Original Contribution

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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

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Summary

Background Skin dryness and an accelerated fragmentation of the collagen network in the dermis are hallmarks of skin aging. Nutrition is a key factor influencing skin health and consequently its appearance. A wide range of dietary supplements is offered to improve skin health. Collagen peptides are used as a bioactive ingredient in nutricosmetic products and have been shown in preclinical studies to improve skin barrier function, to induce the synthesis of collagen and hyaluronic acid, and to promote fibroblast growth and migration. Our aim was to investigate the effect of oral supplementation with specific collagen peptides on skin hydration and the dermal collagen network in a clinical setting.

Methods Two placebo-controlled clinical trials were run to assess the effect of a daily oral supplementation with collagen peptides on skin hydration by corneometry, on collagen density by high-resolution ultrasound and on collagen fragmentation by reflectance confocal microscopy. Human skin explants were used to study extracellular matrix components in the presence of collagen peptides *ex vivo*.

Results Oral collagen peptide supplementation significantly increased skin hydration after 8 weeks of intake. The collagen density in the dermis significantly increased and the fragmentation of the dermal collagen network significantly decreased already after 4 weeks of supplementation. Both effects persisted after 12 weeks. *Ex vivo* experiments demonstrated that collagen peptides induce collagen as well as glycosaminoglycan production, offering a mechanistic explanation for the observed clinical effects.

Conclusion The oral supplementation with collagen peptides is efficacious to improve hallmarks of skin aging.

Keywords: collagen peptides, fragmentation, anti-aging, reflectance confocal microscopy, glycosaminoglycan, hyaluronic acid

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Introduction

Nutrition is known to have a major influence on skin physiology. Thus, a good nutritional status is reflected in good skin health perceived as a surrogate for the person's well-being. A wide range of nutritional supplements have reported to exert benefits on skin health

claiming to lead to a more youthful appearance.[1] Unfortunately, the scientific evidence for those claims is often preliminary.[2]

While young skin is firm, smooth and of radiant appearance, profound changes occur in the structure of the dermis and epidermis over time through processes of intrinsic and extrinsic aging. The collagen density of the dermis decreases with age and is associated with a reduction in dermal thickness.[3] The dermal collagen network becomes increasingly fragmented presenting shorter and less organized fibers and accumulating degraded collagen fragments.[4] An increase in matrix metalloproteinase (MMP) expression accounts for the accelerated collagen degradation.[5] In parallel, the synthesis of new extracellular matrix components by dermal fibroblasts slows down, failing to adequately replace the degraded matrix.[6] The elastic fibers of the papillary dermis loose integrity during aging and reach less far into the dermal–epidermal junction. This overall loss in elasticity and strength leads to sagging and wrinkling.[7,8] The amount of hyaluronic acid, which is abundant in both epidermis and dermis, decreases with age.[9] This is reflected in the reduced capacity to retain moisture, leading to the typically dry skin of aging people and most importantly impairing the epidermal barrier function.[10]

Collagen peptides are natural bioactive ingredients used in many nutricosmetic products, orally taken nutritional supplements which provide skin health and beauty benefits. Collagen peptides present as a mix of specific peptides of different length with high abundance of the amino acids hydroxyproline, glycine, and proline, which are produced by enzymatic hydrolysis of native collagen extracted from animal connective tissues. Hydroxyproline is unique to collagen and can be used analytically to differentiate collagen from other proteins. Collagen peptides are efficiently digested into di- and tripeptides, which are resistant to further intracellular hydrolysis.[11] The peptides are transported across the intestinal mucosa by the transporter PEPT-1.[12] In humans, hydroxyproline containing di- and tripeptides have been shown to appear one hour after ingestion of collagen peptides at nanomolar concentrations in the blood.[13] Investigations using radioactively labeled collagen peptides have demonstrated that the absorbed peptides reach the skin[14] and are retained in the tissue for up to 2 weeks.[15]

A growing body of evidence demonstrates the efficacy of collagen peptides to improve parameters of skin physiology in preclinical studies. Collagen peptides were shown to increase hyaluronic acid production in dermal fibroblasts[16,17] and to improve skin barrier function by increasing the water content of the stratum corneum.[16,18,19] Further, collagen peptides induce the synthesis of collagen on the mRNA and protein level[20,21] as well as the production of stronger collagen fibrils,[22] promote growth of skin fibroblasts,[23] and induce fibroblast migration.[24,25] In contrast, clinical evidence for the efficacy of collagen peptides on human skin is still scarce.[26–30]

Therefore, we have conducted two clinical trials showing the increase of skin hydration and the quantitative and qualitative improvement of the collagen dermal network upon oral supplementation of collagen peptides. Investigations in human *ex vivo* skin biopsies demonstrated that collagen peptides induce collagen as well as hyaluronic acid production. To the best of our knowledge, this is the first report of clinical evidence for the efficacy of collagen peptides to improve the dermal collagen fragmentation and thus to counteract a hallmark of skin aging.

Materials and methods

Test products

The studies were performed using specific collagen peptides of fish origin (Peptan®F) and porcine origin (Peptan®P) with an average molecular weight of 2000–5000 Da. All products were provided by Rousselot.

Clinical study 1

A minimized, placebo-controlled, parallel-group, double-blind, monocentric study was performed in 2008 at SOUKEN Laboratories in Tokyo, Japan. The protocol was conducted according to the guidelines of Good Clinical Practice and the Helsinki declaration. All participants gave their informed consent.

Participants were selected from the SOUKEN laboratory database according to the following eligibility criteria: Japanese women, 40–59 years old, low skin water content, not pregnant, no systemic disease, no intolerance to fish or gluten, no medications, and no food supplements. Sixty women were screened, and 33 were allocated to either a placebo (dextrin) or one of two treatment (Peptan®F [fish] or Peptan®P [porcine]) groups minimizing for variation in skin water content. Participants took a formulated drink at bedtime which contained either 10 g placebo or 10 g Peptan. Treatment took place on 56 consecutive days. Assessment of facial skin parameters was performed at baseline and after 4 and 8 weeks of treatment in a controlled environment with a room temperature of 22 ± 1 °C

and a relative humidity of 50 ± 10% after 30 min of acclimatization. Skin moisture level was measured by conductance with a Corneometer® device (CK electronic, Köln, Germany) at three different application points per subject. The rate of transepidermal water loss (TEWL) was assessed with a Tewameter® device (CK electronic) on the line between the ear lobe and mouth edge at 3 cm distance from the earlobe.

Clinical study 2

A randomized, placebo-controlled, parallel-group, double-blind, monocentric study was performed in 2012 at COSderma Laboratories in Bordeaux, France. The protocol was approved by a French Ethics Committee following the guidelines of the French authorities (AFSSAPS). The study was performed according to the guidelines of Good Clinical Practice and the Helsinki declaration. All participants gave their informed consent.

Participants were selected from the COSderma laboratory database according to the following eligibility criteria: Caucasian women, 40–65 years old, BMI of 18–25 kg/m^2, Fitzpatrick phototype I–IV, all skin types, not pregnant, no systemic disease, no intolerance to fish or gluten, no medications, no food supplements, no changes in dietary habits or hormonal treatment in the last 3 months, no beauty care in the last month, and no extensive sun exposure just before or during the study. One hundred and six women were randomly allocated to either a placebo (maltodextrin) or a treatment (Peptan®F) group. Participants took a formulated powder drink in the morning before breakfast which contained either 10 g placebo or 10 g Peptan. Treatment took place on 84 consecutive days. Assessment of skin parameters was performed at baseline and after 4 and 12 weeks of treatment in a controlled environment with a room temperature of 20 ± 2 °C and a relative humidity of 45 ± 5% after 20 min of acclimatization. Single measurements were performed at the forearm with the same location dedicated to one type of measurement for the duration of the study. Collagen density of the dermis was measured by high-frequency real-time ultrasound of the skin with a Dermcup® device (Atys Medical, Soucieu en Jarrest, France). The echogenic collagen and elastic fibers of the dermis were visualized in a vertical cross section. The echogenicity was quantified by visual scoring on a 10-point scale. The fragmentation of collagen in the reticular dermis was assessed by reflectance confocal microscopy using a Vivascope3000® device (MAVIG GmbH, München, Germany) which provides horizontal optical cross sections of ≤5 μm of the epidermis and the dermis with a maximal depth of 200 μm visualizing the cellular microstructure. The image of the stack positioned 25 μm below the papillary dermis provides information on the fragmentation of the reticular dermis. The images were quantified with the Software ConfoScan (NIDEK Technologies Srl, Padova, Italy). Briefly, the collagen fibers were identified by binarization within a defined region of interest with the same threshold used for all time points per subject. The fragmentation was calculated as the number of objects/average object surface.

Ex vivo experiments

Human skin explants with an average diameter of 10 mm were prepared from a thighplasty sample of a 49-year-old Caucasian woman. Explants were kept in culture at 37 °C and 5% CO_2 in BIO-EC's Explants Medium containing 5% serum for 9 days and incubated with 0.01, 0.1, or 1 mg/mL specific collagen peptides of fish origin (Peptan®F). Experiments were performed in triplicates. Culture medium containing the product was renewed on day 2, 5, and 7. On day 9, the explants were harvested, fixed in Bouin solution, dehydrated, and embedded in paraffin. Sections of 5 μm were prepared, mounted on silanized glass slides, and submitted to different stainings. For the observation of general skin morphology, slides were stained with Masson's Trichrome, Goldner variant. Acidic glycosaminoglycans were stained with Alcian Blue and total collagen with picro-Sirius Red. Microscopic observations were made using a Leica DMLB microscope at a 20× magnification. Ten pictures were taken of each sample of the triplicate with a DP 72 Olympus camera (Olympus Life Science, Hamburg, Germany). For each condition, 15 representative pictures were selected and quantified with the Olympus Cell^D software (Olympus Life Science).

Statistical analysis

Statistical analysis was performed with spss version 17.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 5.04 (GraphPad Software, La Jolla, CA, USA). Normality was tested with the Shapiro–Wilk test. Homogeneity between groups at baseline was tested by anova. Differences intragroup over time and between treatment groups per time point were calculated by anova or ancova with a *post hoc* analysis using the Dunnett's test. Statistical significance was considered when $P < 0.05$.

Results

Peptan improves skin hydration in humans

Aging of the skin is associated with a decrease in water content. In a first clinical pilot study, we evaluated the effect of oral Peptan intake on skin hydration. Thirty-three women were distributed equally on a placebo and two treatment groups by minimizing for variation in skin water content (Fig. 1). The participants took 10 g of placebo or Peptan of fish (Peptan®F) or porcine (Peptan®P) origin orally per day for a duration of 8 weeks. All participants finished the study according to the protocol and no adverse events were reported.

Two parameters of skin hydration were assessed, skin moisture level measured as a function of conductance and TEWL. While there was no change in the placebo group, the oral intake of Peptan F over 8 weeks led to a significant increase of skin moisture level by 12% (Fig. 2). Peptan P significantly increased skin moisture level by 16% already after 4 weeks of treatment and as much as 28% after 8 weeks ($P = 0.003$ vs placebo at 8 weeks). TEWL was not different between both Peptan groups and the placebo (data not shown). Thus, the oral intake of Peptan seems to effectively improve skin hydration without affecting TEWL.

Peptan increases collagen density in the human dermis

To gain extended insight on the efficacy of Peptan to improve skin aging parameters, we performed a randomized, placebo-controlled, double-blind trial. One hundred and six women were screened for eligibility and randomly allocated to take 10 g placebo ($n = 52$) or Peptan ($n = 54$) per day as a flavored powder drink for a duration of 12 weeks. One hundred and one participants completed the study according to the protocol, with 18 subjects (placebo $n = 7$, Peptan $n = 11$) presenting minor deviations from the protocol regarding the product administration (Fig. 3). No adverse events were reported. Three subjects were withdrawn from the placebo group, two on their own request and one for noncompliance. Two subjects were lost to follow-up from the Peptan group. Reflectance confocal microscopy data on collagen fragmentation was not

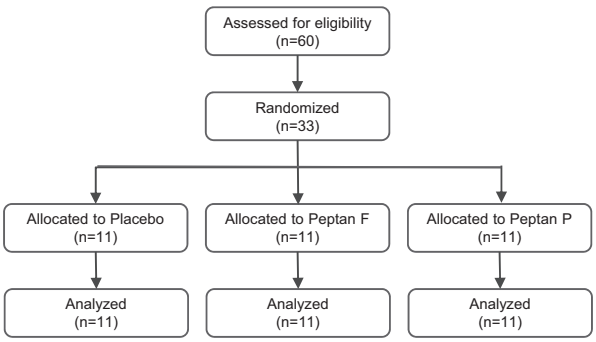


Figure 1 Flowchart of the clinical pilot study performed at SOUKEN laboratories, Japan.

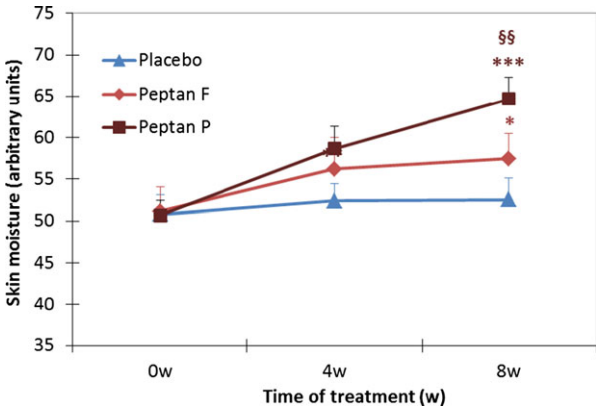


Figure 2 Effect of the daily oral intake of 10 g Peptan compared to placebo on skin hydration determined by Corneometer in women ($n = 11$/group). Values are presented as mean ± SEM. Statistical differences were calculated by ANOVA and Dunnett's *post hoc* test with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; *comparison with baseline, § comparison with placebo.

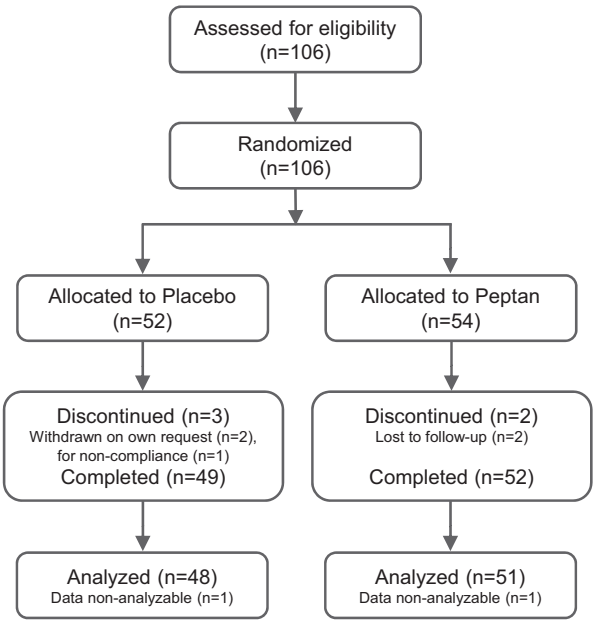


Figure 3 Flowchart of the clinical study performed at COSderma laboratories, France.

Table 1 Baseline characteristics of women randomly allocated to the placebo and the Peptan group. Data are presented as means (SD) or numbers (%).

	Placebo (n = 49)	Peptan (n = 52)
Age (year)	52.4 (2.6)	54.8 (2.6)
Phototype (Fitzpatrick)		
I	0	2 (4%)
II	19 (39%)	18 (35%)
III	29 (60%)	31 (60%)
IV	1 (2%)	1 (2%)
Skin type		
Normal	13 (27%)	13 (25%)
Dry mixed	0	1 (2%)
Dry	36 (73%)	38 (73%)

analyzable for one subject in each group. Hence, the results from 48 subjects of the placebo group and of 51 subjects of the Peptan group are presented below. As shown in Table 1, there were no differences between both groups at baseline regarding age, the distribution of phototype as well as skin type.

A major structural skin parameter that is altered during aging is the density of the collagen layer in the dermis. We evaluated the effect of Peptan intake on this parameter by measuring echogenicity using high-frequency ultrasound. Echogenicity of the dermis did not change in the placebo group during the course of the study. The oral intake of Peptan resulted in a highly significant increase of the dermal echogenicity as early as 4 weeks after the start of the treatment when compared to the baseline value and this effect persisted after 12 weeks of treatment, indicating a consistent increase in collagen density in the dermis over time. After 12 weeks of Peptan intake, the treatment effect (12w-0w) was significantly higher than in the placebo group (8.83% vs. no change, respectively, $P = 0.007$) (Fig. 4).

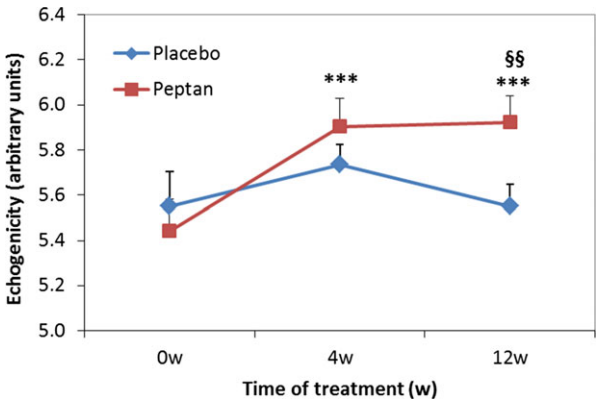


Figure 4 Effect of the daily oral intake of 10 g Peptan compared to placebo on collagen density in the dermis (n = 48–51) determined by high-frequency ultrasound. Values are presented as mean ± SEM. Statistical differences were calculated by ANOVA and Dunnett's *post hoc* test with $^{**}P < 0.01$, $^{***}P < 0.001$; *comparison with baseline, §comparison with placebo.

Peptan reduces collagen fragmentation in the human dermis

During aging, not only the amount of collagen changes in skin but also the quality of the collagen network. Therefore, in the same clinical study (Fig. 3), we investigated the degree of fragmentation of collagen fibers in the reticular dermis using reflectance confocal microscopy by Vivascope. This state-of-the-art technique delivers a stack of confocal images over a stretch of 150 µm from the stratum corneum down with collagen fibers appearing as white structures.

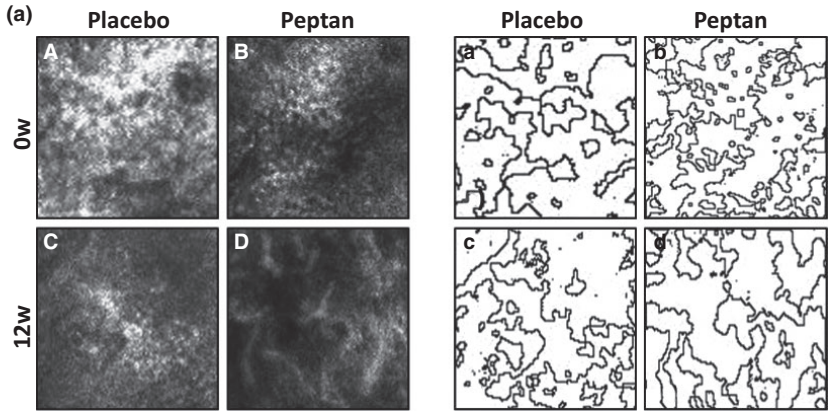
Figure 5a shows exemplary images of the reticular dermis of two subjects at baseline and 12 weeks after the oral intake of placebo or Peptan, respectively. At 12 weeks, the epidermis of the subject taking placebo (Fig. 5a.C) clearly exhibited smaller collagen structures than at baseline (Fig. 5a.A). In contrast, the subject taking Peptan displayed larger collagen fragments after 12 weeks of intake (Fig. 5a.D) compared to baseline (Fig. 5a.B). Thus, the collagen fibers were less fragmented after Peptan intake compared to placebo.

The quantitative analysis of the confocal images confirmed that the collagen fragmentation in the reticular dermis did not change under placebo intake (Fig. 5b). In contrast, intake of Peptan significantly reduced the fragmentation by 17.8% already at 4 weeks and even further, by 31.2%, at 12 weeks. These data clearly demonstrate the efficacy of Peptan to reduce the fragmentation of the dermal collagen layer.

Peptan increases collagen and glycosaminoglycan content in human skin explants

The above presented data show that oral intake of Peptan in humans improves skin hydration and the quality and quantity of the dermal collagen network. To better understand how Peptan exerts these actions, we analyzed the general morphology, glycosaminoglycan, and collagen content of human skin explants in response to different concentrations of Peptan.

The general morphology of the skin explants was not affected by the incubation with Peptan. Both control and Peptan-treated explants exhibited a slight



(b)

	0 weeks	4 weeks			12 weeks		
Fragmentation			Δ vs 0w	p		Δ vs 0w	p
Placebo	12.4 (1.9)	13.4 (1.7)	+8.0%	0.929	13.1 (1.8)	+5.9%	0.933
Peptan	18.8 (2.4)§	15.5 (2.9)	-17.8%	0.036*	12.9 (2.4)	-31.2%	0.001**

Figure 5 Effect of the daily oral intake of 10 g Peptan compared to placebo on the collagen network fragmentation of the reticular dermis (n = 48–51). (a) Exemplary confocal images showing the collagen network of the reticular dermis before and after 12 weeks of daily oral intake of 10 g Peptan compared to placebo. (b) Quantification. Values are presented as mean (SEM). Statistical differences were calculated by ANOVA and Dunnett's *post hoc* test with *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; *comparison with baseline, § comparison with placebo.

parakeratosis, no evidence of stimulation in the epidermis, and a well-cellularized collagen network in the papillary dermis (data not shown).

As depicted in Figure 6, the glycosaminoglycan level in the basal epidermis increased in skin explants in response to Peptan incubation, visible as blue staining in the histological slides (Fig. 6a). The quantification of the images (Fig. 6b) shows that this increase was dose-dependent within the range of 0.01–0.1 mg/mL Peptan with a fivefold and a highly significant 18-fold increase, respectively. At 1 mg/mL Peptan, saturation of the effect was reached with a significant 17-fold increase compared to the control.

In line with the glycosaminoglycan content, Sirius Red staining of human skin explants revealed that the collagen content of the papillary dermis increased in response to incubation with Peptan (Fig. 7a). This increase was dose-dependent with 3% more collagen in the presence of 0.01 mg/mL Peptan and a significant maximal increase of 9% in the presence of 0.1 mg/mL Peptan (Fig. 7b). Again, saturation of the effect was reached at 1 mg/mL Peptan with a significant increase of 5.4%. Given the high basal amount of collagen in the papillary dermis, a 5% increase is considered to be a clear biological difference.

Thus, Peptan is highly efficacious to increase the amount of water-binding glycosaminoglycans and the collagen content of human skin. These effects might underlie the increase in skin moisture (Fig. 2) and collagen density (Fig. 4) observed in the clinical studies.

Discussion

Collagen peptides are a bioactive ingredient used in functional foods and dietary supplements, such as nutricosmetics, to help improve parameters of skin physiology. In the present study, we report that the oral supplementation with specific collagen peptides (Peptan®) improves skin hydration as well as the density and the structure of the collagen network of the dermis. To our knowledge, this is the first clinical study showing an effect of collagen peptides on dermal collagen fragmentation. Analysis of human skin explants cultured *ex vivo* in the presence of specific collagen peptides (Peptan(R)) indicated that an increase in dermal collagen and epidermal GAG content might be responsible for the observed physiological effects.

The properties of skin are defined by the complex architecture of its different layers, which are increasingly degraded during aging. Skin contains up to 70% of collagen, which provides the tissue with tensile strength. However, the collagen content declines with age and this process is accelerated in women by the

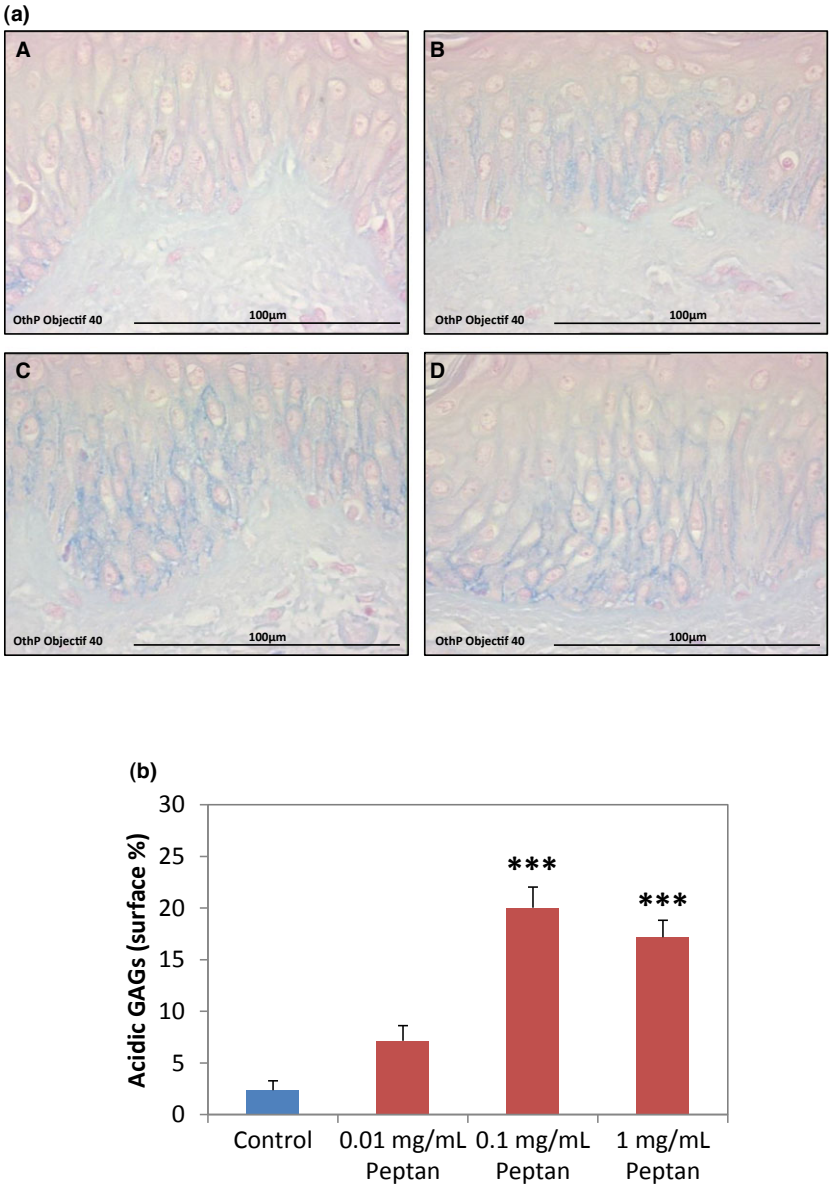


Figure 6 Glycosaminoglycan (GAG) content of the basal epidermis in human skin explants (a) visualized by Alcian Blue staining after incubation with (A) control, (B) 0.01 mg/mL Peptan, (C) 0.1 mg/mL Peptan, (D) 1 mg/mL Peptan for 9 days (representative images). (b) GAG content was quantified using Olympus Cell^{D} Software and expressed as surface %. Values are presented as mean ± SEM. Statistical differences were calculated by ANOVA and Dunnett's *post hoc* test with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

hormonal changes of the menopause.[31] In the first 4 years of menopause, the synthesis of collagen decreases by up to 30%.[32] A weakening of the dermis measured as decreased echogenicity has been related to increased skin wrinkling.[33] We found that oral supplementation with fish collagen peptides (Peptan®F) in women increased the collagen density in the dermis by 9%, as measured by high-frequency ultrasound. In addition, collagen peptides (Peptan®F) dose-dependently increased the amount of collagen in the dermis of human skin explants by up to 5% which can be considered an important biological increase in light of the high basal amount of collagen in the papillary dermis. This finding strongly indicates that collagen peptides strengthen the dermis by inducing collagen synthesis and might thereby reduce skin wrinkling. Our results are in line with two recent studies that have directly shown an induction of collagen synthesis

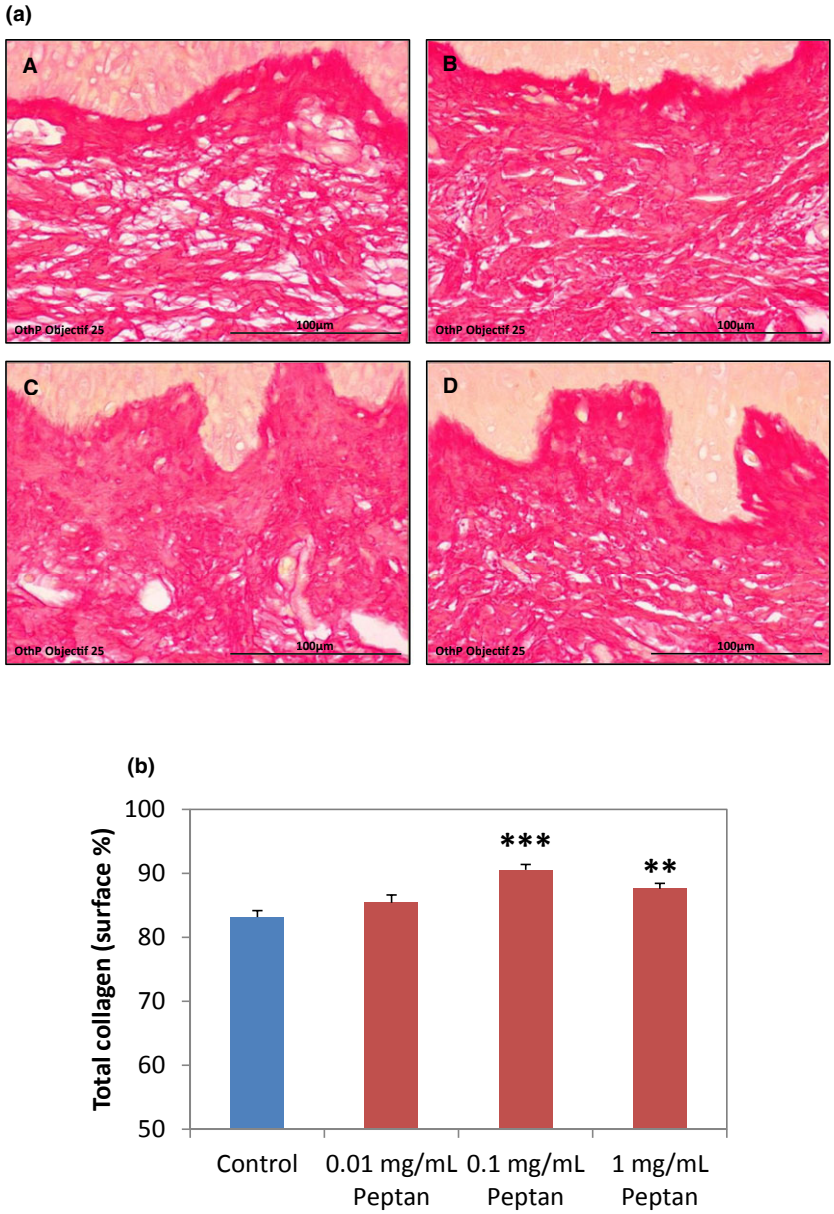


Figure 7 Total collagen content of the papillary dermis of human skin explants (a) visualized by Sirius Red staining after incubation with (A) control, (B) 0.01 mg/mL Peptan, (C) 0.1 mg/mL Peptan, (D) 1 mg/mL Peptan for 9 days (representative images). (b) Total collagen content was quantified using Olympus Cell^D Software and expressed as surface %. Values are presented as mean ± SEM. Statistical differences were calculated by ANOVA and Dunnett's *post hoc* test with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

in response to oral collagen peptide administration. Zague *et al.*[21] reported that bovine collagen peptide feeding to rats increased collagen type I and IV protein content in the skin. Liang *et al.*[20] could link an induction of collagen type I and III mRNA and protein levels upon fish collagen peptide feeding to an increased dermal thickness and hydroxyproline content in rats. Further, porcine collagen peptides were demonstrated to increase the diameter of collagen fibrils in the skin of pigs.[22] Recently, a pilot trial using a fish collagen peptide (Peptan®F) containing formulation showed that collagen density increased after 12 weeks of collagen peptide treatment.[26] Another clinical trial has detected an increase of procollagen and elastin in skin lymph fluid after 8 weeks of oral porcine collagen peptide supplementation indicating an induction of extracellular

matrix synthesis.[27] However, no data were assessed regarding structural changes in the dermal collagen network.

During skin aging, the fragmentation of the dermal collagen network by MMPs has been described to have major consequences not only for the skin's structure but also for the microenvironment of the dermal fibroblasts.[34] The cells detach from the loosening and increasingly disorganized network of shorter and thinner collagen fibers and lack the mechanical stimulus which is a key factor to induce collagen synthesis.[4,32] The induction of different MMPs has been described to be linked to collagen fragmentation.[5] In a model of fibroblasts cultured in a collagen lattice, MMP1 digestion mimicked the negative effect of collagen fragmentation on fibroblast function described for aged skin *in vivo.*[35] In the above reported clinical study, we used a state-of-the-art technology, reflectance confocal microscopy, to assess the effect of oral fish collagen peptide (Peptan®F) supplementation on dermal collagen fragmentation in human skin. Collagen peptide supplementation significantly decreased the fragmentation of the collagen in the reticular dermis. To our knowledge, this is the first clinical evidence for such an anti-aging effect. One previous investigation demonstrated that fish collagen peptide feeding to rats could decrease the collagen fragmentation in skin in accordance with our results.[20] This effect was linked to a reduced expression of MMP1 and an induction of its inhibitor TIMP1.

The water content of the skin depends on the cutaneous evaporation rate and the hydration level of the epidermis, contributing to maintain skin health and a youthful appearance.[7] In the above presented clinical trial, we observed a significant increase in skin moisture level after porcine and fish collagen peptide (Peptan®) supplementation when compared to placebo. However, the TEWL, a measure of stratum corneum integrity, was not affected. As the presence of water-binding glycosaminoglycans, mainly hyaluronic acid, determines the hydration level of skin, we assessed the effect of collagen peptides on glycosaminoglycans in the epidermis of human skin explants. Collagen peptides (Peptan®F) very clearly and significantly increased the amount of glycosaminoglycans in the epidermis in a dose-dependent manner suggesting that an increase in glycosaminoglycan synthesis is responsible for the moisturizing effect. In line with our results, fish collagen peptides in combination with glucosamine and vitamin C have been reported to increase skin moisture and elasticity after 6 weeks in women with dry skin in a noncontrolled study.[30] Another trial showed a dose-dependent increase of skin moisture but not TEWL after fish or porcine collagen peptide supplementation for 4 weeks, comparable to our findings.[29] Interestingly, a recent open-label study with a formulation containing 5 g fish collagen (Peptan®F) reported an improvement in skin dryness and face wrinkles, indicating that a dose of 5 g of collagen peptides might be effective.[26] A recent clinical trial failed to see an effect of porcine collagen peptide supplementation on skin hydration or TEWL after 8 weeks; however, the baseline values of skin hydration were rather low and some of the administered doses were very weak (2.5 g/day).[28] A set of *in vivo* studies further demonstrated that fish collagen peptides and collagen peptide-derived dipeptides could prevent the decrease in water content and the increase in TEWL in mouse models of photoaging[16,18,19] with one study making the link to an increase of the dermal hyaluronic acid content.[16] This proposed mechanism of action is further substantiated by data from an investigation studying the effect of proline–hydroxyproline, one of the major dipeptides present in human blood after collagen peptide ingestion, on human dermal fibroblast function.[36] An increase in hyaluronic acid secretion was observed in response to proline–hydroxyproline, which was linked to an increased expression of hyaluronic acid synthase (HAS)2, the key enzyme of hyaluronic acid synthesis.[17] A link between induced HAS expression and improved skin hydration in humans has been recently established.[37]

The exact mechanisms of action whereby collagen peptides modulate fibroblast function is poorly characterized to date, but certain peptide sequences within the collagen peptides may be specifically recognized by the cell membrane of the fibroblast. A number of studies have been performed on other cell types such as osteoblasts, osteoclasts,[38] and chondrocytes, which originate from the same stem cell line and share certain characteristics, such as the capacity to produce collagen. Further, the dipeptides proline–hydroxyproline and hydroxyproline–glycine, which are abundant in the circulation after collagen peptide ingestion, might contain a certain level of bioactivity in fibroblasts.[17,18]

In conclusion, we provide clinical evidence for the efficacy of specific collagen peptides (Peptan®) to improve skin moisture and, for the first time, to prevent and reduce the fragmentation of the dermal collagen network, thus counteracting one of the hallmarks of skin aging. The improvement of those physiological skin parameters is likely linked to an increase of collagen and glycosaminoglycan synthesis in the respective

skin layers. These data demonstrate that the oral supplementation with specific collagen peptides can improve skin structure and health from within.

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