Review

Effects of hydrolyzed collagen supplementation on skin aging: a systematic review and meta-analysis

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Conflict of interest: None.

Funding source: None.

doi: 10.1111/ijd.15518

Abstract

Skin aging has become a recurring concern even for younger people, mainly owing to increased life expectancy. In this context, the use of nutricosmetics as supplements has increased in recent years. Moreover, numerous scientific studies have shown the benefits of hydrolyzed collagen supplementation in improving the signs of skin aging. The objective of this study was to summarize the evidence on the effects of hydrolyzed collagen supplementation on human skin through a systematic review followed by a meta-analysis of clinical trials focusing on the process of skin aging. A literature search was conducted in the Medline, Embase, Cochrane, LILACS (Latin American and Caribbean Health Sciences Literature), and *Journal of Negative Results in BioMedicine* databases. Eligible studies were randomized, double-blind, and controlled trials that evaluated oral supplementation with hydrolyzed collagen as an intervention and reported at least one of the following outcomes: skin wrinkles, hydration, elasticity, and firmness. After retrieving articles from the databases, 19 studies were selected, with a total of 1,125 participants aged between 20 and 70 years (95% women). In the meta-analysis, a grouped analysis of studies showed favorable results of hydrolyzed collagen supplementation compared with placebo in terms of skin hydration, elasticity, and wrinkles. The findings of improved hydration and elasticity were also confirmed in the subgroup meta-analysis. Based on results, ingestion of hydrolyzed collagen for 90 days is effective in reducing skin aging, as it reduces wrinkles and improves skin elasticity and hydration.

Introduction

Collagen is the main structural protein in connective tissues such as the skin, tendons, cartilage, and bones, constituting 25–30% of all proteins in the body.[1] Collagen is a component of the extracellular matrix of cutaneous tissue, representing up to 75% of its total weight, and its main function is related to providing mechanical support. In association with hyaluronic acid, reticulin, and elastin, which are other fibers found in the extracellular matrix, collagen forms a support network for fibroblasts, keratinocytes, melanocytes, and specialized cells of the skin immune system.[2–4]

The dermal collagen fiber network becomes increasingly fragmented (i.e., presenting shorter and less organized fibers that accumulate as several fragments of degraded collagen) with age.[5] In addition, aging also increases the generation of metalloproteinases, which are enzymes that degrade collagen fibers, thus decreasing the synthesis of new extracellular matrix components, including the type of collagen produced by dermal fibroblasts.[6,7] The overlap of intrinsic aging and extrinsic aging leads to structural and functional changes in the dermis, including volume reduction, elasticity loss, decreased epidermal thickness, increased wrinkles[8], and decreased capacity to retain moisture through the skin owing to decreased hyaluronic acid (a compound responsible for retaining water in skin structures) in the extracellular matrix.[9,10]

A wide range of dietary supplements, aside from the traditional systemic antioxidants, have been used to improve skin health and achieve a younger appearance, such as some marine protein-based macromolecules.[11–16] However hydrolyzed collagen (HC), which has been used as one of the most recent and promising anti-aging systemic supplementation, demonstrated functional and beneficial effects on the skin in several scientific studies, mainly by improving the clinical signs of skin aging.[3,17,18] Some studies have shown that the age-dependent reduction in collagen synthesis can be reversed by the oral administration of bioactive collagen peptides.[19] These peptides are obtained from the enzymatic hydrolysis of natural collagen. Once digested, they are metabolized to dipeptides and tripeptides in the gastrointestinal tract and thereafter transported

through the bloodstream and accumulate in the skin to form new collagen fibers.[20,21]

HC supplements are rich in hydroxyproline, proline, and glycine amino acids. Among these proteins, only hydroxyproline is a component of collagen.[22] Several studies have shown that prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly) are absorbed after ingestion as dipeptides, not as amino acids,[23–27] and become deposited on the skin.[28] These dipeptides increase the bioactivity of dermal fibroblasts by increasing collagen synthesis, thus improving hydration and elasticity[29] and reducing wrinkles.[30]

With the increasing number of scientific publications and clinical studies evaluating collagen supplementation worldwide, the need for compiling and analyzing these data, to assist in decision making concerning supplementation, becomes evident. Therefore, the objective of this study was to summarize the evidence on the effects of HC supplementation on the human skin, as reported in clinical trials focusing on the skin aging process, through a systematic review followed by meta-analysis.

Materials and methods

Search strategy, inclusion criteria, and exclusion criteria

The Medline, LILACS (Latin American and Caribbean Health Sciences Literature), Embase, Cochrane, and *Journal of Negative Results in BioMedicine* databases were searched using various combinations of specific terms from the thesaurus of each database, terms used in the titles or abstracts, and free terms associated with the research question. After the search, studies that evaluated the role of HC supplementation in preventing skin aging in humans were retrieved. The retrieved studies were selected by two independent reviewers (RBM and RCR), who initially considered the eligibility of titles and abstracts, and thereafter checked the eligibility of full texts. In case of disagreements, article selection was decided after a consensus was reached between the two evaluators. The databases were last accessed on October 6, 2020, and the studies considered eligible for inclusion were randomized clinical trials conducted in healthy patients aged >18 years who received oral supplementation with HC. Studies that did not meet the initial criteria and those that did not measure the effect of supplementation on skin aging were excluded.

Data extraction

Data were independently extracted by the two evaluators (RBM and RCR) and recorded in an electronic spreadsheet. The following data were extracted: number of patients, gender, age, groups evaluated in each study (treatment and placebo), duration of the study, intervention, and primary outcomes (hydration, elasticity, wrinkles, and skin density), as well as baseline data of the measured characteristics in the placebo and treatment groups. In studies that presented secondary outcomes, such as the results of immunohistochemical assays, biopsy findings, levels of hyaluronic acid and enzymes, cutaneous pH, and skin color or presence of erythema, these data were also extracted. All quantitative variables were assessed as means ± standard deviations (SDs).

Statistical analysis, sensitivity analysis, and bias assessment

The studies were grouped according to outcome similarity, and independent meta-analyses were conducted for each group. Statistical analysis was performed using the random-effect model (inverse variance) to calculate the mean difference and SD for continuous variables. A probability (P) value of <0.05 was considered statistically significant. The result of the general effect test was reported as a z value corroborating the inference of the 95% confidence interval (CI). The Higgins I (I^2) statistical model was used to assess the heterogeneity of results among the included studies. I^2 values ≤50% corresponded to low and moderate heterogeneity, whereas values >75% indicated high heterogeneity. Subgroup analyses based on result measurement units were performed to identify the sources of heterogeneity. Sensitivity analysis censored by unparalleled measurement units or study size was also conducted to negate the effect of potentially influential studies. The presence of publication bias was graphically presented using a funnel chart. The quality of the included articles was assessed according to the Cochrane guidelines for systematic review and meta-analysis, in which each study was classified according to the five types of bias (selection, performance, detection, attrition, and reporting bias) proposed in the Risk of Bias (RoB)-2 tool.[31] All statistical analyses were performed using the RevMan software (version 5.4; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, 2020).

Results

Research results and study characteristics

The initial search retrieved 365 articles from the databases. After removing studies according to the preestablished criteria in the title and abstract eligibility stage and for being duplicates, 33 articles were considered relevant for a full-text review. Of these, 14 articles were excluded according to the inclusion and exclusion criteria, leaving a total of 19 articles eligible for quantitative analysis. Figure 1 shows the study selection flowchart, divided into the steps of identification, selection, eligibility, and inclusion, according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) recommendations for writing systematic reviews.[32]

Study characteristics

A total of 1,125 patients completed the studies. The mean age of the patients was 49.82 ± 5.68 years in the intervention group and 50 ± 5.63 years in the placebo group ($P = 0.006$). The mean number of female patients was 1,081 (95%) in both

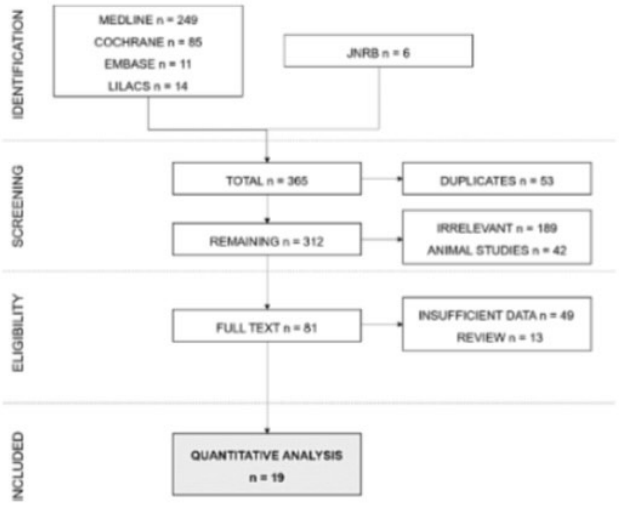

Figure 1 Flowchart of the included studies

groups ($P = 0.05$). The duration of the studies ranged from 4 to 16 weeks. The forms of intervention and outcome assessment criteria widely varied among studies. The main characteristics of the selected studies are described in Table 1.

With respect to the baseline characteristics of the placebo and treatment groups evaluated before the intervention, a comparative analysis showed no differences in the global means between the two groups for skin elasticity (2.16 ± 0.15 vs. 2.01 ± 0.22 MPa, $P = 0.23$), skin wrinkles (32.1 ± 20.9% area vs. 29.7 ± 16.9% area, $P = 0.06$), and transepidermal water loss (TEWL) (14.7 ± 4.6 vs. 14.6 ± 4 g/m^2/h, $P = 0.72$). However, a difference was observed between groups in dermis density (36.5 ± 3.7 vs. 35.4 ± 5.8, $P = 0.0004$), dermis thickness (1435 ± 110 vs. 1564 ± 217 μm, $P < 0.0001$), and skin moisture content (61.9 ± 19.91% vs. 65.5 ± 15.2%, $P = 0.002$).

Meta-analysis results

Pooled analysis of selected studies

The 19 selected studies were initially divided into two groups according to the measured outcome (skin hydration or elasticity) and thereafter subjected to a meta-analysis. Figure 2 shows the forest plot of the meta-analysis of nine studies with respect to combined skin hydration estimates comparing the placebo group with the group of patients supplemented with HC. For this outcome, supplementation resulted in significant improvement ($z = 2.58$, $P = 0.010$), as evidenced by the overall effect size of 1.01 (95% CI 0.24, 1.78). In the meta-analysis with 14 studies (Fig. 3) evaluating skin elasticity, HC supplementation also significantly improved this outcome ($z = 2.31$, $P = 0.02$) in comparison to the placebo group, with a general effect size of 1.27 (95% CI 0.19, 2.35).

The grouped analysis of the studies also showed positive effects of collagen supplementation in terms of a significantly decreased mean skin wrinkle value (−1.11; 95% CI −1.94, −0.28; $P = 0.009$) and increased cutaneous density (0.48; 95% CI 0.09, 0.88; $P = 0.02$), as shown in Figure 4. No significant differences were identified between the treatment and placebo groups in studies evaluating TEWL secondary outcomes (−0.44; 95% CI −0.99, 0.11; $P = 0.12$), skin rash (−0.27; 95% CI −0.61, 0.08; $P = 0.13$), collagen levels (0.74; 95% CI −0.89, 2.36; $P = 0.37$), and melatonin pattern (−0.17; 95% CI −1.20, 0.86; $P = 0.75$). The forest plots of these outcomes, as well as the studies included in the meta-analysis, are shown in Figure S1.

For secondary outcomes extracted as visual analog scale photoaging scores, the serum fibronectin, elastin, hyaluronic acid, and carbonylated protein levels were reported in a single study[33], as well as skin pH[34] and both were not included in the meta-analysis. Comparative data between the HC supplementation group and the placebo group are described in Table S2.

Subgroup analysis based on corresponding measurements

Subgroup analysis for cutaneous hydration was based on the corresponding measurements in micrometres (five studies), percentage of hydration (two studies), and moisture content (arbitrary units [AU], two studies), and showed no significant global differences between the groups ($P = 0.53$). Additionally, the heterogeneity value decreased from $I^2 = 93\%$ to $I^2 = 0\%$. In this subgroup analysis, only the studies that measured hydration in micrometres showed a significant and favorable difference with HC supplementation (0.73; 95% CI 0.19, 1.27; $P = 0.008$), according to the forest plot presented in Figure 5.

Similar to the grouped analysis (Fig. 3), a subgroup analysis for cutaneous elasticity subdivided into MPa, percentage, and AU measurement units demonstrated a significant difference between the HC supplementation group and the placebo group ($P = 0.04$), as shown in Figure 6. This subgroup analysis also showed a decreased heterogeneity value from $I^2 = 98\%$ to $I^2 = 69.1\%$. Separately, only studies that measured elasticity in MPa showed a significant difference in favor of supplementation (4.65; 95% CI 1.11, 8.18; $P = 0.010$). The other studies showed no positive effect of supplementation when measured in percentage (−0.22; 95% CI −1.48, 1.04; $P = 0.73$) and AU (0.28; 95% CI −0.45, 1.01; $P = 0.45$).

Sensitivity analysis

With respect to cutaneous hydration, the study by Žmitek et al. (2020) measured hydration using a different technique compared with the other studies[35], resulting in a higher value, as shown in Figure 5. Thus, a sensitivity analysis was used to assess the influence of this study in the subgroup and grouped analyses, as shown in Figure 7. The exclusion of this study resulted in no significant change, and both the corrected global result (1.13; 95% CI 0.28, 1.97; $P = 0.009$) and the intragroup result by measurement unit (0.86; 95% CI 0.26, 1.46; $P = 0.005$) remained favorable to collagen supplementation.

Table 1 Description of the included studies

Study	Author (year)	Participants	Time	Intervention	Placebo	Outcome extracted	Skin outcomes measurement methods
1	Bolke et al. (2019)[43]	72 healthy women aged >35 years	16 weeks / 12 weeks of intervention	2.5 g collagen peptides (Elasten®)	Yes	Hydration / elasticity / wrinkles / skin density / subjective questionnaire	Corneometry (SH), cutometry (SE), use of silicon skin replicas with optical 3D phase-shift rapid in-vivo measurements (PRIMOS) (SR), and skin ultrasonography (SD)
2	Genovese, Corbo, and Sibilla (2017)[37]	111 healthy women and 9 men aged 40–60 years	12 weeks	5 g HC (Gold Collagen® Forte)	Yes	Elasticity / biopsies / subjective questionnaire	Elasticity probe, SkinLab USB DermaLab® (Young´s elasticity modulus) (SE) and histological analysis
3	Koizumi et al. (2017)[30]	71 healthy women aged 30–60 years	12 weeks	3 g collagen peptides	Yes	Wrinkles / moisture / elasticity / blood tests	Use of silicon skin replicas followed by visiometer assesses (SR), corneometry (SH), and cutometry (SE)
4	Proksch et al. (2014a)[17]	60 healthy women aged 35–55 years, Fitzpatrick* I to IV	12 weeks / 8 weeks of intervention	2.5 g HC / 5 g HC (Verisol®)	Yes	Elasticity / hydration / TEWL / wrinkles	Corneometry (SH), cutometry (SE), use of silicon skin replicas with optical 3D phase-shift rapid in-vivo measurements (PRIMOS) (SR), and TEWL probe, SkinLab USB DermaLab®
5	Žmitek et al. (2020)[35]	31 Caucasian women aged 40–65 years, Fitzpatrick II and III	12 weeks	4 g HC	Yes	Dermal density and thickness / viscoelasticity / hydration / TEWL / wrinkles / moisture / dermal microrelief	Skin ultrasonography (SD and ST), hydration, elasticity, and TEWL probes – SkinLab USB DermaLab® (SE and TEWL)
6	Sugihara, Inoue, and Wang (2015)[45]	53 healthy Chinese women aged 35–55 years	8 weeks	2.5 g HC (Wellnex®)	Yes	Hydration / elasticity / wrinkles	Corneometry (SH), cutometry (SE), and photographic analysis - VisioFace SSA (SR)
7	Yoon et al. (2014)[48]	44 healthy women aged >44 years	12 weeks	3 g HC	Yes	Procollagen type 1, fibrillin 1, metalloproteinases 1 and 12 / biopsies / immunohistochemical staining	RT-PCR and histological analyses
8	Schwartz et al. (2019)[41]	113 healthy white women aged 36–59 years, Fitzpatrick I to IV	12 weeks	0.6 g HC (Bio Cell®)	Yes	Erythema / hydration / TEWL / elasticity / wrinkles / dermal collagen / subjective questionnaire	Visual analyses (Erythema), TEWL probe – Vapometer® (TEWL), MoistureMeterSC probe (SH), cutometry (SE), and spectrophotometric intracutaneous analysis (dermal collagen)
9	Laing et al. (2020)[49]	60 healthy women aged 40–70 years	12 weeks	2.5 g collagen peptides (Elasten®)	Yes	Dermal collagen fragmentation / subjective questionnaire	Confocal laser scanning microscopy (dermal collagen fragmentation)
10	Czajka et al. (2018)[50]	120 healthy people aged 21–70 years	12 weeks	4 g HC (Gold Collagen® Active)	Yes	Elasticity / biopsies / self-perception questionnaire	Elasticity probe, SkinLab USB DermaLab® (Young´s elasticity modulus) (SE) and histological analysis

Table 1 Continued

Study	Author (year)	Participants	Time	Intervention	Placebo	Outcome extracted	Skin outcomes measurement methods
11	Di Cerbo et al. (2014)[33]	30 healthy women	4.5 weeks of intervention	372 mg HC (Viscoderm®)		Cutaneous pH, hydration, sebum, elasticity and skin tone / elastin, elastase 2, fibronectin, hyaluronic acid, and carbonyl proteins	Skin Tester (pH, SH, sebum SE, and Skin tone) and ELISA (elastin, elastase 2, fibronectin, hyaluronic acid, and carbonyl proteins)
12	Proksch et al. (2014b)[18]	107 healthy women aged 45–65 years	12 weeks / 8 weeks of intervention	2.5 g collagen peptides	Yes	Wrinkles / biopsy / procollagen type 1, elastin, and fibrillin	Use of silicone skin replicas with optical 3D phase-shift rapid in-vivo measurements (PRIMOS) and ELISA (procollagen type 1, elastin, and fibrillin)
13	Campos et al. (2015)[38]	60 healthy women aged 40–50 years	12 weeks	9 g HC	Yes	Corneal stratum hydration / skin viscoelasticity / dermal echogenicity / high-resolution photography	Corneometry (SH), cutometry (SE), ultrasonography (skin echogenicity), and photographic analysis - VisioFace SSA (SR)
14	Asserin et al. (2015)[51]	134 healthy Japanese women aged 40–59 years and Caucasian women aged 40–65 years	8 weeks / 12 weeks	10 g pig Peptan 10 g fish Peptan 10 g Peptan	Yes	Skin moisture / TEWL / dermal density / dermal echogenicity / dermal collagen fragmentation	Corneometry (SH), TEWL probe – Tewameter® (TEWL), ultrasonography (skin echogenicity), and microscopy (collagen fragmentation)
15	Ito, Seki, and Ueda (2018)[34]	21 healthy Japanese people aged 30–50 years	8 weeks	10 g fish collagen peptides	Yes	Elasticity / moisture / TEWL / skin pH / spots, wrinkles, skin pores, texture / density and collagen score / GH, IGF-1	Cutometry (SE), corneometry (SH and TEWL), pH meter (skin pH), ultrasonography (SD), photographic analysis (spots, wrinkles, and skin pores), and blood analyses (GH, IGF-1)
16	Choi et al. (2014)[39]	32 women undergoing fractional laser treatment	5 weeks	Collagen peptides	Yes	Skin hydration / elasticity / TEWL / erythema / satisfaction questionnaire	Corneometry (SH), TEWL probe – Tewameter® (TEWL), cutometry (SE), and Mexameter® (erythema index)
17	Inoue, Sugihara, and Wang (2016)[46]	80 people aged 35–55 years	8 weeks	2.5 g Collagen peptides with different concentrations of Pro-Hyp and Hyp-Gly	Yes	Skin moisture / elasticity / wrinkles	Corneometry (SH), cutometry (SE), and photographic analysis - VisioFace SSA (SR)
18	Sangsuwan and Asawanonda (2020)[52]	36 healthy women aged 50–60 years	8 weeks / 4 weeks of intervention	5 g HC	Yes	Elasticity	Cutometry (SE)
19	Nomoto and Iizaka (2020)[40]	39 people aged >65 years hospitalized for <5 months	8 weeks	12 g collagen peptides	No	Stratum corneum hydration / Elasticity	Corneometry (SH) and cutometry (SE)

ELISA, enzyme-linked immunosorbent assay; HC, hydrolyzed collagen; IGF-1, insulin-like growth factor 1; RT-PCR, reverse transcriptase polymerase chain reaction; SD, skin density; SE, skin elasticity; SH, skin hydration; SR, skin roughness; ST, skin thickness; TEWL, transepidermal water loss.
*Fitzpatrick skin phototype.

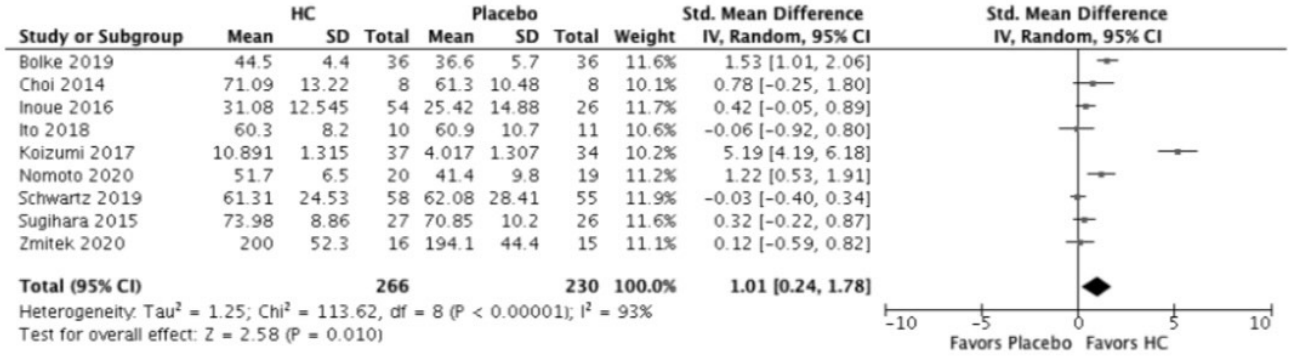

Figure 2 Forest plot for the combined estimate of the included studies evaluating skin hydration in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond

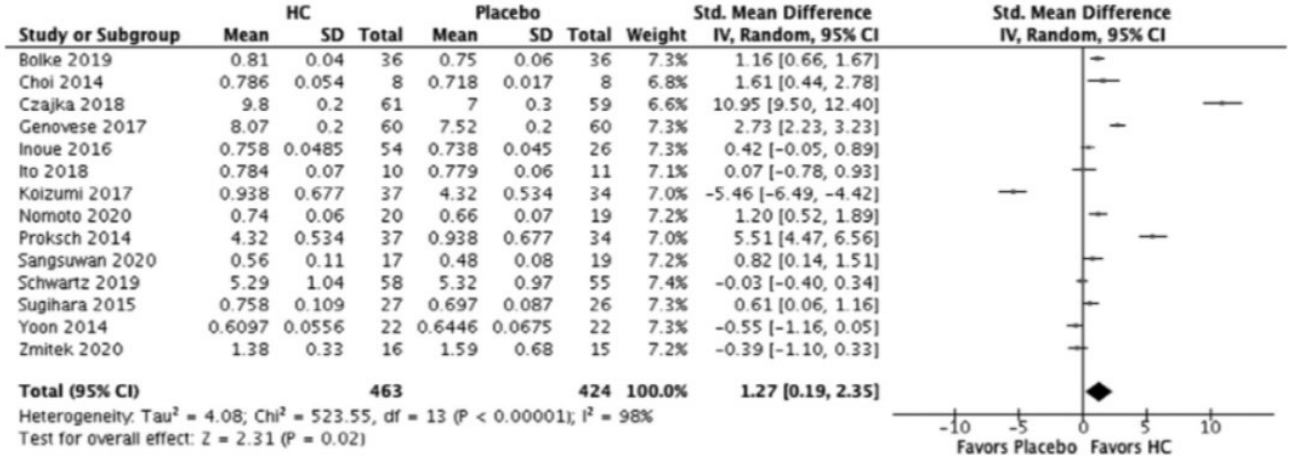

Figure 3 Forest plot for the combined estimate of the included studies evaluating skin elasticity in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond

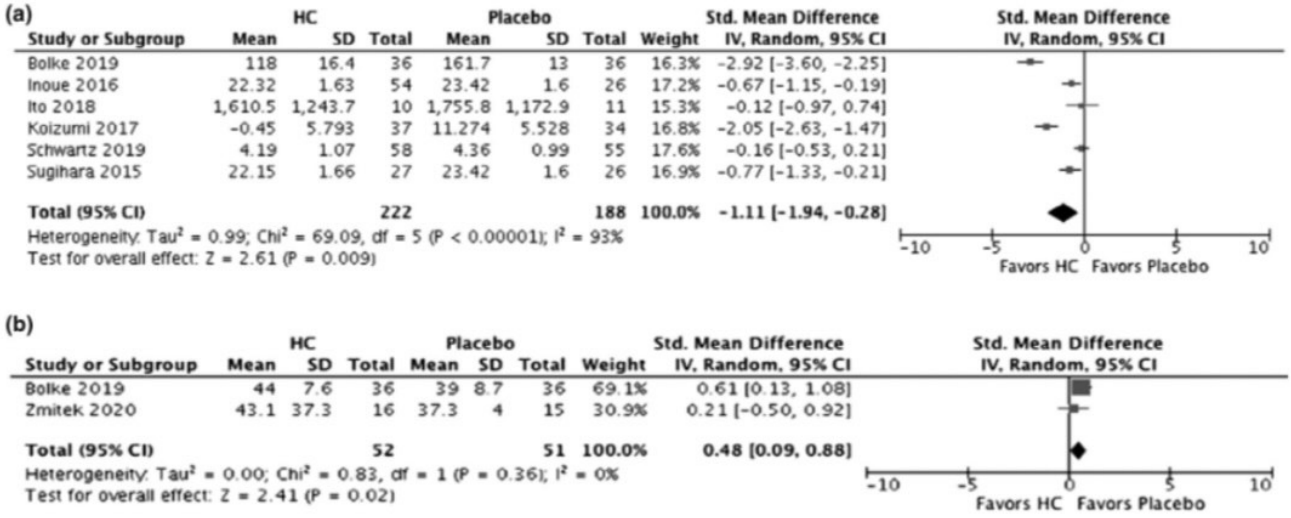

Figure 4 Forest plot for the combined estimate of the included studies evaluating skin roughness in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond

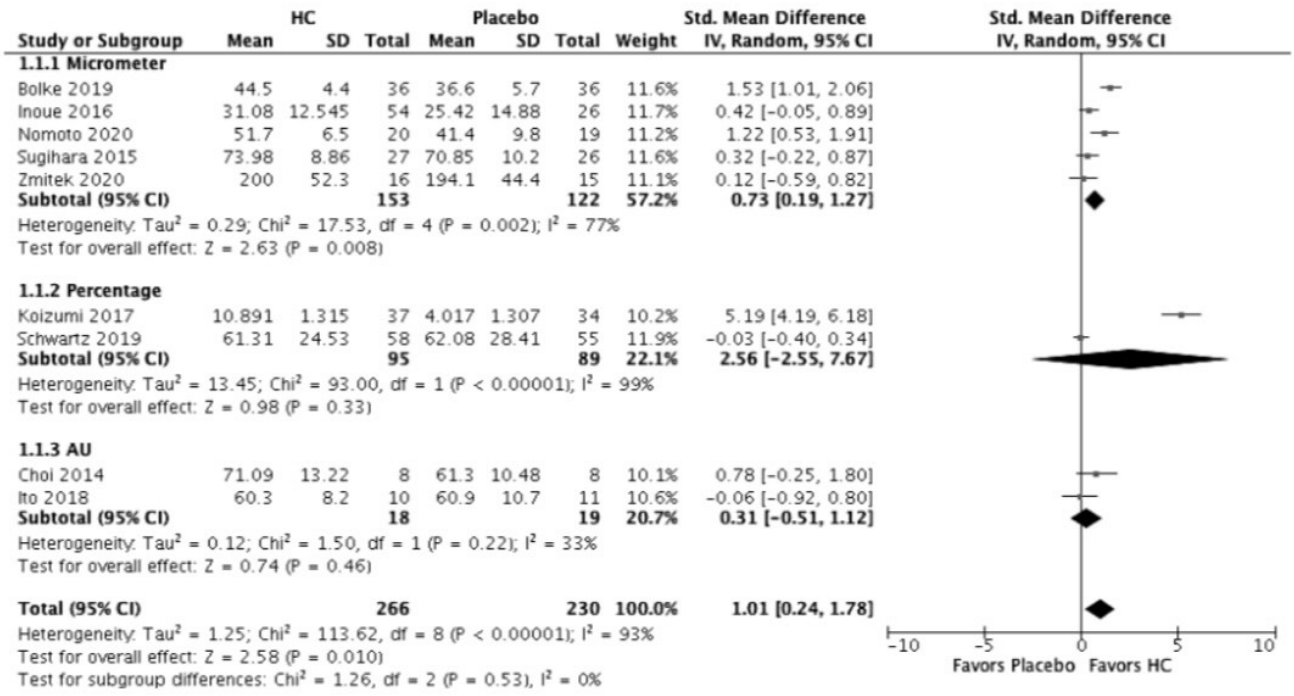

Figure 5 Forest plot for subgroup analysis of skin hydration expressed as micrometers, percentage, and arbitrary units (AU) in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds

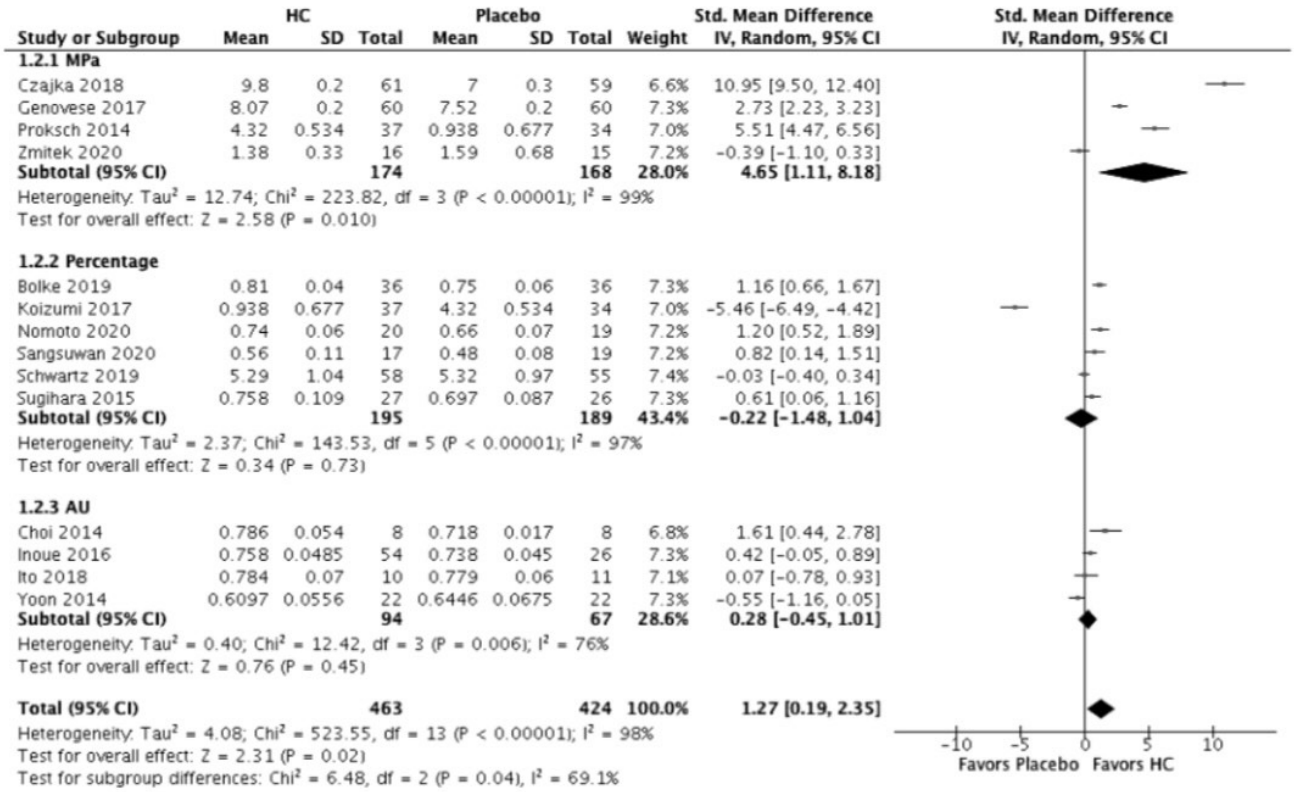

Figure 6 Forest plot for subgroup analysis of cutaneous elasticity expressed as MPa, percentage, and arbitrary units (A.U.) in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds

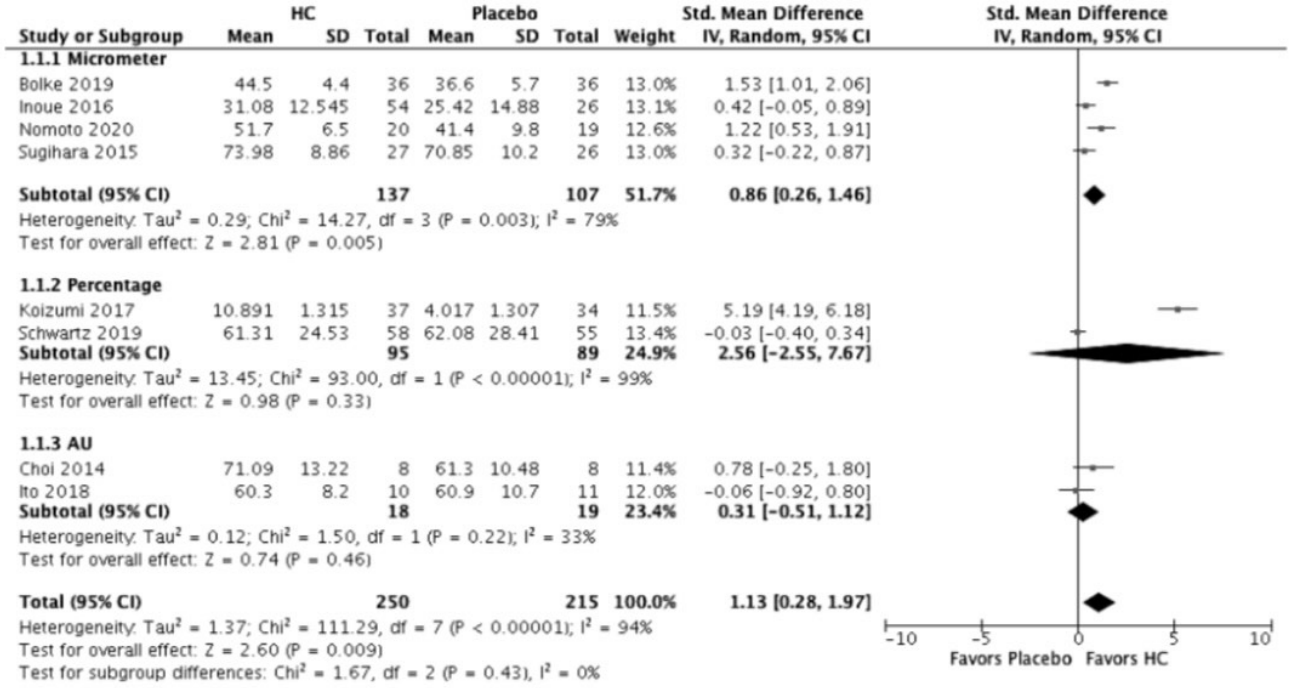

Figure 7 Forest plot of the sensitivity analysis based on the exclusion of the study by Žmitek et al. (2020) for cutaneous hydration: patients supplemented with hydrolyzed collagen (HC) vs. patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds

In addition, this sensitivity analysis considered an additional factor because a different measurement instrument was used for hydration and HC was administered in association with coenzyme Q10.[35] Coenzyme Q10 supplementation had positive effects on skin aging, such as reduced wrinkles and skin smoothing in a clinical trial conducted by the same research group.[36] In this context, coenzyme Q10 could act complementarily or synergistically with collagen, mitigating the effects of skin aging, with the sensitivity analysis confirming the direct influence of this study on the overall result.

Of the studies that evaluated the effect of supplementation on skin elasticity, only Genovese, Corbo, and Sibilla (2017) expressed the result in millimeters,[37] resulting in a different value compared with the other studies. Thus, a sensitivity analysis was conducted for elasticity, in which this study was removed. Figure 8 shows that after removing the study, the overall effect remained favorable to collagen supplementation (1.16; 95% CI 0.04, 2.27; $P = 0.04$).

Bias

A methodological assessment of the quality of RoB was performed using the RoB-2 tool. The assessment of RoB at the domain level revealed a low RoB for most studies, as shown in Figure 9. At the study level, an RoB was found in the blinding of participants and researchers in the studies by Campos et al. (2015), Choi et al. (2014), and Nomoto and Iizaka (2020),[38–40] and with respect to incomplete data in the results by Nomoto and Iizaka (2020), Proksch et al. (2014a), and Schwartz et al. (2019),[17,40,41] as shown in Figure 10.

Publication bias

In the visual evaluation, the funnel chart showed symmetry (Fig. 11), indicating that the limited dispersion occurred because of sample variation and not because of publication bias. The vertical axis of the graph used standard error to estimate the sample size of the study, plotting large population studies at the top and smaller studies at the bottom. The horizontal expansion showed the power and effect size of the included studies.

Discussion

Despite the heterogeneity among studies, which used different collagen peptide concentrations, formulations, origins (pigs, fish, chicken, etc.), and forms of administration (liquid and solid) of the oral supplement, most of the studies reported improved skin hydration and elasticity, increased dermal density, and reduced facial wrinkles. The beneficial effects were evident at 60 and 90 days after the start of supplementation and were maintained for 30 days after the end of the intervention. Thus, the benefits of this supplementation on the aspect of skin are related to its maintenance period.

Several clinical studies evaluated the effects of oral HC and observed improved dermal collagen synthesis, increased

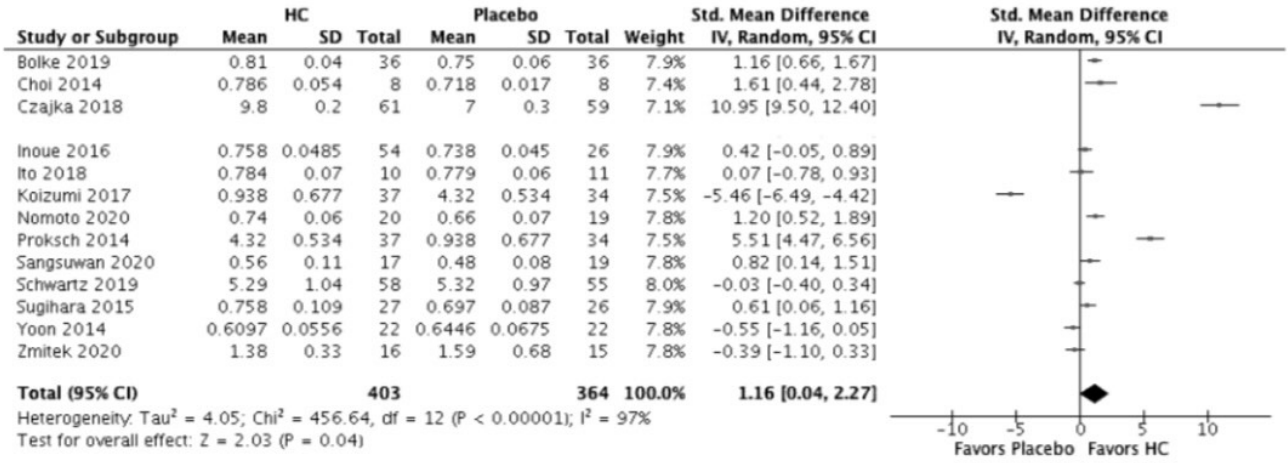

Figure 8 Forest plot of the sensitivity analysis based on the exclusion of the study by Genovese, Corbo, and Sibilla (2017) for cutaneous elasticity: patients supplemented with hydrolyzed collagen (HC) vs. patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds

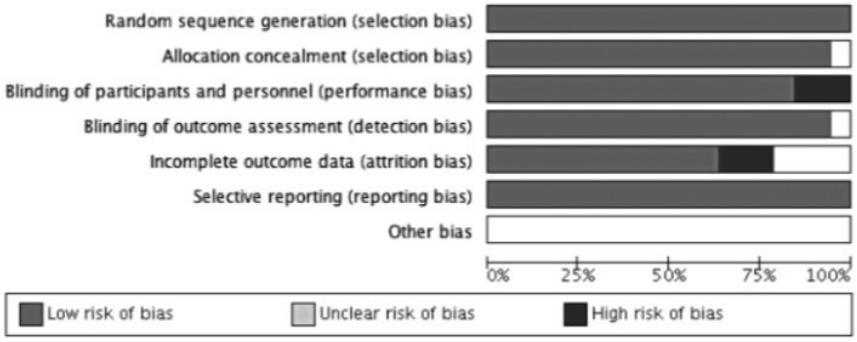

Figure 9 Graph of risk of bias for each study according to the five domains defined by RoB-2

collagen synthesis by fibroblasts, improved skin hydration and elasticity, and decreased wrinkles.[6,16,18,42]

The study by Bolke et al. (2019) showed that a daily intake of 2.5 g collagen peptides increased the level of hydration, skin elasticity, and dermal density, and decreased the area of the wrinkles in women aged >35 years after 90 days of supplementation.[43] These findings were proven both by objective and subjective assessment methods (questionnaires). In addition, the observed results persisted for 30 days after the intervention. Corroborating these findings, similar results were obtained in clinical trials that administered 5 g collagen peptides, demonstrating that supplementation of 2.5 g collagen peptides for 90 days is enough to obtain beneficial effects. The study by Proksch et al. (2014) showed no improvement in skin hydration with 2.5 or 5 g HC (Verisol®), and the difference may be attributable to the measurement site (inner side of the arm).[17] As the measurement site is a region that is normally protected from solar radiation, its skin aging process is less accelerated than that of the outer side. That is, positive effects can be more easily observed when evaluating areas that are more exposed to extrinsic factors such as radiation and pollution.

Previous studies have shown that the Pro-Hyp and Hyp-Gly dipeptides have advanced effects on dermal fibroblasts, stimulating their metabolism, migration, and proliferation by producing collagen fibers in the dermis.[23,42,44] The clinical trial conducted by Koizumi et al. (2017), in which the intervention was ingestion of 3 g collagen peptides derived from tilapia fish scales (high content of Hyp, Gly, and Pro) for 90 days, resulted in effective reduction of periorbital wrinkles and improved skin moisture (hydration) and elasticity in women.[30] Additionally, the study by Sugihara, Inoue, and Wang (2015) demonstrated that the ingestion of 2.5 g HC peptides, also derived from fish scales and containing the Pro-Hyp and Hyp-Gly bioactive dipeptides, improved hydration and elasticity, and smoothed facial skin wrinkles in 4 weeks.[45] The effects of the Pro-Hyp and Hyp-Gly concentrations were measured in the study by Inoue, Sugihara, and Wang (2016), which showed effectiveness in both groups; however, the clinical improvement of the parameters was more

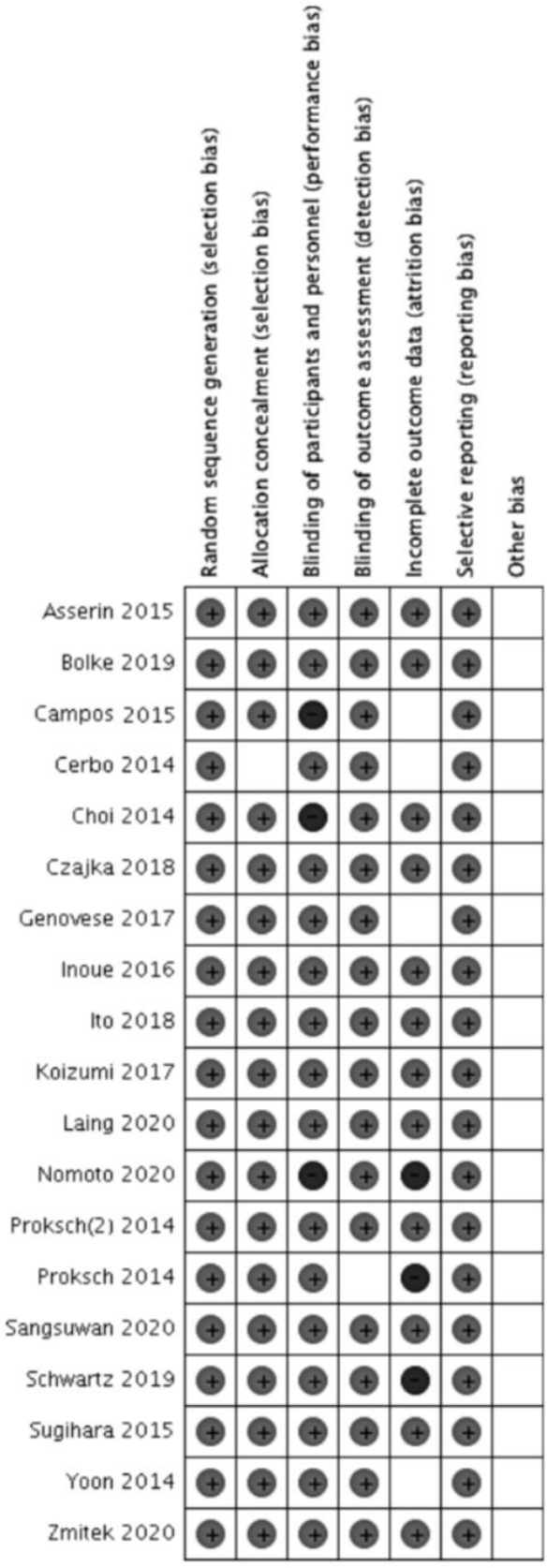

Figure 10 Graph of risk of bias at the study level

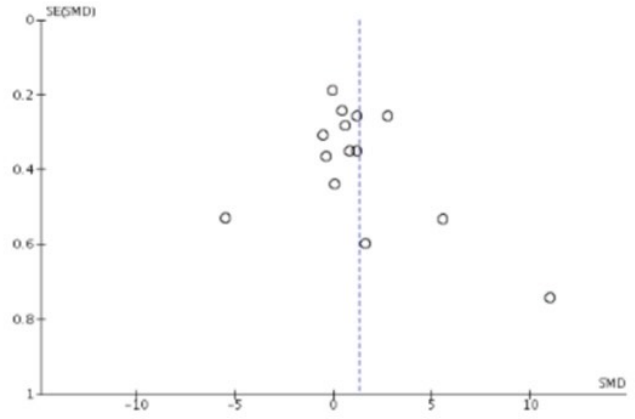

Figure 11 Funnel chart for publication bias

quickly observed in the group supplemented with a higher concentration of dipeptides, reinforcing the presumption that the composition directly affects the results.[46]

Collagen peptides have been shown to be effective in improving the skin, regardless of origin (fish, pigs, cattle, or chickens) but depending on peptide composition and concentration.

Some studies that administered type II HC mentioned effectiveness in improving the structure of dermal collagen, with reduction of wrinkles and elasticity, and obtained positive results in subjective questionnaires. This shows that both type I and II HC supplements can promote beneficial effects on the skin. However, the structural modification of the dermal collagen cannot be attested by the studies evaluated, considering that a limited number of studies performed biopsy analyses of skin samples (n = 3). In the reported cases, based on the short intervention time of the studies, the reduction of wrinkles and in increasing elasticity may be associated with an increase in the degree of skin hydration and not exactly the modification of the collagen structure.

The study by Nomoto and Iizaka (2020), which included hospitalized older and unhealthy adults, showed that the intake of HC peptide supplement was beneficial and reduced skin vulnerability to traumatic injuries caused by procedures in bedridden older patients.[40] The effects of HC supplementation on skin recovery after laser treatment were evaluated in the study by Choi et al. (2014), and the main results showed an improved healing process in supplemented patients.[39]

Most of the selected studies used commercial HC supplementation in ready-to-consume preparations as the intervention (Table 1). These commercial brands had different percentage compositions, containing (in addition to collagen peptides) vitamins, minerals, antioxidants, coenzyme Q10, hyaluronic acid, and chondroitin sulfate. In these studies, the positive outcomes of supplementation were solely attributed to collagen and the effect of the formulation vehicle was not comparatively

evaluated. Thus, the beneficial effects achieved may have occurred owing to the synergism of these substances with collagen. Coenzyme Q10, for example, has an important antioxidant function, neutralizing the damage caused by free radicals generated in the skin aging process,[12] thus improving the signs of aging. Other vitamins, such as vitamin C, and hyaluronic acid participate in and stimulate collagen biosynthesis, respectively.[47] Nevertheless, studies using collagen in its isolated form demonstrated its effectiveness.

None of the studies reported adverse effects related to the dietary supplement. Furthermore, in the evaluated studies, the use of HC in liquid and solid forms (capsules and powder supplements available for solubilization) showed good patient acceptability owing to easy swallowing and the safety of administration.

Limitations

The limitations of this study were related to the large heterogeneity of the studies, mainly because of the composition of the supplementation, methods used to verify the results, and different measurement units, making it difficult to compare them in terms of both intervention and outcomes. Nevertheless, HC and HC peptides are effective in reducing skin aging and are safe for consumption, with benefits related to maintaining supplementation. Another important and not detailed factor in all studies is related to the patients' lifestyle habits. Patients with healthy lifestyle habits such as a balanced diet and adequate water intake could present more evident and faster results in improving the appearance of the skin with collagen supplementation than patients with unhealthy lifestyle habits.

Conclusion

Based on the results of this study, HC supplements or collagen peptides can delay and improve the signs of skin aging by decreasing facial wrinkles and improving skin hydration and elasticity, while the supplementation is maintained. The time required to delay skin aging in most studies was 90 days, and the result was maintained for 4 weeks after the end of supplement administrations. Studies using supplements with higher concentrations of Pro-Hyp and Hyp-Gly dipeptides showed visible improvements of the evaluated parameters after 4 weeks. Supplement intake is effective and safe because no adverse effects were reported in any of the analyzed studies. Further studies are needed to evaluate the long-term use of HC peptides, as the longest intervention among the studies lasted for 90 days, with 120 days of evaluation of the effects. Further studies are needed to evaluate the effect of the vehicle and of other substances co-administered with collagen, mainly vitamins and coenzyme Q10, which can act in association or synergistically with collagen to significantly improve the measured effects.

Questions (answers provided after references)

1 Is collagen the second most abundant structural protein in connective tissues?
a Yes
b No

2 Is collagen usually supplemented as a protein structure?
a Yes
b No

3 One of the main expected effects, when supplementing with collagen peptides, is the improvement in the appearance of the skin.
a True
b False

4 Is all the hydrolyzed collagen supplemented converted into the cutaneous extracellular matrix?
a Yes
b No

5 Can the presence of other components in the formulation of collagen supplements, such as enzymatic cofactors (CoQ10) and vitamins, hinder the comparative analysis between results of clinical studies?
a Yes
b No

6 Do randomized clinical studies evaluating the effects of collagen supplementation on the skin present homogeneity in relation to the clinical parameters evaluated (for example, skin roughness and hydration)?
a Yes
b No

7 After 2 weeks, is it possible to observe the significant effect of hydrolyzed collagen supplementation on the skin?
a Yes
b No

8 Are clinical studies heterogeneous in terms of patient age?
a Yes
b No

9 Are hydrolyzed collagen supplements administered mainly in solid forms?
a Yes
b No

10 Does collagen obtained from fish scales have promising effects because of the presence of the peptides Pro-Hyp and Hyp-Gly?
a Yes
b No

References

1 Squire JM, Parry DAD. Fibrous protein structures: Hierarchy, history and heroes. In *Subcellular Biochemistry*. 2017; 1–33.

2 Benson HAE, Watkinson AC. *Transdermal and Topical Drug Delivery: Principles and Practice. Transdermal and Topical Drug Delivery: Principles and Practice*. Hoboken: John Wiley & Sons, 2012: 1–446.
3 Dini I, Laneri S. Nutricosmetics: a brief overview. *Phyther Res*. 2019; **33**: 3054–3063.
4 Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol* 2011; **3**: a004978.
5 Varani J, Dame MK, Rittie L, *et al*. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *Am J Pathol* 2006; **168**: 1861–1868.
6 Quan T, Little E, Quan H, Qin Z, Voorhees JJ, Fisher GJ. Elevated matrix metalloproteinases and collagen fragmentation in photodamaged human skin: Impact of altered extracellular matrix microenvironment on dermal fibroblast function. *J Invest Dermatol* 2013; **133**: 1362–1366.
7 Jin HC, Jin YS, Hai RC, *et al*. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. *J Invest Dermatol*. 2001; **117**: 1218–1224.
8 Calleja-Agius J, Brincat M, Borg M. Skin connective tissue and ageing. *Best Pract Res Clin Obstet Gynaecol* 2013; **27**: 727–740.
9 Calleja-Agius J, Muscat-Baron Y, Brincat MP. Skin ageing. *Menopause Int* 2007; **13**: 60–64.
10 Verdier-Sévrain S, Bonté F. Skin hydration: a review on its molecular mechanisms. *J Cosmet Dermatol* 2007; **6**: 75–82.
11 Schagen SK, Zampeli VA, Makrantonaki E, Zouboulis CC. Discovering the link between nutrition and skin aging. *Dermatoendocrinol* 2012; **4**: 298–307.
12 Madhere S, Simpson P. A market overview of nutricosmetics. *Cosmet Dermatology* 2010; **23**: 268–274.
13 Costa A, Pereira ESP, Favaro R, *et al*. Treating cutaneous aging in women, with an oral supplement based on marine protein, concentrated acerola, grape seed extract and tomato extract, within 360 days. *Surg Cosmet Dermatology* 2011; **3**: 302–311.
14 Costa A, Arruda LHF, Ota FS, *et al*. Clinical, biometric and ultrasound assessment of the effects of daily use of a nutraceutical composed of lycopene, acerola extract, grape seed extract and Biomarine Complex in photoaged human skin. *An Bras Dermatol* 2012; **87**: 52–61.
15 Suleria HAR, Osborne S, Masci P, Gobe G. Marine-based nutraceuticals: an innovative trend in the food and supplement industries. *Mar Drugs* 2015; **13**: 6336–6351.
16 De Luca C, Mikhal'chik EV, Suprun MV, Papacharalambous M, Truhanov AI, Korkina LG. Skin antiageing and systemic Redox effects of supplementation with marine collagen peptides and plant-derived antioxidants: a single-blind case-control clinical study. *Oxid Med Cell Longev* 2016; **2016**: 1–14.
17 Proksch E, Segger D, Degwert J, Schunck M, Zague V, Oesser S. Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: a double-blind, placebo-controlled study. *Skin Pharmacol Physiol* 2014; **27**: 47–55.
18 Proksch E, Schunck M, Zague V, Segger D, Degwert J, Oesser S. Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. *Skin Pharmacol Physiol* 2014; **27**: 113–119.
19 Sato K. The presence of food-derived collagen peptides in human body-structure and biological activity. *Food Funct* 2017; **8**: 4325–4330.
20 Zague V, de Freitas V, da Rosa M, de Castro GA, Jaeger RG, Machado-Santelli GM. Collagen hydrolysate intake increases skin collagen expression and suppresses matrix metalloproteinase 2 activity. *J Med Food* 2011; **14**: 618–624.
21 Choi FD, Sung CT, Juhasz MLW, Mesinkovsk NA. Oral collagen supplementation: a systematic review of dermatological applications. *J Drugs Dermatology* 2019; **18**: 9–16.
22 Liu C, Sugita K, Nihei KI, Yoneyama K, Tanaka H. Absorption of hydroxyproline-containing peptides in vascularly perfused rat small intestine in situ. *Biosci Biotechnol Biochem* 2009; **73**: 1741–1747.
23 Ohara H, Ichikawa S, Matsumoto H, *et al*. Collagen-derived dipeptide, proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. *J Dermatol* 2010; **37**: 330–338.
24 Iwai K, Hasegawa T, Taguchi Y, *et al*. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J Agric Food Chem* 2005; **53**: 6531–6536.
25 Ohara H, Matsumoto H, Ito K, Iwai K, Sato K. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J Agric Food Chem* 2007; **55**: 1532–1535.
26 Shigemura Y, Akaba S, Kawashima E, Park EY, Nakamura Y, Sato K. Identification of a novel food-derived collagen peptide, hydroxyprolyl-glycine, in human peripheral blood by pre-column derivatisation with phenyl isothiocyanate. *Food Chem* 2011; **129**: 1019–1024.
27 Sugihara F, Inoue N, Kuwamori M, Taniguchi M. Quantification of hydroxyprolyl-glycine (Hyp-Gly) in human blood after ingestion of collagen hydrolysate. *J Biosci Bioeng* 2012; **113**: 202–203.
28 Oesser S, Adam M, Babel W, Seifert J. Oral administration of 14C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *J Nutr* 1999; **129**: 1891–1895.
29 Ohara H, Ito K, Iida H, Matsumoto H. Improvement in the moisture content of the stratum corneum following 4 weeks of collagen hydrolysate ingestion. *J Japanese Soc Food Sci Technol* 2009; **56**: 137–145.
30 Koizumi S, Inoue N, Shimizu M, Kwon C, Kim HY, Park KS. Effects of dietary supplementation with fish scales-derived collagen peptides on skin parameters and condition: a randomized, placebo-controlled, double-blind study. *Int J Pept Res Ther* 2017; **24**: 397–402.
31 Cochrane. Rob 2: risk of bias tools [Internet]. Current version of Rob 2. 2019. Available from https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2
32 Liberati A, Altman DG, Tetzlaff J, *et al*. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 2009; **6**.
33 Di Cerbo A, Laurino C, Palmieri B, Iannitti T. A dietary supplement improves facial photoaging and skin sebum, hydration and tonicity modulating serum fibronectin, neutrophil elastase 2, hyaluronic acid and carbonylated proteins. *J Photochem Photobiol B Biol* 2015; **2014**: 94–103.
34 Ito N, Seki S, Ueda F. Effects of composite supplement containing collagen peptide and ornithine on skin conditions and plasma IGF-1 levels: a randomized, double-blind, placebo-controlled trial. *Mar Drugs* 2018; **16**: 1–12.
35 Žmitek K, Žmitek J, Butina MR, Pogačnik T. Effects of a combination of water-soluble coenzyme Q10 and collagen on skin parameters and condition: results of a randomised, placebo-controlled, double-blind study. *Nutrients* 2020; **12**: 1–13.

36 Žmitek K, Pogačnik T, Mervic L, Žmitek J, Pravst I. The effect of dietary intake of coenzyme Q10 on skin parameters and condition: results of a randomised, placebo-controlled, double-blind study. *BioFactors* 2017; **43**: 132–140.
37 Genovese L, Corbo A, Sibilla S. An insight into the changes in skin texture and properties following dietary intervention with a nutricosmeceutical containing a blend of collagen bioactive peptides and antioxidants. *Skin Pharmacol Physiol* 2017; **30**: 146–158.
38 Campos MBGPM. An oral supplementation based on hydrolyzed collagen and vitamins improves skin elasticity and dermis echogenicity: a clinical placebo-controlled study. *Clin Pharmacol Biopharm* 2015; **04**.
39 Choi SY, Ko EJ, Lee YH, *et al.* Effects of collagen tripeptide supplement on skin properties: a prospective, randomized, controlled study. *J Cosmet Laser Ther* 2014; **16**: 132–137.
40 Nomoto T, Iizaka S. Effect of an oral nutrition supplement containing collagen peptides on stratum corneum hydration and skin elasticity in hospitalized older adults: a multicenter open-label randomized controlled study. *Adv Ski Wound Care* 2020; **33**: 186–191.
41 Schwartz SR, Hammon KA, Gafner A, *et al.* Novel hydrolyzed chicken sternal cartilage extract improves facial epidermis and connective tissue in healthy adult females: a randomized, double-blind, placebo-controlled trial. *Altern Ther Health Med* 2019; **25**: 12–29.
42 Postlethwaite AE, Seyer JM, Kang AH. Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. *Proc Natl Acad Sci USA* 1978; **75**: 871–875.
43 Bolke L, Schlippe G, Gerß J, Voss W. A collagen supplement improves skin hydration, elasticity, roughness, and density: results of a randomized, placebo-controlled, blind study. *Nutrients* 2019; **11**: 7–11.
44 Shigemura Y, Iwai K, Morimatsu F, *et al.* Effect of prolyl-hydroxyproline (Pro-Hyp), a food-derived collagen peptide in human blood, on growth of fibroblasts from mouse skin. *J Agric Food Chem* 2009; **57**: 444–449.
45 Sugihara F, Inoue N, Wang X. Clinical effects of ingesting collagen hydrolysate on facial skin properties: A randomized, placebo-controlled, double-blind trial. *Jpn Pharmacol Ther.* 2015;**43**:67–70.
46 Inoue N, Sugihara F, Wang X. Ingestion of bioactive collagen hydrolysates enhance facial skin moisture and elasticity and reduce facial ageing signs in a randomised double-blind placebo-controlled clinical study. *J Sci Food Agric* 2016; **96**: 4077–4081.
47 Lodish H, Berk A, Zipursky SL. Collagen: The Fibrous Proteins of the Matrix. In: Lodish H, Arnold Berk S, Zipursky L, Matsudaira P, Baltimore D and Darnel J, eds. *Molecular Cell Biology.* 4th ed. New York: W. H. Freeman; 2000.
48 Yoon HS, Cho HH, Cho S, Lee SR, Shin MH, Chung JH. Supplementating with dietary astaxanthin combined with collagen hydrolysate improves facial elasticity and decreases matrix metalloproteinase-1 and -12 expression: a comparative study with placebo. *J Med Food* 2014; **17**: 810–816.
49 Laing S, Bielfeldt S, Ehrenberg C, Wilhelm KP. A dermonutrient containing special collagen peptides improves skin structure and function: a randomized, placebo-controlled, triple-blind trial using confocal laser scanning microscopy on the cosmetic effects and tolerance of a drinkable collagen suppleme. *J Med Food* 2020; **23**: 147–152.
50 Czajka A, Kania EM, Genovese L, *et al.* Daily oral supplementation with collagen peptides combined with vitamins and other bioactive compounds improves skin elasticity and has a beneficial effect on joint and general wellbeing. *Nutr Res* 2018; **57**: 97–108.
51 Asserin J, Lati E, Shioya T, Prawitt J. The effect of oral collagen peptide supplementation on skin moisture and the dermal collagen network: evidence from an ex vivo model and randomized, placebo-controlled clinical trials. *J Cosmet Dermatol* 2015; **14**: 291–301.
52 Sangsuwan W, Asawanonda P. Four-weeks daily intake of oral collagen hydrolysate results in improved skin elasticity, especially in sun-exposed areas: a randomized, double-blind, placebo-controlled trial. *J Dermatolog Treat* 2020; **9**: 1–6.

Questionnaire responses

1-B, 2-B, 3-A, 4-B, 5-A, 6-B, 7-B, 8-A, 9-A, 10-A.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Search strategy.

Table S2. Data extracted from secondary outcomes analyzed in a single study for the HC supplemented group and the placebo group.

Figure S1. Forest plots for combined estimate of included studies evaluating (a) transepidermal water lost (TEWL), (b) cutaneous erythema, (c) collagen levels, and (d) melatonin pattern of patients supplemented with hydrolyzed collagen (HC) and patients of placebo group.