucosamine and chondroitin sulphate) could have a beneficial effect on skin properties, skin texture and joint health. We investigated the changes in skin elasticity; and to expand the

in vivo observations, a microscopic examination of skin biopsies was carried out in a pilot study. In addition, joint health, mobility and general wellbeing were also measured as secondary outcomes.

2. Methods and materials

2.1. *Study design, subject selection, and randomization*

A double-blind, randomized, placebo-controlled monocentric study was conducted by an independent aesthetic clinic in Rome (MedicalSpa Education, Rome, Italy). The sample size was calculated using DanielSoper statistical calculator (https://www.danielsoper.com/statcalc/category.aspx).

In this study, 122 subjects were recruited to participate and randomly allocated into two groups (Fig. 1). One group had 61 volunteer subjects between 21 and 70 years of age, who were instructed to consume the test product daily for 90 days, and the other group had 61 volunteer subjects, within the same age range, who consumed a placebo daily for 90 days.

Two subjects in the placebo group were lost to follow-up due to personal reasons, therefore they were excluded from the analysis at the end of study. The remaining subjects (61 in the test product group and 59 in the placebo group) came back for all three follow-up visits (at 30 days, 60 days and 90 days) and confirmed they had taken one bottle of the test product or placebo every day, according to the protocol, meaning that the compliance was 100%.

The subjects were randomized between the test product and the placebo groups on the basis of the following characteristics: age, sex, BMI, ethnic background (mainly Caucasian), smoking habits, alcohol intake, nutritional level and skin type assessed using Fitzpatrick scale. 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 [36]. 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). The nutritional level was assessed by a qualified nutritionist in relation to the nutrients introduced with the diet, in a scale of 1–10.

The sample size was chosen after consultation with a statistician in order to reach statistically significant results for each group when the final analysis was performed. Ingredients for the test product and placebo are listed in Table 1. MINERVA Research Labs generated the random allocation sequence using GraphPad Quick Calcs (www.graphpad.com/quickcalcs/randomize/1). Both the placebo and the test product were distributed in white boxes containing clear, unlabeled bottles, with a coding system known only to the sponsor. All investigators, research personnel and participants remained blinded to these codes until statistical analyses were complete.

The inclusion criteria were as follows: healthy female or male volunteers between 21 and 70 years of age, any body mass index (BMI), of any ethnic type, who had a balanced diet, and had the ability to understand the study related information and to give written informed consent. Subjects with the characteristic hallmarks of skin photo-aging (i.e., wrinkling,

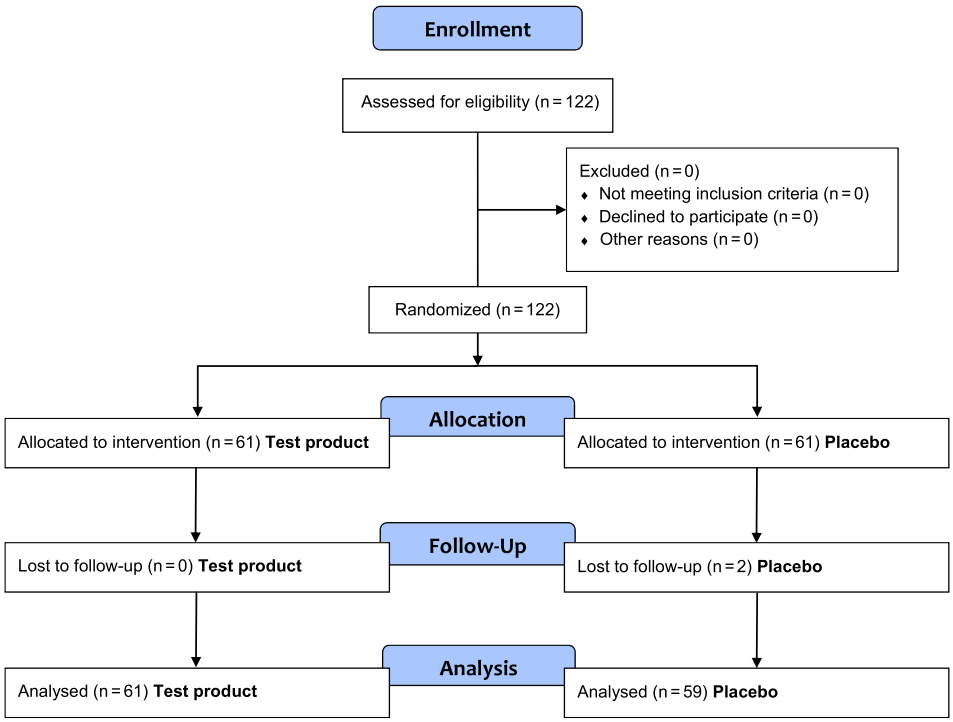

Fig. 1 – Flow diagram of subjects' selection for the study.

Table 1 – Composition of the test product and placebo

Test product	Ingredients	Per 50 ml
Patented complex [a]	Hydrolyzed Collagen (from Fish)	8%
	Glucosamine HCl	2%
	L-Carnitine	0.4%
	Black Pepper and dried Maca extracts	0.042%
	Hyaluronic Acid	0.004%
Minerals	Zinc	0.003% (15% NRV [b])
	Copper	0.0003% (15% NRV [b])
Vitamins	Vitamin C	0.2% (100% NRV [b])
	Vitamin B3	0.005% (15% NRV [b])
	Vitamin B6	0.003% (100/% NRV [b])
	Biotin	0.0001% (100% NRV [b])
	Vitamin D	0.00001% (100% NRV [b])
	Vitamin B12	0.000001% (15%NRV [b])
Additional active ingredients	Chondroitin Sulphate	1%
	N-acetylglucosamine	0.01%
Other ingredients	Water	87%
	citric acid, malic acid,	1.2%
	Stevia (natural sweetener)	0.04%
	Flavoring (apple and mango)	0.3%
Placebo	**Ingredients**	
	Water	99.08%
	Stevia	0.02%
	Apple and mango flavoring	0.4%
	Citric acid, malic acid	0.5%

[a] Patent granted in Italy No. 0001413152; European patent application pending.
[b] NRV = Nutrient Reference Value.

orthokeratotic hyperkeratosis, irregular acanthosis, vascular ectasia and dermal elastosis) were also recruited in the study.

The following exclusions were implemented: female subjects who were pregnant, had a recent pregnancy (past 6 months), were planning a pregnancy or breastfeeding, had allergies to fish, had a significant past medical history which in the opinion of the investigator would compromise the safety of the subjects, had severe skin-related pathologies (i.e., skin cancer, psoriasis, eczema, melasma), and/or were using collagen-based food supplements. An initial eligibility screening was performed by telephone prior to conducting the clinical evaluation procedures at the study site.

Subjects continued to use their usual facial skin care and make-up products for the duration of the entire study. Physical activity of all participants remained constant during the trial. This research study was a double blind, randomized, placebo-controlled trial. Neither the investigator nor the subjects were aware of treatment assignments. At the end of the study only the Sponsor was able to allocate the batch number to a specific product.

2.2. Test product and placebo

The product tested in this study was GOLD COLLAGEN® ACTIVE (referred to in the text as test product), a liquid dietary supplement manufactured by MINERVA Research Labs (London, UK). This liquid supplement contains a patented complex (patent granted in Italy: 0001413152 and European patent application pending), which includes the following active ingredients in a 50 ml bottle (1 dose): hydrolyzed fish collagen type I (4,000 mg), molecular weight of 0.3–8 kDa, hyaluronic acid, glucosamine hydrochloride, L-carnitine, black pepper and maca extracts. The test product also contains chondroitin sulphate, vitamins, and minerals for the maintenance of healthy connective tissues (Table 1). The test product is stable at room temperature and has a shelf-life of 24 months from the day it was manufactured. The placebo contains water (which replaces the active ingredients present in the test product), natural preservatives (malic acid and citric acid), flavoring (mango and apple) and a natural sweetener (stevia).

2.3. Ethics

The study conformed to the requirements of the 1964 Declaration of Helsinki and its subsequent amendments [37]. The study was carried out within the spirit of the ICH Guidelines on Good Clinical Practice, 1996 [38] at MedicalSpa Education in Rome. Subjects were informed of the nature, purpose, and known risk of the study both orally and in writing and gave their written informed consent before participating in the study. Minerva Research Labs was the Sponsor and monitored the clinical trial throughout its duration. The clinicians and medical personnel at MedicalSpa Education recruited the patients and conducted the study.

2.4. Primary Outcome Measures

2.4.1. Skin elasticity

The primary outcome of interest was a change in skin elasticity (expressed as Young's modulus), which was assessed with a

SkinLab USB Elasticity Module (DermaLab® Series, Cortex Technology, Hadsund, Denmark) [39] and histological examination of the skin. Skin elasticity was measured in the inner part of the ventral forearm, at a distance of 10 cm from the wrist of each subject recruited in the trial as described previously [27]. The test site was the same for all participants and areas with any type of skin lesion were disregarded (i.e., moles, scars, tattoos). Skin elasticity was measured at baseline (Day 0), after 4 weeks (Day 30), after 8 weeks (Day 60) and at the end of the treatment (Day 90) in all subjects recruited for the trial who were allocated with either the test product or placebo.

2.4.2. *Histological examination*

This exam was performed by incisional biopsy (Punch 4.0 mm) in the inner forearm. The histological examination was performed by staining the paraffined sections (4.0 μm) with Hematoxylin Eosin stain for the qualitative analysis of skin structures [40,41], with Weigert's elastic stain to identify elastic fibers and with Masson's trichrome stain to distinguish collagen fibers from the surrounding connective tissue [42,43]. Biopsies were taken at the beginning (Day 0) and at the end of the trial (Day 90) from 4 volunteer subjects who took either the test product or placebo. The study protocol was reviewed and approved by an internal medical committee. The Principal Investigator performed the biopsies after the volunteer subjects agreed to undergo this procedure and signed an informed consent.

2.5. *Secondary outcome measures*

2.5.1. *Comparative photography*

A photographic record of the face of all subjects who gave their consent was taken at the beginning (Day 0) and at the end of the study (Day 90) by a professional photographer, with particular focus given to the crow's feet area and nasolabial folds. DermaVision software (OptoBioMed™, South Korea), a digital skin image analysis system, was used to analyze the skin texture in the areas of interest, as previously described, and is based on the cross-evaluation of facial skin [44-46].

2.5.2. *Self-perception questionnaires*

Self-perception questionnaires (SPQs) consisted of questions related to skin, hair, nails, joints, mood, wellbeing, and questions regarding the subjects' appreciation of the product. In addition, subjects were asked questions about their fitness, sports activities, energy levels and nutrition. Questionnaires were completed by each participant at the beginning (Day 0) and at the end (Day 90) of the trial.

2.5.3. *Questionnaires to assess knee pain and quality of life*

In order to assess the stiffness, pain, and disability of joints, all subjects suffering from joint discomfort and/or mild osteoarthritis were asked to fill in the joint pain and Lysholm scoring questionnaires under the guidance of qualified clinicians and medical personnel. Both, joint pain and Lysholm indexes are patient-completed questionnaires that are considered to be a valid and responsive measure of outcome [47] and can be used for the assessment of joint pain, joint stiffness/discomfort, and physical function related to hip and knee osteoarthritis. Higher scores in Lysholm index indicate a better outcome with fewer symptoms and disability, while in the joint pain scoring system, higher values correlate with functional limitations.

2.6. *Statistical analyses*

Statistical analyses of the data were carried out using GraphPad Prism 7. Data distribution was determined using the Kolmogorov–Smirnov test and histograms and parametric tests were used on raw data. Data were expressed as means ± standard errors of the means (SEM) or standard deviations (SD). Groups were compared using Independent t-test, paired t-test (2 groups), or one-way ANOVA/repeated measures ANOVA with post hoc Tukey's multiple comparison test (>2 groups). P values <.05 were considered to be significant.

3. Results

3.1. *Demographic data and baseline skin characteristics*

122 subjects (61subjects in each group) were enrolled in this study; 2 subjects from the placebo group were lost to follow-up and therefore were excluded from the analysis at the end of the study (Fig. 1). The gender ratio was 82% and 70% women in the test product and placebo groups, respectively. There was no significant difference between the test product and placebo groups in the mean age of the subjects (43 ± 13.1 and 43 ± 12.3, respectively) and in the BMI (21 ± 2.6 and 21 ± 2.7, respectively). There was no significant difference between the groups in the subjects' nutritional level, alcohol intake, smoking habits, caffeinated drinks intake (Table 2), use of medicines, or diet (data not shown). Among all subjects, the most represented Fitzpatrick skin classification was type III (cream white skin, fair, with any hair or eye color, Table 3).

No adverse events were reported in this study. Compliance during the trial was excellent, with no subject withdrawing from the study and all subjects taking the test product or placebo every day.

Table 2 – Demographics of subjects

	Test product (n = 61)	Placebo (n = 59)
Age, years	43 ± 13.1	43 ± 12.3
Sex, n (%)		
Female	50 (82)	41 (70)
Male	11 (18)	18 (30)
BMI	21 ± 2.6	21 ± 2.7
Ethnic background, n (%)		
Asian	5 (8.2)	3 (5.1)
Caucasian	54 (88.5)	53 (89.8)
Latin-American	2 (3.3)	1 (1.7)
Middle-Eastern	0 (0)	2 (4)
Subjects smoking, n (%)	26 (42.6)	23 (39)
Subjects drinking alcohol, n (%)	34 (55.7)	33 (55.9)
Nutritional level [a]	7/10	7/10

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

[a] The nutritional level was assessed by a qualified nutritionist in relation to the nutrients introduced with the diet, in a scale of 1–10.

Table 3 – Skin phototype of subjects in the test product and placebo groups

	Test product (n = 61)	Placebo (n = 59)
Fitzpatrick[a], n (%)		
I	8 (13.1)	6 (10.2)
II	11 (18)	16 (27.1)
III	36 (59)	31 (52.5)
IV	6 (9.6)	5 (8.5)
V	0 (0)	1 (1.7)

[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).

3.2. *Increase in skin elasticity*

Skin elasticity was analyzed between the test product and placebo groups at day 0, day 30, day 60, and day 90. There was a statistically significant difference ($P < .05$) in skin elasticity at baseline between the test product (7.9 ± 0.2, n = 61) and placebo (6.9 ± 0.3, n = 59) groups, which further increased at day 30, day 60, and day 90 (9.8 ± 0.2 and 7.0 ± 0.3, respectively; with an overall increase of +40%, $P < .001$, Fig. 2A). Due to the significant difference between the tested groups at baseline, a repeated measures ANOVA was conducted between day 0 and day 90 in each group (test product and placebo) to further investigate the difference between the two groups. A highly significant increase in skin elasticity was measured in the subjects consuming the test product at each consecutive measurement time point (day 30, day 60, and day 90) when compared to the baseline (day 0) and with an overall increase of 24% at day 90 ($P < .001$, Fig. 2A). No such statistically significant differences were observed in the placebo group ($P > .05$, Fig. 2A).

In order to see if there were age-related differences in skin elasticity, the data were stratified into two different age groups: 21–50 years and 51–70 years. In younger subjects, skin elasticity was significantly higher ($P < .05$) at baseline between the test product group (7.8 ± 0.2, n = 45) and the placebo group (6.6 ± 0.3, n = 41), which further significantly increased at day 30, day 60, and day 90 (9.6 ± 0.2 vs. 6.7 ± 0.5, respectively, with an overall increase of +43%, $P < .001$, Fig. 2B). When a repeated measures ANOVA was performed, a statistically significant increase ($P < .001$) in skin elasticity was detected at each consecutive measurement point among the subjects in the test product group, when compared to baseline (day 0) and with an overall increase of +23% at day 90. No statistically significant differences in skin elasticity during the entire duration of the trial were observed in the placebo group ($P > .05$, Fig. 2B).

We did not detect any significant differences at baseline between the test product (8.0 ± 0.3) and placebo (7.7 ± 0.8) groups in older subjects (age range 51–70 years old, $P > .05$). Skin elasticity measured in this age group increased in a statistically significant way only at day 90 (9.8 ± 0.5, n = 16), when compared to the placebo group (7.3 ± 0.8, n = 18) with an overall increase of +34% ($P < .001$, Fig. 2C). Moreover, when compared to baseline within each group, there was a highly significant increase in skin elasticity at day 60 (+10% increase, $P < .001$) and day 90 (+22% increase, $P < .001$. Fig. 2C) in the test product group. No such statistically significant differences were detected in the placebo group.

3.3. *Histological analysis and comparative photography*

Histological biopsies of healthy skin from 4 different subjects taken before the treatment with the test product or the placebo showed the following skin characteristics that are usually associated with photo-damaged and aging skin: orthokeratotic hyperkeratosis, irregular acanthosis, vascular ectasia, and dermal elastosis. Poor collagen content, poor arrangement of collagen fibers, and flattening of dermal papillae were also visible. In the biopsies from the subjects consuming the test product for 90 days, an improvement in the structure and stratification of the

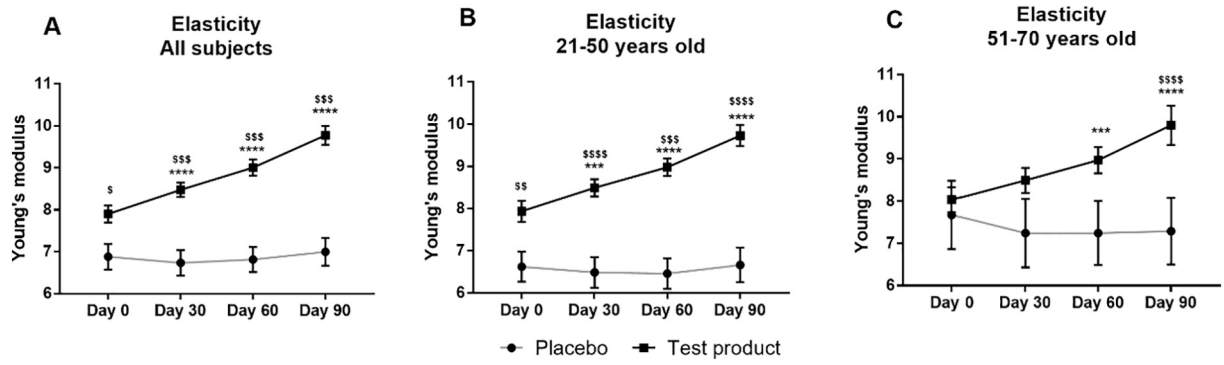

Fig. 2 – Skin elasticity measurements. A) Significant increase in skin elasticity in all subjects consuming the test product for 90 days (test product n = 61, placebo n = 59). B) Significant increase in skin elasticity in younger subjects consuming the test product between day 0 and day 90 (test product n = 44, placebo n = 41). C) Significant increase in skin elasticity in elderly subjects consuming the test product, at day 60 and day 90, when compared to baseline (test product n = 17, placebo n = 18). Data are presented as means ± SEM and analyzed by two-way ANOVA, followed by Tukey's multiple comparison test $^{\$}P < .05$, $^{\$\$}P < .01$, $^{***}/^{\$\$\$}P < .001$, $^{****}/^{\$\$\$\$}P < .0001$. *indicates changes vs. baseline (day 0), $^{\$}$ indicates test product compared to placebo.

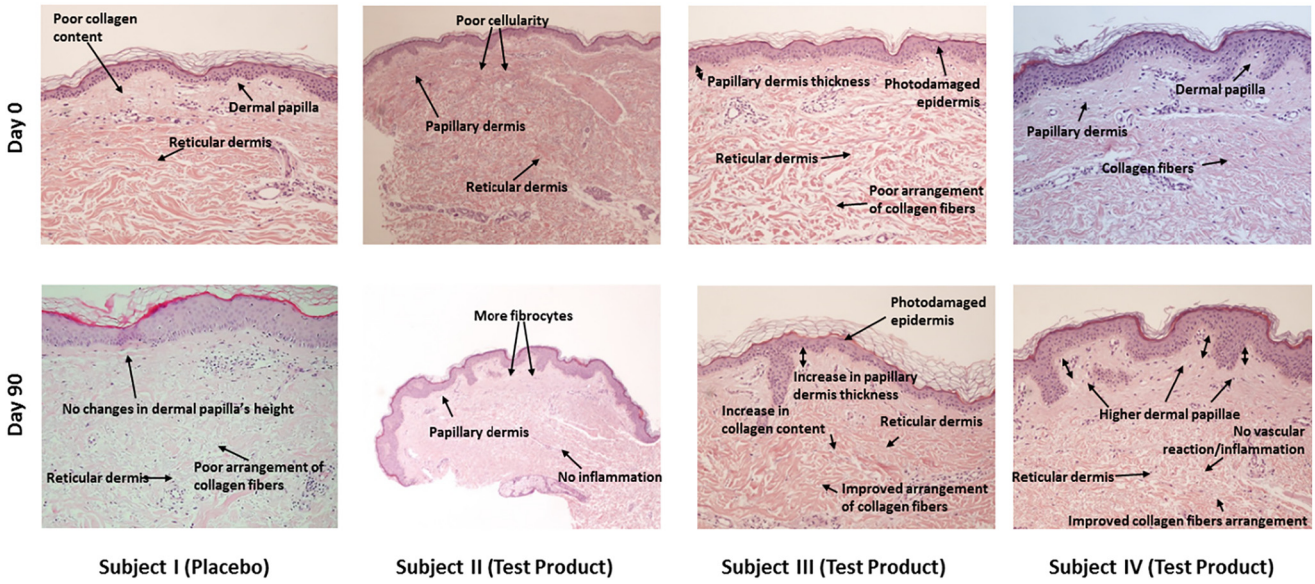

Fig. 3 – Supplementation with the test product improved skin structure and characteristics. Histological examination of skin biopsies in 4 subjects consuming the test product (subjects II-IV) or the placebo (subject I) for 90 days. Day 0 (top panel), day 90 (bottom panel).

epidermal layers (absence of the architectural disorder of Malpighian layer) and an improvement in the collagen fiber organization within the dermis (increase in the fiber width and parallel collagen running fibers) were observed (Fig. 3). Moreover, an increase in the papillary dermis thickness, an increase in the number of fibrocytes, a reduction in the elastosis, and an overall reduction in the photo-damage-related inflammation were also visible in these samples. No such improvement was present in the samples taken from the subject using placebo (Fig. 3). A photographic record of the subjects' faces was acquired before, as well as after the treatment as shown in Fig. 4. The skin of subjects consuming the test product was smoother and more supple; the texture and the appearance of fine lines in the crow's feet area and nasolabial folds were also improved (Fig. 4). When calculated by using DermaVision software the reduction in wrinkles was –4.5% and –3.8% in the crow's feet area and –4.5% and –5.3% in the nasolabial wrinkles in subject III and subject IV, respectively (data not shown).

3.4. *Joint health questionnaires results*

A subset of subjects (18 out of 34) in the 51–70 year group reported problems in their joints, knees, or hip function, and their responses were therefore analyzed at the beginning and at the end of the study (Fig. 5). Joint pain score in the test product group was significantly reduced (–43%; $P < .05$, n = 10) from a score of 29 ± 5.6 at baseline to 12.4 ± 3.7 at day 90 (Fig. 5A), whilst LYSHOLM score was significantly increased (+39%;

Fig. 4 – Oral supplementation with the test product for 90 days improved the appearance of fine lines in the crow's feet areas and nasolabial folds; skin texture was also improved when compared to baseline (day 0).

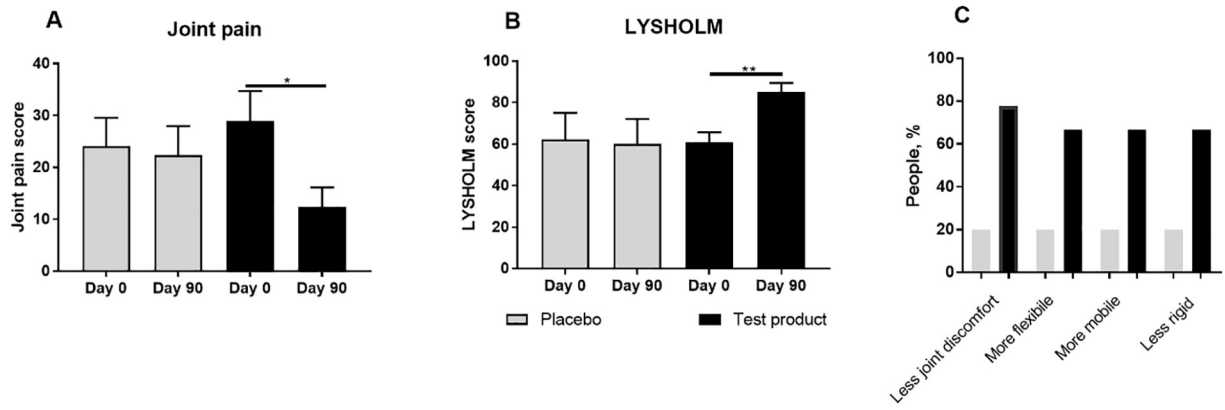

Fig. 5 – Improvement in joint health. Effects of 90 day supplementation with the test product on A) joint pain (–43%, n = 10) and B) LYSHOLM (+39%, n = 9) scores in 51–70 years old subjects. C) Self-perception questionnaires completed by the subjects (placebo vs. test product) regarding their joints. Data are presented as means ± SEM (A and B) and as percentage of positive responses (C). Paired t-test, *P < .05, **P < .01.

P < .05, n = 9) from a score of 61 ± 4.6 to 85 ± 4.5 at day 90, when compared to day 0 (Fig. 5B). In the self-perception questionnaires results, 78% of subjects in the test product group reported to have less joint discomfort, and more than 60% of the subjects agreed their joint health improved by increasing joint flexibility, mobility, and reducing joint stiffness (Fig. 5C). No significant improvement or change in the responses among subjects consuming placebo was recorded (Fig. 5A-C).

3.5. *Self-perception questionnaires results*

This analysis was performed to compare placebo and test product questionnaire results at day 0 and day 90 for skin, nails, hair, general wellbeing and product appreciation. At baseline, both groups reported similar perceptions for the parameters considered (data not shown). Following the 90-day supplementation with the test product, the subjects reported favorable improvements in their skin, hair and nails properties. In addition, 83% of the subjects claimed to have more energy and 70% felt that the supplementation with the test product improved their general wellbeing, with more than 93% of the subjects willing to continue taking the product. The analysis of the results related to the placebo group revealed a significantly lower appreciation for all considered parameters (Fig. 6).

4. Discussion

In this study, we reported for the first time a positive effect of the test product on skin appearance, with a significant increase in skin elasticity, reduction in skin photo-aging, and an improvement in general wellbeing, thus confirming our initial hypothesis. We also observed a significant improvement in joint health with reduced joint discomfort and an increase in joint mobility in a subgroup of elderly subjects (51–70 years old).

The test product used in this study is a multicomponent dietary supplement comprised of collagen bioactive peptides, hyaluronic acid, glucosamine, chondroitin sulphate, L-carnitine, vitamins, and minerals. The activity of the formulation of the test product might be enhanced by the presence of maca and black pepper extracts. Maca has been shown to have a chondroprotective function in human cartilage [48] and black pepper extract has been clinically proven to enhance cell bioavailability, and consequently, to increase the absorption of a variety of nutrients (up to 60%) [49].

Skin aging is associated with an increased degradation of collagen and elastin fibers, which form the main network to support the skin's structure, and consequently, contribute to its smooth appearance. We have recently reported a significant improvement in skin texture and skin properties in subjects who consumed a hydrolyzed collagen-based dietary supplement combined with antioxidants [27]. In this study, the results from the histological examination of skin biopsies showed an improvement in the structure of the dermis and in the stratification of the epidermal layers in the test product group. The reduction in solar elastosis and the improvement in the collagen fibers organization may have been enhanced by the mix of vitamins and minerals present in the test product.

Vitamin C is a natural antioxidant [50], which stimulates collagen production in the skin acting as a co-factor in the hydroxylation of lysine and proline, which are two main amino acids forming the collagen fibers [51,52]. Vitamin C has also been shown to have a photo-protective effect when administered orally for 3 months, by reducing a thymine dimer formation in the DNA of skin cells [53]. Moreover, consumption of food rich in vitamin C has been shown to be correlated with reduced dermal elastosis [54], possibly due to the direct effect of this antioxidant on the biosynthesis of elastin [55].

Oral supplementation with zinc and copper, essential micronutrients, may not only contribute to healthy skin, hair and nails, but also enhance the anti-oxidative protection [56,57]. Both copper and zinc play an important role in the activation and regulation of many enzymes, e.g. Cu/Zn superoxide dismutase which possesses anti-inflammatory and antioxidant activity. Zinc itself is a component of 300

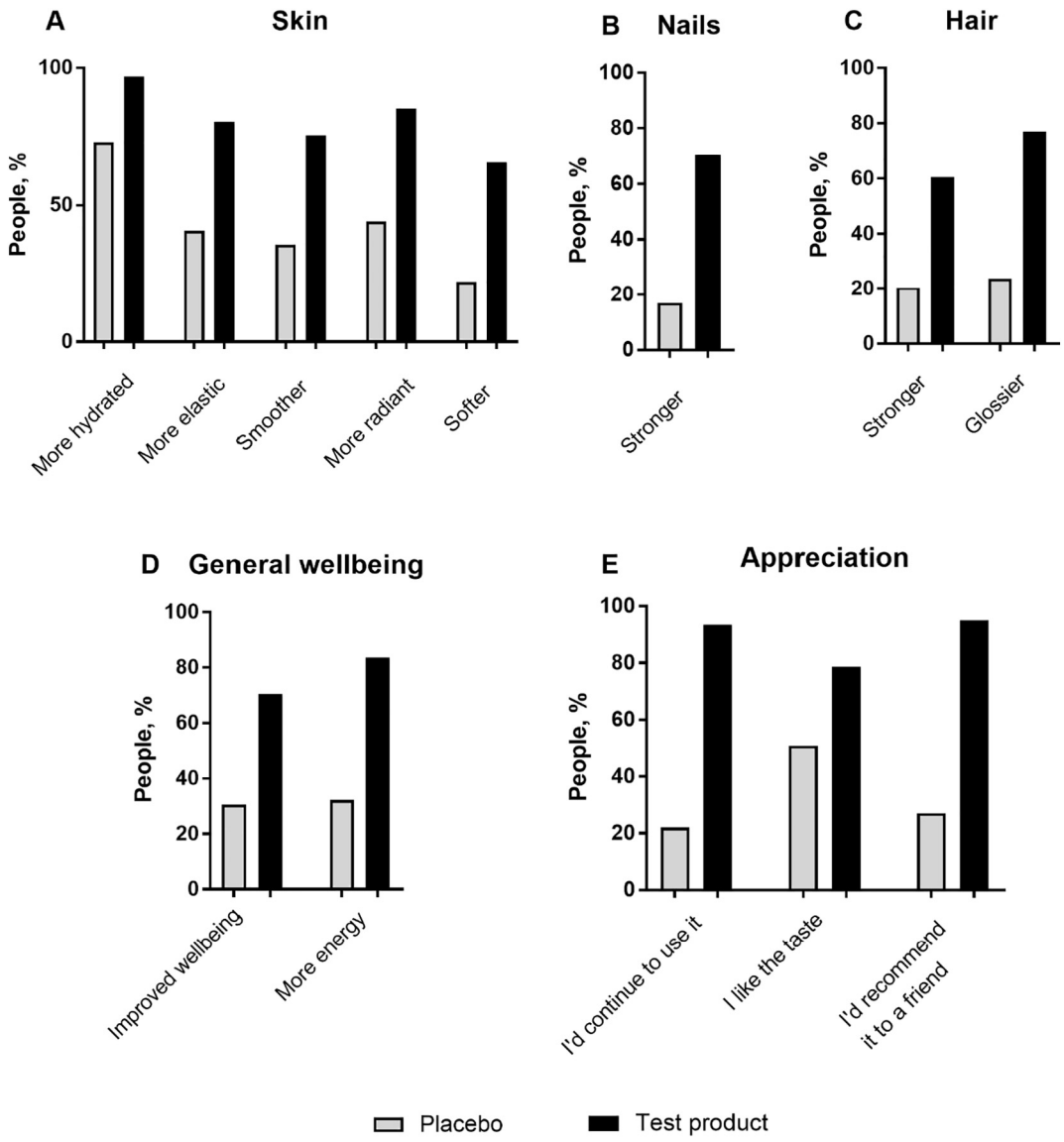

Fig. 6 – Results of the self-perception questionnaires completed by all subjects (placebo vs. test product) regarding their A) skin, B) nails, C) hair, D) general wellbeing, and E) appreciation of the test product or placebo after 90 days of treatment, n = 61 (test product), n = 59 (placebo), Data presented as percentage of positive responses.

enzymes, some of them playing an important part in muscle metabolism and energy production [58].

In addition to improvement in skin architecture, we also observed a significant increase in skin elasticity following consumption of the test product, which might be a direct effect of hydrolyzed collagen peptides. Hydrolyzed collagen, as demonstrated in clinical, *in vivo*, and *ex vivo* studies, improves skin hydration, dermal collagen content, elastin and glycosaminoglycans production, all contributing to the improvement in the mechanical properties of the skin [16,59-61]. The bioactive properties of collagen peptides are based on their direct absorption, distribution, and action on skin cells, which has been confirmed in animal [22] and human studies [62]. The improvement in skin elasticity among elderly participants was observed later in this study (after 60 days of supplementation) when compared to the younger subjects. Moreover, their skin elasticity, when calculated against the placebo results, was lower by ~10% at the end of the trial when compared to younger participants. These results are not surprising as one of the hallmarks of aging is a loss in skin elasticity due to the up-regulation of elastase activity [63], and therefore, it might take longer for the aged skin to improve. In addition, an impaired absorption of micronutrients observed in the elderly [34] might also have had an impact on this study outcome.

Aging is also associated with a decline in motor function, a decrease in muscular performance, and the deterioration of joints. In the current report, both the joint pain and Lysholm scores showed a significant improvement in the test product group, which was confirmed by the results from the SPQs. Subjects in the test product group felt less pain and observed an increased flexibility in their joints, which contributed to the improvement in their everyday activities. These data are in accordance with other studies in which the combination of

glucosamine and/or chondroitin sulphate, together with hyaluronic acid, hydrolyzed collagen, and other nutrients, were shown to have promising benefits on joint cartilage, synovial fluid, and overall clinical outcome in OA patients [64,65]. In an animal model of post-traumatic osteoarthritis, the oral consumption of hydrolyzed collagen decreased the inflammatory response and had a protective effect against cartilage loss by increasing the number of chondrocytes and stimulating the production of proteoglycans [66]. In addition, supplementation with hydrolyzed collagen reduced the number of additional treatments required to reduce activity-related knee pain [67]. Hyaluronic acid, which plays an essential role in tissue hydration and moisture retention, along with N-acetylglucosamine, chondroitin sulphate, and/or glucosamine sulphate, is shown to reduce pain and to improve physical function of joints and cartilages [68-70]. Both, chondroitin sulphate and glucosamine are natural compounds considered as Symptomatic Slow Acting Drugs for Osteoarthritis (SYSADOA) [71] and are often used together for the treatment and prevention of OA [72-74]. Oral supplementation with N-acetyl-d-glucosamine was also shown to significantly enhance the prevention of joint damage [75-77], in a similar manner as glucosamine hydrochloride. Finally, intake of vitamin D was shown to improve the quality of life and reduce joint pain in patients with OA following 12 months of supplementation [78].

Taking collagen-based nutraceuticals may also positively influence energy levels and physical wellbeing. Reports by other research groups have shown that oral supplementation with hydrolyzed collagen, alone or in combination with resistance training, can have a positive effect on muscle mass and body composition in elderly subjects [67,79]. Our group previously observed a positive change in the heart rate (reduction) and body composition (increase in muscle mass and decrease in body fat) after 14 weeks of supplementation with the test product [14] in a preliminary pilot study, but no such statistically significant changes were recorded in this study (data not shown). The discrepancy between the current and the pilot studies may be due to the differences in age, sex, and physical activity of the enrolled subjects. In the previous pilot study, subjects were younger, physically more active men, while in this report subjects were significantly older, mixed sexes, and varied in their levels of physical activity. However, in the current report, more than 70% of the subjects agreed that their physical wellbeing improved, and we observed a positive trend in the increase in strength and fitness level among subjects in the test product group (data not shown). Subjects consuming the test product also reported an increase in energy levels and an improvement in their mood, which may due to the mix of B vitamins (B3, B6, B7, and B12) and L-carnitine, with all playing an important role in the cell energy metabolism and in supporting an active lifestyle [80].

The analysis of the SPQs showed an overall significant perceived improvement in skin, hair, nails, and general wellbeing in the test product group at the end of the treatment, which was not recorded to such extent in the placebo group. Subjects in the test product group were also happy to continue with the oral supplementation of the test product after the end of the study. Analysis of the clinical data suggests that daily oral supplementation with collagen-derived bioactive peptides combined with chondroitin sulphate, glucosamine, L-carnitine, maca, black pepper, vitamins and minerals has beneficial effects on clinical parameters related to skin elasticity, skin photo-aging, and joint health. No adverse events were reported during this study and the compliance of the subjects was excellent.

The major limitations of this study were the low number of biopsies taken to assess skin structural changes and photodamage, as well as the low number of subjects suffering from joint discomfort. Further, more in-depth studies are needed to have a better understanding of the mechanism of action and the protective effect of nutrients and nutraceuticals on age-related changes occurring in the body, including skin architecture, joint health, body composition, and in promoting an improvement in the overall wellbeing and quality of life.

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