The Effectiveness of Oral Fish Collagen Supplementation on Skin Aging: A Systematic Review and Meta-analysis of Skin Hydration, Skin Elasticity, and Skin Wrinkles

Imelda Gunawan[1]*, Retno Indar Widayati[1], Puguh Riyanto[1], Asih Budiastuti[1], Diah Adriani Malik[1], Muslimin[1], Widyawati[1], and Suhartono[2]

[1]Department of Dermatology, Venereology, and Aesthetic, Faculty of Medicine, Diponegoro University/Dr. Kariadi General Hospital Medical Center, Dr. Sutomo Street No. 16, 50244, Semarang, Indonesia.[2]Department of Environmental Health, Faculty of Public Health, Diponegoro University, Prof. Soedarto Street, Tembalang, 50275, Semarang, Indonesia.

ABSTRACT

Introduction: Skin aging is a complex biological process with cumulative structural and physiological changes, progressive changes in each layer of the skin, and changes in the appearance of the skin. Human skin undergoes 2 types of skin aging, that are intrinsic and extrinsic skin aging. Skin aging is characterized by a decrease in skin hydration and elasticity, as well as an increase in skin wrinkles. Anti-aging strategies are still being developed. A new anti-aging therapy modality that has been widely used is nutraceutical supplementation, such as fish collagen. Fish collagen is a new alternative source of collagen with a high degree of homology to human collagen structure and high bioavailability. The aim of this study was to assess the efficacy of oral fish collagen supplementation on skin aging.

Methods: This study was an analytic observational study, with systematic review and meta-analysis. Data searching was conducted online at Pubmed-MEDLINE, EBSCO (CINAHL), Scopus, SpringerLink, ProQuest, Cochrane Library, ClinicalTrials.gov, Cambridge Core, and Google Scholar, as well as hand searching from libraries in Indonesia. Twelve studies were included in the qualitative analysis (systematic review) (n=753 subjects) and 6 of them were included in the quantitative analysis (meta-analysis) (n=426 subjects).

Results: The results of the meta-analysis showed that the increase in skin hydration in the group given oral fish collagen sup-

*Correspondence to Author:
Imelda Gunawan, Department of Dermatology, Venereology, and Aesthetic, Faculty of Medicine, Diponegoro University/Dr. Kariadi General Hospital Medical Center, Dr. Sutomo Street No. 16, 50244, Semarang, Indonesia

-plementation was greater significantly than in the placebo group, with standardized mean difference (SMD) (95% CI)=0.56 (0.32 to 0.81), Z=4.47 (p<0.00001), I2=24%. The increase in skin elasticity in the group given oral fish collagen supplementation was greater significantly than in the placebo group, with SMD (95% CI)=0.33 (0.17 to 0.49), Z=4.05 (p<0 ,0001), I2=21%. The reduction in skin wrinkles in the group given oral fish collagen supplementation was greater significantly than in the placebo group, with SMD (95% CI)=-0.47 (-0.74 to -0.19), Z=3.34 (p=0.0008), I2=49%. Adverse events were not found in the included studies.

Conclusion: Oral fish collagen supplementation is effective against skin aging, mainly in increasing skin hydration and skin elasticity, and reducing skin wrinkles.

Keywords: skin aging, nutraceutical supplementation, fish collagen, skin hydration, skin elasticity, skin wrinkles

Introduction

Skin aging is a complex biological process with cumulative structural and physiological changes, progressive changes in each layer of the skin, and changes in the appearance of the skin, especially in areas of the skin exposed to sunlight.[1] Human skin undergoes 2 types of aging, that are intrinsic and extrinsic skin aging. Skin aging is characterized by a decrease in skin hydration and elasticity, as well as an increase in skin wrinkles.[2-5] The population over the age of 60 is increasing significantly worldwide. This population will reach 1.4 billion in 2030, 2.1 billion in 2050, even 3.1 billion in 2100.[6,7] Aging-related skin diseases have become a burden finance in many countries, and become an important issue in the following year.[3]

Anti-aging strategies are still being developed with the aim of achieving healthy aging, treating cutaneous disorders, and delaying skin aging.[1] Some of these therapeutic modalities until now are not satisfying, thus several new therapeutic modalities have been developed.[1,8] A new anti-aging therapy modality that has been widely used is nutraceutical supplementation, such as collagen (fish, bovine, porcine collagen).[7,9-11] Fish collagen is the newest alternative source of collagen derived from fish skin, bones, cartilage, and scales.[12]

Several studies had investigated the benefits of oral fish collagen supplementation on skin aging. The results of these studies showed that fish collagen improved skin aging.[13-15] Fish collagen can increase dermal fibroblast bioactivities, human collagen synthesis, prevent degradation and fragmentation of human collagen.[15,16] Fish collagen can also increase hyaluronic acid production, improve skin barrier function, increase skin turnover, and also has potential as an antioxidant, therefore through these mechanisms, fish collagen can increase skin hydration and elasticity, and reduce skin wrinkles.[16-20]

Oral fish collagen supplementation is one of the latest promising anti-aging therapies, so that researchers are interested in evaluating the effectiveness of oral fish collagen supplementation on skin aging. Several experimental studies have investigated the effectiveness of oral fish collagen supplementation on skin aging, but systematic review and meta-analysis have not been conducted.

Materials and Methods

Literature Search

This study was an analytic observational study, with systematic review and meta-analysis. The study began with searching and collecting suitable articles online in electronic databases, such as Pubmed-MEDLINE, EBSCO (CINAHL), Scopus, SpringerLink, ProQuest, Cochrane Library, ClinicalTrials.gov, Cambridge Core, and Google Scholar. Other sources were reference lists, conference proceedings, researchers in the field, and journals based on hand searching results. The search for sources of information was carried out until the time of data analysis.

The following Medical Subject Headings (MeSH) terms were used to create three citation subgroups (1) fish collagen; (2) supplementation; (3) skin aging. The literature search was conducted based on the PRISMA 2009 flowchart.

Inclusion criteria were studies that using oral fish collagen supplementation without being combined with other active ingredients as a therapy for skin aging, study design was randomized controlled clinical trial, study subjects were female or male with all skin color types, age range was 21-70 years, study subjects had signs of skin aging, either intrinsic or extrinsic skin aging, the duration of follow-up was at least 4 weeks, and the study outcome was the mean difference of at least one of the following outcomes: skin hydration, skin elasticity, or skin wrinkles.

Exclusion criteria were case reports, case series, letters, systematic reviews, meta-analyses, literature reviews, studies written in languages other than Indonesian and English if the translated versions were not available, study subjects suffered from skin diseases, systemic diseases, connective tissue diseases, local infections, were pregnant and lactating, had an intolerance or allergy to fish, gluten, or pharmaceutical products, took other dietary supplements, had a history of taking oral retinoids, oral steroids, or other anti-aging drugs in the last 6 months, history of ablative or non-ablative laser resurfacing in the last 6 months, history of hormonal therapy, chemical peels, aesthetic surgery, or other aesthetic procedures for anti-aging in the last 3 months, history of using skin care products for anti-aging in the last 1 month, and excessive sun exposure right before the study began.

Study Selection

Three reviewers conducted the study selection independently. Duplicated articles were removed. Title and abstract, as well as full-texts, were reviewed for eligibility using the predefined inclusion and exclusion criteria. Differences in opinion were resolved between all reviewers to reach a consensus.

Data Extraction

Data extraction was performed independently by three reviewers using The Cochrane Collaboration data collection form for RCTs only. Every disputes in determining studies and data extraction were resolved with consensus.

Assessment of Risk of Bias

Risk of bias assessments were performed independently by three reviewers using The Cochrane Collaboration data collection form for RCTs only and The Cochrane Collaboration's tool for assessing the risk of bias in randomized clinical trials.

Data Synthesis

Meta-analysis of difference in weighted mean was conducted using *The Cochrane systematic review software (Review Manager [RevMan] Version 5.4.1, 2020)*. If data were not available to enable pooling, a descriptive synthesis was performed.

Results

The search for research articles was conducted based on the 2009 Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart (Figure 1).

Study Characteristics

The studies were conducted in South Korea (n=4), followed by Japan (n=2), France (n=2), China (n=2), Canada (n=1), and Thailand (n= 1). All studies were randomized controlled trials. The total sample of 12 studies was 753 subjects.

Ten studies selected a female population, while the other 2 studies selected male and female populations as study subjects, with varying age intervals between 21-70 years. All studies subjects were healthy and had signs of skin aging, both intrinsic and extrinsic skin aging (photoaging). All twelve studies used oral fish collagen supplementation from various fish species without being combined with other active ingredients. The study outcomes were skin hydration in 11 studies, skin elasticity in 10 studies, and skin wrinkles in 7 studies. Oral fish collagen supplementation was given once per day in 11 studies, but in 1 study it was given twice per day, with doses varying between 1-10 grams/day. Treatment duration was 4 weeks in 1 study, 8 weeks in 4 studies, and 12 weeks in 7 studies. The baseline characteristics of the 12 studies are summarized in Table 1.
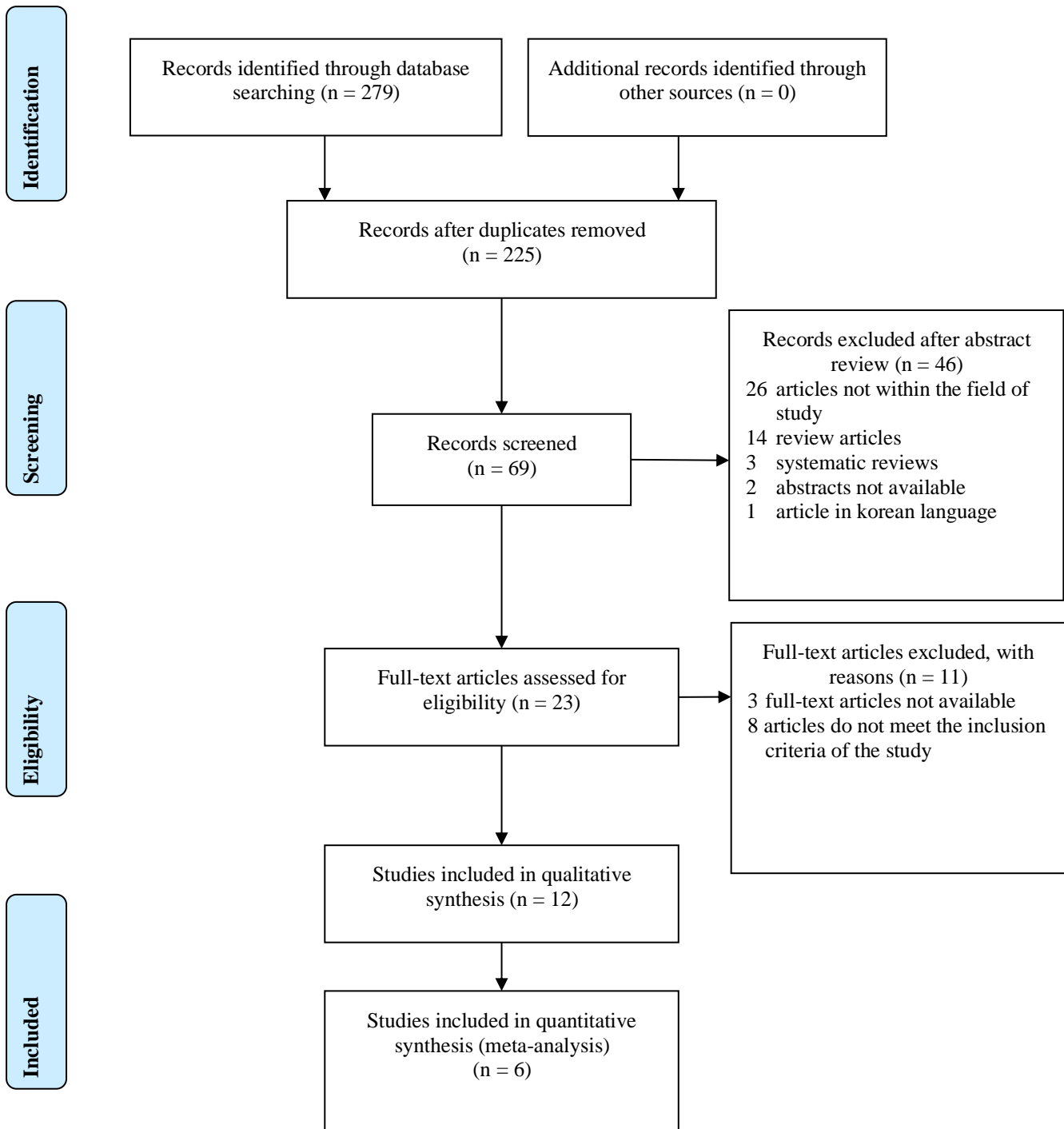

Figure 1. PRISMA Flow Diagram

Table 1. Characteristics of included studies

No	Author and Year	Country	Sample Size	Study Population	Interventions — Fish Collagen Group	Interventions — Placebo Group	Study Outcome	Frequency	Duration of Intervention	Study Design
1	Asserin et al. 2015	Japan	33	Japanese women, age 40-59 years, healthy, with low water content in the skin	Oral supplementation of fish collagen peptide, in the form of powder dissolved in water	Placebo (dextrin)	Skin hydration	Once daily with a dose of 10 grams/day	56 days (8 weeks)	Minimized, placebo-controlled, parallel-group, double-blind, monocentric study
2	Sugihara et al. 2015	China	56	Chinese women, age 30-55 years, healthy, BMI < 30 kg/m², with dry and rough skin	Oral supplementation of fish collagen hydrolysate derived from fish scale gelatin, in the form of powder	Placebo (maltodextrin)	Skin hydration, elasticity, and roughness	Once daily every night with a dose of 2.5 grams/day	8 weeks	Randomized, placebo-controlled, double-blind study
3	Duteil, Queille-Roussel, Maubert, et al. 2016	France	60	Women, age 45-70 years, Fitzpatrick skin phototype II-V, healthy, with signs of facial skin aging (periorbital wrinkles)	Oral supplementation of type 1 fish collagen hydrolysate (BPMG, HPMG, and 1000MG), in the form of powder in a sachet, dissolved in 20 mL of water	Placebo (maltodextrin)	**Primary:** skin biomechanics/elasticity, hydration, periorbital wrinkle (crow's feet); **Secondary:** investigator global efficacy appreciation (IGEA), satisfactory level	Once daily before breakfast with a dose of 5 grams/day	8 weeks	Monocentric, double-blinded, randomized, placebo-controlled supplementation study
4	Inoue et al. 2016	China	85	Chinese women, age 35-55 years, BMI < 30 kg/m², healthy, with dry or rough skin	Oral supplementation of fish collagen hydrolysate with low (L-CP) and high (H-CP) ratios of Pro-Hyp and Hyp-Gly dipeptides, in the form of powder in an aluminum sachet, dissolved in hot milk, coffee, or other drinks	Placebo (maltodextrin)	**Primary:** skin hydration, elasticity, wrinkle (number, area, depth of wrinkles, and skin roughness); **Secondary:** side effect	Once daily every night with a dose of 5 grams/day	8 weeks	Randomized double-blind placebo-controlled study
5	Duteil, Queille-Roussel, Bruno-Bonnet, et al. 2018	France	57	Mature women, healthy, with signs of skin aging	Oral supplementation of fish collagen peptide type 1, in the form of powder	Placebo (maltodextrin)	**Primary:** skin elasticity, hydration, wrinkle (crow's feet), skin radiance, homogeneity, skin tone, skin thickness and echogenicity; **Secondary:** satisfactory level	Once daily with a dose of 2.5 grams/day	12 weeks	Monocentric, double-blinded randomized, placebo-controlled supplementation study
6	Kim et al. 2018	South Korea	64	Women, age 40-60 years, with crow's feet score between 2-6	Oral supplementation of fish collagen hydrolysate derived from the skin of sutchi catfish (*Pangasius hypophthalmus*), in the form of low molecular weight collagen peptide (LMWCP) in 50 mL of drink	Placebo (drinks that had the same taste, except that they did not contain LMWCP and were replaced with water)	**Primary:** skin hydration, skin wrinkles, skin elasticity; **Secondary:** subjective assessment in the form of a questionnaire, the level of safety through blood laboratory tests	Once daily with a dose of 1000 mg/day	12 weeks	Randomized, double-blind, placebo-controlled design

7	Koizumi et al. 2018	South Korea	77	Women, age 30-60 years, healthy, with grade 2-6 periorbital wrinkles	Oral supplementation of fish collagen peptides derived from Tilapia fish scales (*Oreochromis mosambicus*), in the form of 20 mL of drink	Placebo (drinks that did not contain fish collagen peptide, with the color and smell of the placebo product modified by fragrance and coloring agents resembling the fish collagen product)	**Primary:** periorbital wrinkles, skin hydration, skin elasticity; **Secondary:** side effects and level of safety through blood laboratory tests	Once daily with a dose of 3000 mg/day	12 weeks	Randomized, placebo-controlled, double-blind study
8	Evans et al. 2020	Canada	50	Women, age 45-60 years, BMI 20-29.9 kg/m^2, with signs of intrinsic and extrinsic skin aging (photoaging) on the face	Oral supplementation of fish collagen hydrolysate from *Pangasius hypohthalmus*, in the form of powder, dissolved in 100 mL of water	Placebo (maltodextrin)	**Primary:** skin wrinkles, skin elasticity; **Secondary:** skin quality with the Visual Analogue Scale (VAS) questionnaire	Once daily with a dose of 10 grams/day	12 weeks	Randomized, triple-blind, placebo-controlled, parallel study
9	Jung et al. 2021	South Korea	50	Men and women, aged 35-60 years, with dry skin, skin moisture content ≤ 49%, or degree of wrinkles in the crow's feet area > 3	Oral supplementation of AP collagen peptide derived from *Nemipterus virgatus* scales, in the form of 25 mL ampoules	Placebo (ampoules with the same taste, but did not contain AP collagen peptide)	Skin hydration, TEWL, skin texture (skin roughness, skin gloss, stratum corneum flexibility), NMF in the stratum corneum (amino acids, amino acid derivatives), and ceramide content in the stratum corneum	Once daily with a dose of 1000 mg/day	12 weeks	Double-blind, randomized, parallel, and placebo-controlled study
10	Miyanaga et al. 2021	Japan	99	Women, age 35-50 years, healthy, with dry skin and decreased skin elasticity	Oral supplementation of fish collagen peptide at a dose of 1 or 5 grams dissolved in 50 mL of water	Placebo (similar sweetened, soured, and flavored beverages, but did not contain fish collagen peptides)	**Primary:** hydration of the stratum corneum; **Secondary:** epidermis and dermis water content, TEWL, skin elasticity, skin thickness, NMF levels in the stratum corneum (free amino acids, carboxylic acids, urea, lactic acid, and urocanic acid)	Once daily every night with a dose of 1 or 5 grams/day	12 weeks	Randomized, placebo-controlled, double-blind trial
11	Sangsu-wan et al. 2021	Thailand	38	Healthy women, postmenopau-sal, age 50-60 years, BMI: 18.5-23 kg/m^2	Oral supplementation of fish collagen hydrolysate (fish skin and scales), in the form of powder	Placebo (maltodextrin)	**Primary:** skin elasticity; **Secondary:** long-term therapeutic effect on skin elasticity after 4 weeks of no treatment, side effects	Once daily with a dose of 5 grams/day	4 weeks	Prospective, randomized, double-blind placebo-controlled trial
12	Tak et al. 2021	South Korea	84	Men and women, aged 40-60 years, healthy, with a TEWL score ≥ 4	Oral supplementation of fish collagen tripeptides from the skin of Nile Tilapia fish (*Oreochromis niloticus*), in the form of capsule (each capsule contained 250 mg collagen tripeptide)	Placebo (capsules containing 170 mg of maltodextrin or 80 mg of dextrin)	**Primary:** TEWL; **Secondary:** skin hydration, skin elasticity, skin wrinkles, perception through a questionnaire	2 capsules every 12 hours (total 4 capsules/day [1000 mg/day])	12 weeks	Randomized, placebo-controlled, double-blind clinical trial

Result of Qualitative Data Analysis (Systematic Review)

1. Asserin et al, 2015[10]

This study was a minimized, placebo-controlled, parallel-group, double-blind, monocentric study that evaluated the effectiveness of oral supplementation of fish collagen peptides compared to porcine collagen peptides and placebo against skin aging. This study was conducted in Tokyo, Japan on 33 Japanese women, aged 40-59 years, with low skin hydration, divided randomly into 3 groups, that were the *Peptan* F group which received 10 grams of fish collagen peptide, the *Peptan* P group which received 10 grams of porcine collagen peptide, and the placebo group. The treatment products were in the form of a drink and consumed once a day for 56 consecutive days. The results showed *Peptan* improved human skin hydration. Skin hydration did not change in the placebo group, but skin hydration increased significantly by 12% over baseline in *Peptan* F group, by 16% at week 4 and by 28% at week 8 (p=0.003) in *Peptan* P group. TEWL did not differ between the *Peptan* and placebo groups, therefore, oral *Peptan* supplementation was effective in increasing skin hydration without affecting TEWL. Asserin et al. in this study concluded that the results of the study provided clinical evidence for the effectiveness of specific collagen peptides (*Peptan*) derived from fish in increasing skin hydration, thereby reducing signs of skin aging. These data suggested that oral supplementation with fish-specific collagen peptides can improve skin structure and health from within.

2. Sugihara et al, 2015[21]

This study was a randomized, placebo-controlled, double-blind study that evaluated the effectiveness of oral supplementation of fish collagen hydrolysate compared to placebo against facial skin aging. This study was conducted in Shanghai, China on 56 healthy Chinese women, aged 30-55 years, with dry and rough skin, divided randomly into 2 groups, that were the collagen hydrolysate (CH) group which received 2.5 grams of hydrolyzed fish collagen and the placebo group which received 5 grams of maltodextrin. The treatment products were in the form of powder in sachets and consumed once a day for 8 weeks. The results showed that skin hydration and skin elasticity increased significantly (p<0.01, p<0.01 respectively), and skin roughness decreased significantly in the CH group at week 4 (p<0.05) and 8 (p<0.01). Sugihara et al. in this study concluded that oral supplementation of fish collagen hydrolysate of 2.5 grams per day could improve hydration, elasticity, and roughness of facial skin.

3. Duteil, Queille-Roussel, Maubert, dkk, 2016[22]

This study was a monocentric, double-blinded, randomized, placebo-controlled supplementation study that evaluated the effectiveness of oral supplementation of type 1 fish collagen peptide against skin aging. This study was conducted in France on 60 healthy women, aged 45-70 years, with periorbital wrinkles, divided randomly into 4 groups, that were the *Naticol* BPMG (CH1), *Naticol* HPMG (CH2), *Naticol* 1000 mg (CH3) group which received 5 grams of fish collagen peptides with different molecular weights and the placebo group which received 5 grams of maltodextrin. The treatment products were in the form of powder in 20 mL of water and consumed once a day for 8 weeks. The results showed that skin biomechanics/elasticity were significantly improved and periorbital wrinkles were significantly reduced by -11% in the *Naticol* group at week 8 (p=0.014 in CH2 group and p=0.023 in CH3 group) over the baseline, however, skin hydration was not significantly different between the *Naticol* and placebo groups. Duteil, Queille-Roussel, Maubert, et al. in this study concluded that oral supplementation of type 1 hydrolyzed fish collagen had a beneficial effect on skin quality, especially the CH2 group showed the greatest effect on improving skin biomechanics, reducing wrinkles, and increasing satisfaction of study

subjects. Adverse events were not found during the study.

4. Inoue dkk, 2016[23]

This study was a randomized double-blind placebo-controlled study that evaluated the effectiveness of oral supplementation of fish collagen hydrolysate compared to placebo against skin aging. This study was conducted in Shanghai, China on 85 healthy Chinese women, aged 35-55 years, with dry and rough skin, divided randomly into 3 groups, that were the H-CP group which received 5 grams of fish collagen hydrolysate with a high dipeptide ratio, the group L-CP which received 5 grams of fish collagen hydrolysate with a low dipeptide ratio, and the placebo group which received 5 grams of maltodextrin. The treatment products were in the form of powder in sachets and consumed once a day for 8 weeks. The results showed that hydration and skin elasticity increased significantly, and skin wrinkles significantly decreased in the H-CP and L-CP groups (p<0.05). The skin improvements in the H-CP group were significantly greater than in the L-CP group. Inoue et al. in this study concluded that fish L-CP and H-CP were effective supplements in increasing hydration and improving skin roughness in women with dry and rough skin. Hydrolyzed collagen with a high dipeptide content (H-CP) showed a greater improvement in increasing skin elasticity and reducing facial skin wrinkles. Side effects were not found during the study.

5. Duteil, Queille-Roussel, Bruno-Bonnet, dkk, 2018[24]

This was a monocentric, double-blinded, randomized, placebo-controlled supplementation study which evaluated the effectiveness of oral supplementation of type I fish collagen peptides either in combination with silicone or not in combination against skin aging. This study was conducted in France on 57 adult women with signs of skin aging, divided randomly into 3 groups, that were the CP group which received 2.5 grams of fish collagen peptide, the CP/Si group which received a combination of 2.5 grams of fish collagen peptide and placebo, and the placebo group which received 2.5 grams of maltodextrin for 12 weeks. The results showed that skin biomechanics/elasticity improved significantly and crow's feet wrinkles decreased significantly by -16.5% in the CP group over the baseline. Improvements in crow's feet at week 12 were 74% in the CP group and 60% in the CP/Si group. However, skin hydration was not significantly different between the CP and placebo groups. Duteil, Queille-Roussel, Bruno-Bonnet, et al. in this study concluded that oral supplementation of low-dose fish collagen peptide with or without silicone combination once daily for 3 months could improve skin quality.

6. Kim dkk, 2018[25]

This study was a randomized, double-blind, placebo-controlled study that evaluated the effectiveness of oral low-molecular-weight fish collagen peptide (LMWCP) supplementation against skin aging. The study was conducted in South Korea on 64 healthy women, aged 40-60 years, with crow's feet, divided randomly into 2 groups, that were the LMWCP group which received 1000 mg of LMWCP and the placebo group. The treatment products were in the form of 50 mL of drink and consumed once a day for 12 weeks. The results showed that hydration and skin elasticity increased significantly (p=0.000, p=0.002 respectively), and skin wrinkles decreased significantly in the LMWCP group at week 12 (p=0.000). Kim et al. in this study concluded that fish LMWCP was a functional healthy food ingredient that was safe and effective as anti-skin photoaging, which could effectively increase skin hydration, skin elasticity, and improve human skin wrinkles with a dose of 1000 mg once daily. Side effects were not found during the study.

7. Koizumi dkk, 2018[26]

This study was a randomized, placebo-controlled, double-blind study that evaluated the effectiveness of oral supplementation of fish collagen peptides compared to placebo against

skin aging. This study was conducted in South Korea on 77 healthy women, aged 30-60 years, with periorbital wrinkles, divided randomly into 2 groups, that were the collagen peptide (CP) group which received 3000 mg of fish collagen peptide and the placebo group. The treatment products were in the form of 20 mL of drink and consumed once a day for 12 weeks. The results showed that skin wrinkles decreased significantly (p<0.05), skin hydration and skin elasticity (p<0.05, p<0.05 over the baseline, p<0.001 compared to the placebo group respectively) increased significantly in the CP group at week 12. Koizumi et al. in this study concluded that oral supplementation of 3000 mg of collagen peptide derived from fish scales for 12 weeks was effective in reducing periorbital wrinkles, and increasing skin hydration, and skin elasticity. The beneficial effects of fish collagen peptides on skin health were resulted from the high absorption of specific amino acid contents with high concentrations of Hyp, Gly, and Pro. Side effects were not found during the study.

8. Evans dkk, 2020[27]

This study was a randomized, triple-blind, placebo-controlled, parallel study that evaluated the effectiveness of oral supplementation of fish collagen hydrolysate compared to placebo against skin aging. The study was conducted in Canada on 50 women, aged 45-60 years, with signs of skin aging, divided randomly into 2 groups, that were the *Vinh Wellness Collagen* (VWC) group which received 10 grams of fish collagen hydrolysate and the placebo group which received 10 grams of maltodextrin. The treatment products were in the form of a powder which was dissolved in 100 mL of water and consumed once a day for 12 weeks. The results showed that skin wrinkles decreased significantly on the left-sided face (17%, P=0.009) and right-sided face (35%, P=0.005 over the baseline, 24%, p=0.035 compared to the placebo group) and skin hydration increased but not significantly in the VWC group at week 12, and skin elasticity was not significantly different between the VWC and placebo groups.

Evans et al. in this study concluded that study subjects who were given VWC supplementation for 12 weeks showed a decrease in the score of wrinkles on both sides of the face, an increase in the hydration of the skin of the cheeks and the score of the research subject's self-assessment. This study showed that VWC was safe and well tolerated. The results of this study supported the use of fish-derived hydrolyzed collagen for improving skin health in an aging population.

9. Jung dkk, 2021[28]

This study was a double-blind, randomized, parallel, and placebo-controlled study that evaluated the effectiveness of oral supplementation of enzymatically decomposed AP collagen peptide (APCP) from fish against skin aging. This study was conducted in South Korea on 50 men and women, aged 35-60 years, with dry skin and crow's feet, divided randomly into 2 groups, that were the APCP group which received 1000 mg of APCP and the placebo group. The treatment products were in the form of a powder which was suspended in 25 mL of water and consumed once a day for 12 weeks. The results showed that skin hydration increased significantly by 2.71% ± 3.19% (p=0.002) in the APCP group at week 12. The increase in skin hydration in the APCP group (7.33%) was significantly greater than that in the placebo group (2.83%) at week 12 (p=0.031). However, TEWL was not significantly different between the APCP and placebo groups. Jung et al. in this study concluded that APCP supplementation on dry skin could increase skin hydration and improve skin barrier function by increasing natural moisturizing factor (NMF) and ceramide content in the stratum corneum. Adverse events were not found during the study.

10. Miyanaga dkk, 2021[29]

This study was a randomized, placebo-controlled, double-blind trial that evaluated the effectiveness of oral supplementation of fish collagen peptides against skin aging. The study was conducted in Tokyo, Japan on 99 healthy women, aged 35-50 years, with dry skin, decreased skin elasticity, divided randomly into

3 groups, that were group CP1 which received 1 gram of fish collagen peptide, CP5 which received 5 grams of fish collagen peptide, and the placebo group. The treatment products were in the form of 50 mL of drink and consumed once a day for 12 weeks. The results showed that skin hydration increased significantly and TEWL decreased significantly in the CP1 and CP5 groups at week 12. The hydration of the stratum corneum of the cheeks in the CP1 and CP5 groups improved significantly at 8 and 12 weeks compared to the placebo group (CP1 group: $p<0.01$ and $p<0.001$, CP5 group: $p<0.001$ respectively). Hydration of the stratum corneum of the forearm increased significantly in the CP1 group ($p<0.05$, $p<0.01$) at week 8 and 12, and in the CP5 group ($p<0.05$) at week 12 compared to the placebo group. However, skin elasticity was not significantly different between the three groups. Miyanaga et al. in this study concluded that improved skin hydration after oral supplementation of fish collagen peptides contributed to increased levels of NMF in the stratum corneum.

11. Sangsuwan dkk, 2021[30]

This study was a prospective, randomized, double-blind placebo-controlled trial that evaluated the effectiveness of oral supplementation of fish collagen hydrolysate compared to placebo against skin aging. This study was conducted in Bangkok, Thailand on 38 healthy, postmenopausal women, aged 50-60 years, divided randomly into 2 groups, that were the CH group which received 5 grams of fish collagen hydrolysate and the placebo group which received 5 grams of maltodextrin. The treatment products were in the form of powder in sachets and consumed once a day for 4 weeks. The results showed that skin elasticity of left and right cheeks increased significantly in the CH group at weeks 4 ($p=0.006$, $p=0.03$) and 8 ($p=0.01$, $p=0.004$), however, forearm skin elasticity was not significantly different between the CH and placebo groups. Sangsuwan et al. in this study concluded that oral supplementation of hydrolyzed fish collagen for 4 weeks could significantly increase skin elasticity in sun-exposed areas. Improvement in skin elasticity persisted 4 weeks after discontinuation of the test product. Side effects were not found during the study.

12. Tak dkk, 2021[31]

This study was a randomized, placebo-controlled, double-blind clinical trial that evaluated the effectiveness of fish collagen tripeptide oral supplementation against skin aging. This study was conducted in Yangsan-si, South Korea on 84 healthy women, aged 40-60 years, TEWL score ≥ 4, randomly divided into 2 groups, that were the collagen tripeptide (CTP) group which received 1000 mg of fish collagen tripeptide and the placebo group which received 170 mg of maltodextrin and 80 mg of dextrin for 12 weeks. The treatment products were in the form of capsules (250 mg per capsule) and consumed 2x2 capsules every day for 12 weeks. The results showed that skin hydration increased greater in the CTP group than in the control group although the difference was not significant at week 12 ($p=0.325$ at adjusted humidity and temperature, $p=0.335$ at adjusted UVA, $p=0.278$ at adjusted temperature, high temperature, and UVA). The difference in TEWL was statistically significant in study subjects aged more than 50 years at week 12 between the CTP group and the control group after adjusting for humidity, temperature and UVA ($p=0.034$). However, skin elasticity and wrinkles were not significantly different between the CTP and placebo groups. Tak et al. in this study concluded that oral fish CTP supplementation was well tolerated and helped reduce TEWL in middle-aged adult women. Temperature, humidity, and UVA could affect human skin health.

Quantitative Data Result (Meta-Analysis)

Meta-analysis of the parameter skin hydration with fixed effect model showed a heterogeneity test with $Chi^2=5.25$, df=4 ($p=0.26$); $I^2=24\%$. Standardized mean difference was 0.56 with 95% confidence interval (CI) of 0.32 to 0.81, and the test results for the overall effect showed

Z=4.47 (p<0.00001). The results of this meta-analysis showed that the increase in skin hydration was greater significantly in fish collagen group compared to the placebo group (p<0.00001) (Figure 2).
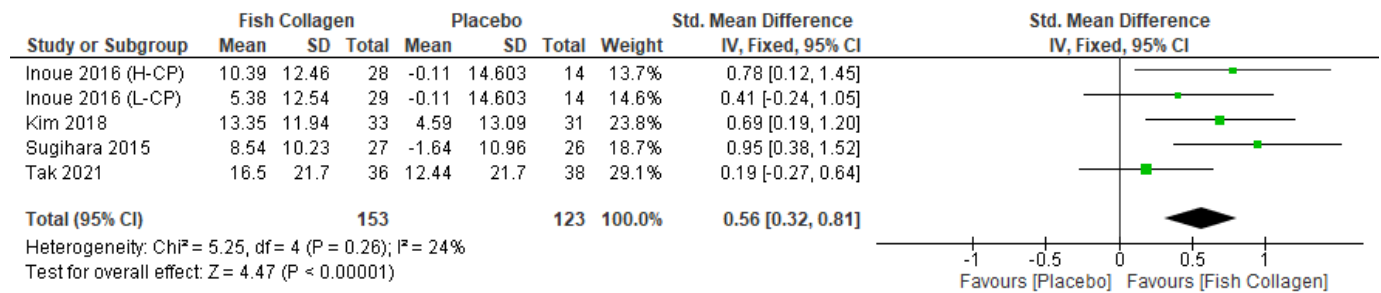

Figure 2. The results of a meta-analysis of the effectiveness of oral fish collagen supplementation compared to the placebo on skin hydration

Meta-analysis of the parameter skin elasticity with fixed effect model showed a heterogeneity test with $Chi^2=15.25$, df=12 (p=0.23); $I^2=21\%$. The results of the combined meta-analysis of parameters R2, R5, and R7 showed that the standardized mean difference was 0.33 with 95% confidence interval (CI) of 0.17 to 0.49, and the test results for the overall effect showed Z=4.05 (p<0.0001). The results of this meta-analysis showed that the increase in skin elasticity was greater significantly in fish collagen group compared to the placebo group (p<0.0001) (Figure 3).
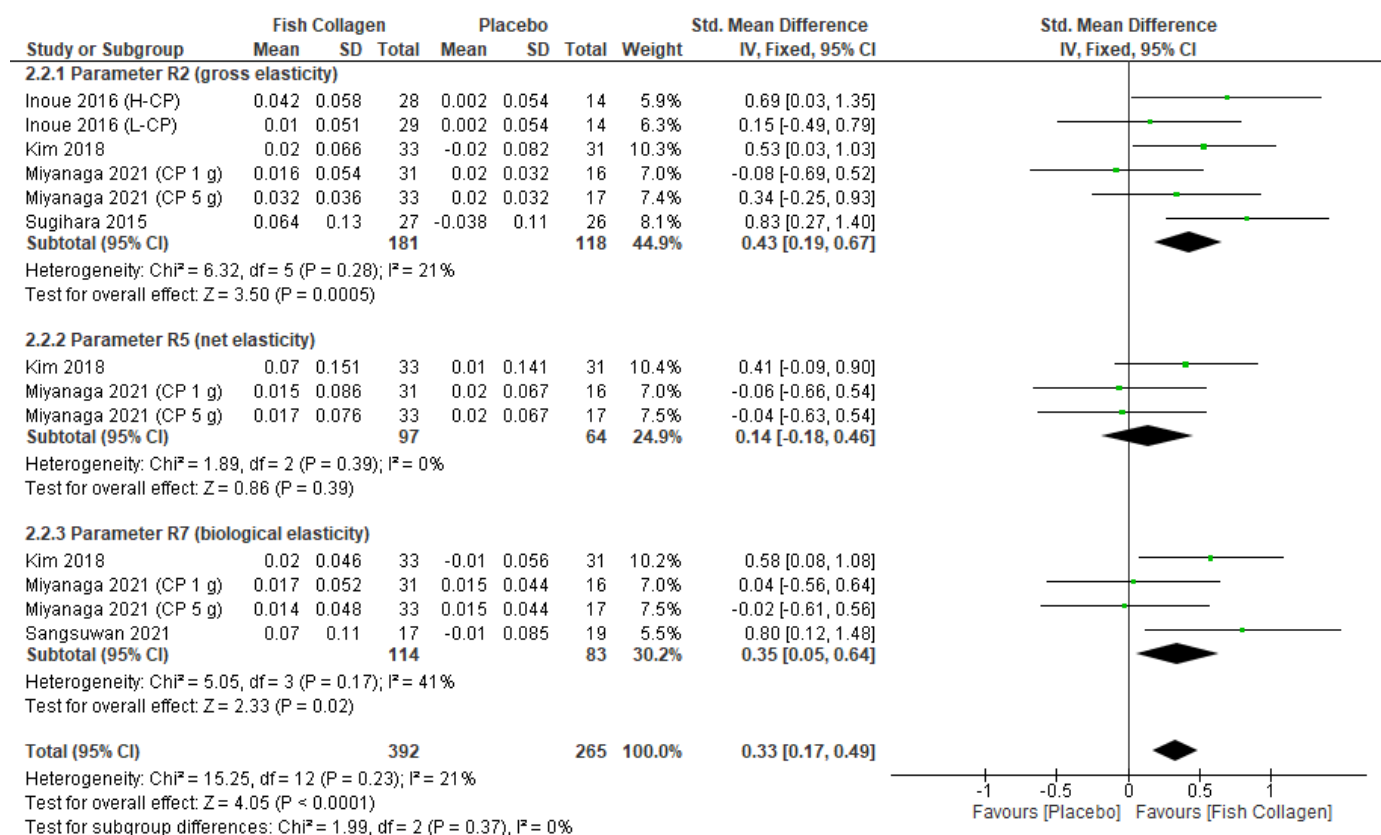

Figure 3. Results of a meta-analysis of the effectiveness of fish collagen oral supplementation compared to placebo on skin elasticity

Meta-analysis of the parameter skin wrinkles with fixed effect model showed a heterogeneity test with Chi^2=5.88, df=3 (p=0.12); I^2=49%. Standardized mean difference was -0.47 with 95% confidence interval (CI) of -0.74 to -0.19, and the test results for the overall effect showed Z=3.34 (p=0.0008). The results of this meta-analysis showed that the reduction in skin wrinkles was greater significantly in fish collagen group compared to the placebo group (p=0.0008) (Figure 4).
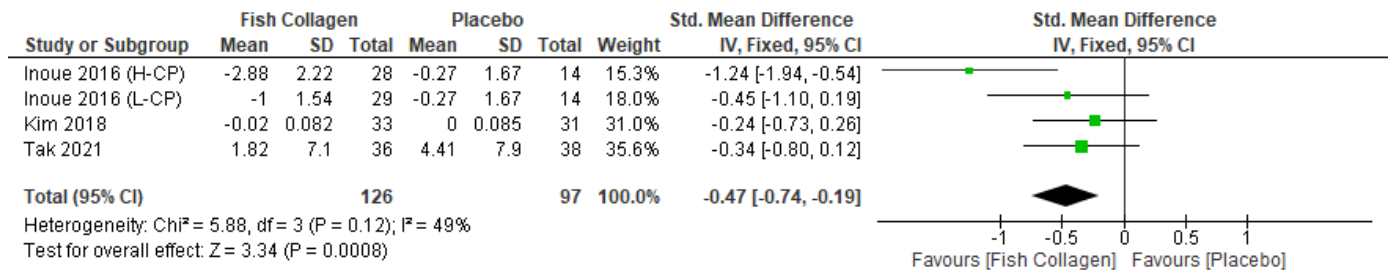

Figure 4. Results of a meta-analysis of the effectiveness of fish collagen oral supplementation compared to placebo on skin wrinkle

Risk of Bias in Included Studies

The risk of bias from the studies included in the analysis, both qualitative and quantitative analyses, was assessed using the Cochrane Risk of Bias Tool for Randomized Controlled Trials, including random sequence generation, allocation concealment, blinding participants and personnel, blinding outcome assessment, incomplete outcome data, selective reporting, and other bias (Table 2).

Discussion

This study was an analytic observational study, with systematic review and meta-analysis that evaluating the effectiveness of oral fish collagen supplementation on skin aging with parameter of skin hydration, skin elasticity, and skin wrinkles. Twelve studies were included in the qualitative analysis (systematic review) and 6 of them were included in the quantitative analysis (meta-analysis).

Studies included in the systematic review and meta-analysis had a varied age of study subjects, which was between 21-70 years. According to the literature, oral fish collagen supplementation is given effectively starting at the age of 20 years because collagen levels in the body begin to decrease at the age of 30 years. Evans et al, 2020 reported that the increase in skin elasticity was not significantly different between the population group aged 45-54 years and the population group aged 55-60 years after oral fish collagen supplementation. Tak et al, 2021 study reported that the reduction in TEWL after oral fish collagen supplementation was greater significantly than the placebo group, both in population groups aged over 50 years and under 50 years.[27,31] Skin aging is divided into 2 types, that are intrinsic and extrinsic skin aging.[5] Oral fish collagen supplementation can improve skin quality, both in intrinsic and extrinsic skin aging.[15,18]

All studies included in both the systematic review and the meta-analysis used fish collagen, not collagen from mammals. According to the literature, the use of fish collagen compared to collagen from mammals is growing faster due to several unique characteristics, such as not transmitting disease, no religious prohibitions, efficient cost, low molecular weight, high biocompatibility, and absorbed more easily by the human body.[10,31,32] The fish collagen used in the studies came from various fish species. Collagen derived from fish according to the literature is rich in type I collagen. The amino acid content of collagen is similar among several fish species, such as glycine, alanine, proline, glutamic acid, and hydroxyproline.[33] Each

sequence of 3 amino acids in each helical chain triple fish collagen consists of amino acids glycine (Gly-X-Y), proline (Pro at the X or Y position), and hydroxyproline (Hyp at the Y position). Hydroxyproline (Hyp) is one of the main amino acids from fish collagen which plays a role in collagen stability. The most commonly used fish collagen sources are catfish (*Pangasius*) and nile tilapia (*Oreochromis*).[12,15]

Table 2. Risk of bias of included studies in the systematic review and meta-analysis

	Random Sequence Generation	Allocation Concealment	Blinding Participants and Personnel	Blinding Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias	Overall
Asserin et al, 2015	?	+	?	?	+	+	+	−
Sugihara et al, 2016	+	+	?	+	+	+	+	+
Duteil, Queille-Roussel, Maubert, et al, 2016	?	+	+	+	+	+	+	+
Inoue et al, 2016	+	+	+	+	+	+	+	+
Duteil, Queille-Roussel, Bruno-Bonnet, et al, 2018	?	+	+	?	+	+	+	+
Kim et al, 2018	?	?	+	?	+	+	+	−
Koizumi et al, 2018	+	+	+	?	+	+	+	+
Evans et al, 2020	+	+	+	+	+	+	+	+
Jung et al, 2021	+	+	+	+	+	+	+	+
Miyanaga et al, 2021	?	+	+	?	+	+	+	+
Sangsuwan et al, 2021	+	+	+	+	+	+	+	+
Tak et al, 2021	+	+	?	+	+	+	+	+

Fish collagen supplementation was given orally in all studies, either in the form of a powder dissolved in water, powder in capsules, or in the form of a drink. Several studies have investigated the absorption of fish collagen peptides. Fish collagen given via the oral route is well absorbed into the body in the form of amino acids, dipeptides, and tripeptides. Small peptides derived from fish collagen (tripeptide [Gly-Pro-Hyp], dipeptide [Pro-Hyp, Hyp-Gly], or free Hyp) after ingestion of fish collagen can be detected in human blood circulation as early as 10 minutes after ingestion, with peak concentrations are reached within 1-2 hours after ingestion. Gly-Pro-Hyp can be absorbed through the intestine into the human blood circulation. The absorbed Gly-Pro-Hyp remains in the plasma for several hours and is detectable in various tissues, especially the skin, where this tripeptide persists longer (96 hours) in the skin than in other tissues. Dipeptides, such as Pro-Hyp and tripeptides are excreted via the urine after ingestion of fish collagen peptides. The results of this study are consistent with the rationale that amino acids, dipeptides, and tripeptides derived from fish collagen peptides, as well as their related metabolites, can reach the skin and contribute to improve skin quality.[10,25,29,34]

Oral fish collagen preparations according to the literature consist of powder collagen and liquid

collagen. Liquid collagen contains a lower dose of collagen per serving than powder collagen, which is less than 3 grams per beverage product, while powder collagen contains a higher dose of collagen per serving of up to 15 grams, so that the cost of collagen powder is more efficient if higher dose of collagen supplementation is needed. Liquid collagen contains other additional ingredients, while powder collagen only contains collagen particles. Liquid collagen is easier and faster to serve, while powder collagen takes longer and must be dissolved slowly until all the powder is completely dissolved before consumption. Powder and liquid collagen both use collagen peptides, therefore they have similar bioavailability and absorption. Scientific evidence shows that none has better absorption between liquid and powder collagen.[35,36] Powder collagen in the form of powder in sachets or powder in pills (tablets or capsules) has comparable validity, effectiveness, absorption, and health benefits. The difference between the two is in the dosage of collagen per serving, cost, and convenience.[37]

Studies included in the systematic review and meta-analysis had a varied dosage and duration of oral fish collagen supplementation, which was between 1-10 grams/day for 4-12 weeks. According to the literature, the safe and effective dose of oral fish collagen supplementation is 2.5-15 grams/day.[19] Several further clinical studies regarding the benefits of oral fish collagen supplementation on skin aging have been conducted. The dosage and duration of oral fish collagen supplementation based on various clinical studies varies, which is 1-10 grams/day for 4-24 weeks, with a frequency of 1-2x/day for anti-aging therapy, which can increase skin hydration and skin elasticity, and reduce skin wrinkles. Various clinical studies have also reported that oral supplementation of collagen up to 15 grams/day is safe and non-toxic.[38,39]

The results of this meta-analysis showed that the increase in skin hydration was greater significantly in fish collagen group compared to the placebo group. According to the literature, oral fish collagen supplementation can increase skin hydration. A recent histological study analyzed biomarker expression in mice to evaluate the cutaneous hydration effect of oral supplementation of fish collagen tripeptides. Increased expression levels of ceramide kinase, hyaluronic acid, collagen 1A, hyaluronan synthase-2 (HAS2), and decreased levels of hyaluronidase-1 (HYAL1) and CD44 were observed in human dermal fibroblasts after application of fish collagen tripeptides. Significant reductions in TEWL, scratching behavior, HYAL1, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and increases in water content and HAS2 levels were also observed. The results of this study indicate that fish collagen tripeptides can increase skin hydration and become a potential skin hydration agent in humans.[31]

Other studies had shown that the levels of natural moisturizing factor (NMF) constituents, such as pyrrolidone carboxylic acid (PCA) and urocanic acid (UCA), increased significantly after ingestion of fish collagen peptides, and total amino acids tended to increase. These results are consistent with the results that oral supplementation of fish collagen peptides triggers filaggrin expression in mice. Filaggrin has a role in connecting keratin fibers from stratum granular to the lower stratum corneum in the process of keratinization. Filaggrin is also involved in maintaining the internal structure of keratinocytes and skin barrier function. The decrease in TEWL and increase in NMF levels may be due to increased filaggrin expression. The results of this study indicate that oral ingestion of fish collagen peptides can increase skin hydration due to increased levels of NMF in the stratum corneum and decreased TEWL.[29]

Ingestion of fish collagen peptide can also increase the water content of the dermis. An in vitro study using human dermal fibroblast cells by Ohara et al reported that Pro-Hyp increased cell proliferation and hyaluronic acid synthesis by upregulating HAS2 mRNA levels, resulting in

water retention. Pro-Hyp also stimulates phosphorylation of signal transducer and activator of transcription 3 (STAT3), which is an important intracellular signaling factor.[25,29,40]

The results of this meta-analysis showed that the increase in skin elasticity was greater significantly in fish collagen group compared to the placebo group. According to the literature, oral fish collagen supplementation can increase skin elasticity. Borumand et al compared the effects of hydrolyzed collagen in oral, topical, and placebo forms. Oral hydrolyzed collagen resulted in significantly improved dermal morphology, collagen fiber production, and increased dermal density detected by ultrasonography. Sangsuwan et al, 2021 showed that ingestion of hydrolyzed collagen could also reduce some matrix metalloproteinases (MMP), such as MMP-1 and 12. Proline and hydroxyproline derived from collagen are the main building blocks in collagen synthesis in the dermis, resulting in skin elasticity 4 weeks after discontinuation oral fish collagen supplementation remains the same as when they were still given oral fish collagen supplementation.[30]

The mechanical properties of human skin, such as elasticity, resilience, and strength, are mainly influenced by collagen and elastic fibers in the extracellular matrix of the dermis. MMP-2 and MMP-9 degrade insoluble elastin into soluble fragments. MMP-2, MMP-9, and MMP-13 catabolize microfibril fibers, therefore downregulation of these proteins will affect skin elastic fibers. Oral fish collagen supplementation can increase the production of collagen and elastic fibers in the skin by downregulating MMP and increasing the expression of tissue inhibitor matrix metalloproteinase (TIMP), especially TIMP-1. Skin hydration also affects skin elasticity because conformational changes in elastin only occur in hydrated proteins.[25] Increased skin hydration will increase skin elasticity.[25]

Hyaluronic acid besides playing a role in skin hydration, also plays an important role in skin elasticity. Pro-Hyp and Hyp-Gly derived from fish collagen, as previously explained, can stimulate the production of hyaluronic acid in the dermis. Several experimental animal studies have also shown that oral supplementation of fish collagen can increase levels of procollagen types I and III thereby triggering biosynthesis of collagen types I and III, and maintaining the ratio of collagen type III to type I. Fish collagen can upregulate the TGF-β/Smad signaling pathway which directly triggers the expression of procollagen types I and III. Fish collagen can increase the production, growth, and migration of fibroblasts, thus triggering the synthesis of collagen fibers, elastic fibers, and other extracellular matrices.[15,18,41]

The results of this meta-analysis showed that the reduction in skin wrinkles was greater significantly in fish collagen group compared to the placebo group. According to the literature, oral fish collagen supplementation can reduce skin wrinkles. Previous studies in experimental animals showed that expression and activation of MMPs (MMP-1, MMP-2, MMP-3, and MMP-9) were downregulated after oral administration of fish collagen peptides containing Pro-Hyp and Hyp-Gly in irradiated mice with UVB, thereby increasing the collagen content in the skin and decreasing the formation of wrinkles.[25] Previous studies had also shown that the synthesis of type I procollagen and elastin, components of the dermal extracellular matrix, increased after oral administration of fish collagen peptides, thereby effectively reducing the number and depth of wrinkles.[23] Oral supplementation of fish collagen has antioxidant activity and can repair endogenous collagen and elastin fibers.[21] Hydrolyzed fish collagen has been confirmed as a potential antioxidant.[17]

Side effects of oral fish collagen supplementation, include gastrointestinal disturbances (nausea, vomiting, diarrhea, constipation), decreased appetite, increased calcium levels, kidney stones, allergic reactions, and mood changes. However, severe side effects have not been reported until now from

several clinical studies. Toxicity tests have been conducted on fish collagen, and obtained negative results, so it can be concluded that oral fish collagen supplementation is safe and has good tolerance.[42]

Conclusion

Oral fish collagen supplementation is effective against skin aging, mainly in increasing skin hydration and skin elasticity, and reducing skin wrinkles. The supplementation dose used for skin aging in most of the studies was 5 grams/day. The duration of supplementation needed to inhibit skin aging in most studies was 12 weeks, and the effects persisted for 4 weeks after supplementation was discontinued. Study using oral fish collagen supplementation with high concentrations of Pro-Hyp dipeptide showed better skin quality improvement after 4 weeks than oral supplementation of fish collagen with low concentrations of Pro-Hyp dipeptide. Oral fish collagen supplementation is safe and well tolerated because no side effects or adverse events were observed in the studies analyzed. Further studies with bigger sample sizes and longer observational periods are needed to confirm effectiveness of oral fish collagen supplementation on skin aging.

Abbreviations:

BMI: Body Mass Index

CI: Confidence interval

CINAHL: Cumulative Index to Nursing and Allied Health Literature

CH: Collagen hydrolysate

CP: Collagen peptide

CTP: Collagen tripeptide

Gly: Glycine

HAS2: Hyaluronan synthase-2

HYAL1: Hyaluronidase-1

Hyp: Hydroxyproline

IGEA: Investigator global efficacy appreciation

IL-6: Interleukin-6

LMWCP: Low molecular weight collagen peptide

MMP: Matrix metalloproteinase

NMF: Natural moisturizing factor

Pro: Proline

RCT: Randomized Controlled Trial

SMD: Standardized mean difference

TIMP: Tissue inhibitor matrix metalloproteinase

TNF-α: Tumor necrosis factor-alpha

MeSH: Medical Subject Headings

PRISMA: Preferred Reporting Items for Systematic Review and Meta Analysis

VAS: Visual Analogue Scale

VWC: *Vinh Wellness Collagen*

References

1. Zouboulis CC, Ganceviciene R, Liakou AI, Theodoridis A, Elewa R, Makrantonaki E. Aesthetic Aspects of Skin Aging, Prevention, and Local Treatment. Clin Dermatol. 2019;37:365-72.

2. Mohiuddin AK. Skin Aging & Modern Age Anti-aging Strategies. Glob J Med Res: Pharma, Drug Discovery, Toxicology & Medicine. 2019;19(2):1-47.

3. Wang L, Jiang Y, Wang X, Zhou J, Cui H, Xu W, et al. Effect of Oral Administration of Collagen Hydrolysates from Nile Tilapia on the Chronologically Aged Skin. J Funct Foods. 2018;44:112-7.

4. Zhang S, Duan E. Fighting against Skin Aging: The Way from Bench to Bedside. Cell Transplantation. 2018;27(5):729-38.

5. Kerns ML, Chien AL, Kang S. Skin Aging. In: Kang S, Amagai M, Bruckner AL, Enk AH, Morgolis DJ, McMichael AJ, et al., editors. Fitzpatrick's Dermatology. Volume 1. 9th ed. New York: McGraw-Hill Education; 2019. p. 1779-91.

6. Strnadova K, Sandera V, Dvorankova B, Kodet O, Duskova M, Smetana K, et al. Skin Aging: the Dermal Perspective. Clin Dermatol. 2019;37:326-35.

7. Campa M, Baron E. Anti-aging Effects of Select Botanicals: Scientific Evidence and Current Trends. Cosmetics. 2018;5(54):1-15.

8. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH, Choi HR, et al. Molecular Mechanisms of Dermal Aging and Antiaging Approaches. Int J Mol Sci. 2019;20(9).

9. Czajka A, Kania EM, Genovese L, Corbo A, Merone G, Luci C, 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.

10. 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(4):291-301.

11. Choi SY, Ko EJ, Lee YH, Kim BG, Shin HJ, Seo DB, et al. Effects of Collagen Tripeptide Supplement on Skin Properties: A Prospective, Randomized, Controlled Study. J Cosmet Laser Ther. 2014;16:132-7.

12. Alves AL, Marques ALP, Martins E, Silva TH, Reis RL. Cosmetic Potential of Marine Fish Skin Collagen. Cosmetics. 2017;4(39):1-16.

13. Abuine R, Rathnayake AU, Byun H-G. Biological Activity of Peptides Purified from Fish Skin Hydrolysates. Fish Aquatic Sci. 2019;22(10):1-14.

14. Venkatesan J, Anil S, Kim S-K, Shim MS. Marine Fish Proteins and Peptides for Cosmeceuticals: A Review. Mar Drugs. 2017;15(143):1-18.

15. Liang J, Pei X, Zhang Z, Wang N, Wang J, Li Y. The Protective Effects of Long-Term Oral Administration of Marine Collagen Hydrolysate from Chum Salmon on Collagen Matrix Homeostasis in the Chronological Aged Skin of Sprague-Dawley Male Rats. J Food Sci. 2010;75(8):H230-H8.

16. Miranda RBd, Weimer P, Rossi RC. Effects of Hydrolized Collagen Supplementation on Skin Aging: a Systematic Review and Meta-analysis. Int J Dermatol. 2021;60(12):1449-61.

17. Aguirre-Cruz G, Leon-Lopez A, Cruz-Gomez V, Jimenez-Alvarado R, Aguirre-Alvarez G. Collagen Hydrolysates for Skin Protection: Oral Administration and Topical Formulation. Antioxidants (Basel). 2020;9(2).

18. Kang MC, Yumnam S, Kim SY. Oral Intake of Collagen Peptide Attenuates Ultraviolet B Irradiation-Induced Skin Dehydration In Vivo by Regulating Hyaluronic Acid Synthesis. Int J Mol Sci. 2018;19(11).

19. 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(10).

20. Kumar MH, Spandana V, Poonam T. Extraction and Determination of Collagen Peptide and Its Clinical Importance from Tilapia Fish Scales (*Oreochromis niloticus*). Int Res J Pharm. 2011;2(10):97-9.

21. 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(1):67-70.

22. Duteil L, Queille-Roussel C, Maubert Y, Esdaile J. Specific Natural Bioactive Type 1 Collagen Peptides Oral Intake Reverse Skin Aging Signs in Mature Women. J Aging Res Clin Practice. 2016;5(2):84-92.

23. 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(12):4077-81.

24. Duteil L, Roussel CQ, Bruno-Bonnet C, Lacour JP. Effect of Low Dose Type I Fish Collagen Peptides Combined or not with Silicon on Skin Aging Signs in Mature Women. JOJ Case Stud. 2018;6(4):001-5.

25. Kim D-U, Chung H-C, Choi J, Sakai Y, Lee B-Y. Oral Intake of Low-Molecular-Weight Collagen Peptide Improves Hydration, Elasticity, and Wrinkling in Human Skin: A Randomized. Double-Blind, Placebo-Controlled Study. Nutrients. 2018;10(826):1-13.

26. Koizumi S, Inoue N, Shimizu M, Kwon C-j, Kim H-y, Park KS. Effects of Dietary Supplementation with Fish Scales-Derived Collagen Peptides on Skin Parameters and Condition: A Randomized, Placebo-Controlles, Double-Blind Study. Int J Pept Res Ther. 2018;24(3):397-402.

27. Evans M, Lewis ED, Zakaria N, Pelipyagina T, Guthrie N. A Randomized, Triple-blind, Placebo-controlled, Parallel Study to Evaluate the Efficacy of a Freshwater Marine Collagen on Skin Wrinkles and Elasticity. J Cosmet Dermatol. 2020;20(3):825-34.

28. Jung K, Kim SH, Joo KM, Lim SH, Shin JH, Roh J, et al. Oral Intake of Enzymatically Decomposed AP Collagen Peptides Improves Skin Moisture and Ceramide and Natural Moisturizing Factor Contents in the Stratum Corneum. Nutrients. 2021;13(12).

29. Miyanaga M, Uchiyama T, Motoyama A, Ochiai N, Ueda O, Ogo M. Oral Supplementation of Collagen Peptides Improves Skin Hydration by Increasing the Natural Moisturizing Factor Content in the Stratum Corneum: A Randomized,

Double-Blind, Placebo-Controlled Clinical Trial. Skin Pharmacol Physiol. 2021;34(3):115-27.

30. 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 Dermatol Treat. 2021;32(8):991-6.

31. Tak YJ, Shin DK, Kim AH, Kim JI, Lee YL, Ko HC, et al. Effect of Collagen Tripeptide and Adjusting for Climate Change on Skin Hydration in Middle-Aged Women: A Randomized, Double-Blind, Placebo-Controlled Trial. Front Med (Lausanne). 2021;7:608903.

32. Jafari H, Lista A, Siekapen MM, Ghaffari-Bohlouli P, Nie L, Alimoradi H, et al. Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. Polymers (Basel). 2020;12(10).

33. Tylingo R, Mania S, Panek A, Piatek R, Pawlowicz R. Isolation and Characterization of Acid Soluble Collagen from the Skin of African Catfish (*Clarias gariepinus*), Salmon (*Salmo salar*) and Baltic Cod (*Gadus morhua*). J Biotechnol Biomater. 2016;6(2):1-6.

34. Silva TH, Moreira-Silva J, Marques AL, Domingues A, Bayon Y, Reis RL. Marine origin collagens and its potential applications. Mar Drugs. 2014;12(12):5881-901.

35. Schneider J, Ferira AJ. Liquid Collagen vs. Powder: Experts Explain Which Form is More Effective 2022 [Available from: https://www.mindbodygreen.com/articles/liquid-collagen-vs-powder.

36. Cate. Liquid Collagen vs Powder: The Complete Comparison Guide 2022 [Available from: https://cbsupplements.com/cc/liquid-collagen-vs-powder/.

37. Cate. Collagen powder vs Pills: The Complete Comparison Guide. 2022.

38. Al-Atif H. Collagen Supplements for Aging and Wrinkles: A Paradigm Shift in the Fields of Dermatology and Cosmetics. Dermatol Pract Concept. 2022;12(1):e2022018.

39. Choi FD, Sung CT, Juhasz ML, Mesinkovsk NA. Oral Collagen Supplementation: A Systematic Review of Dermatological Applications. J Drugs Dermatol. 2019;18(1):9-16.

40. Ohara H, Ichikawa S, Matsumoto H, Akiyama M, Fujimoto N, Kobayashi T, et al. Collagen-derived dipeptide, proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. J Dermatol. 2010;37(4):330-8.

41. Barati M, Jabbari M, Navekar R, Farahmand F, Zeinalian R, Salehi-Sahlabadi A, et al. Collagen supplementation for skin health: A mechanistic systematic review. J Cosmet Dermatol. 2020;19(11):2820-9.

42. Yamamoto K, Igawa K, Sugimoto K, Yoshizawa Y, Yanagiguchi K, Ikeda T, et al. Biological safety of fish (tilapia) collagen. Biomed Res Int. 2014;2014:630757.