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Supplementating with Dietary Astaxanthin Combined with Collagen Hydrolysate Improves Facial Elasticity and Decreases Matrix Metalloproteinase-1 and -12 Expression: A Comparative Study with Placebo

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ABSTRACT Photoaging accounts for most age-related changes in skin appearance. It has been suggested that both astaxanthin, a potent antioxidant, and collagen hydrolysate can be used as antiaging modalities in photoaged skin. However, there is no clinical study using astaxanthin combined with collagen hydrolysate. We investigated the effects of using a combination of dietary astaxanthin and collagen hydrolysate supplementation on moderately photoaged skin in humans. A total of 44 healthy subjects were recruited and treated with astaxanthin (2 mg/day) combined with collagen hydrolysate (3 g/day) or placebos, which were identical in appearance and taste to the active supplementation for 12 weeks. The elasticity and hydration properties of facial skin were evaluated using noninvasive objective devices. In addition, we also evaluated the expression of procollagen type I, fibrillin-1, matrix metalloproteinase-1 (MMP-1) and -12, and ultraviolet (UV)-induced DNA damage in artificially UV-irradiated buttock skin before and after treatment. The supplement group showed significant improvements in skin elasticity and transepidermal water loss in photoaged facial skin after 12 weeks compared with the placebo group. In the supplement group, expression of procollagen type I mRNA increased and expression of MMP-1 and -12 mRNA decreased compared with those in the placebo group. In contrast, there was no significant difference in UV-induced DNA damage between groups. These results demonstrate that dietary astaxanthin combined with collagen hydrolysate can improve elasticity and barrier integrity in photoaged human facial skin, and such treatment is well tolerated.

KEY WORDS: • ***anti-aging*** • ***astaxanthin*** • ***collagen hydrolysate*** • ***photoaging***

INTRODUCTION

PHOTOAGING IS CAUSED BY the superpositioning of chronic ultraviolet (UV)-induced damage on the intrinsic aging process and accounts for the majority of age-associated changes in skin appearance. Aged skin, especially photoaged skin, manifests as a decrease of skin thickness and elasticity, skin dryness, epidermal barrier dysfunction, and changes in pigmentation.[1] Reactive oxygen species (ROS) likely contribute to this process.[2]

UV radiation has numerous direct and indirect effects on the skin.[2] The indirect damage induced by UV irradiation is suggested to be initiated by ROS,[2] which are involved in connective tissue alterations.[3] Numerous antioxidants have been tested for their ability to prevent or reverse clinical signs associated with photoaging secondary to ROS. Strategies utilizing endogenous skin antioxidants as well as plant-derived or synthetic compounds have been examined.[2]

Although both topical and systemic application of antioxidants can significantly increase antioxidant levels in skin, systemically applied supplements and topical agents are often combined to enhance efficacy through their synergistic affects.[4] Furthermore, the systemic application of antioxidants has produced more pronounced and sustained effects than topical application.[5] Thus, antioxidants are one of the most popular categories of nutraceutical ingredients used to improve skin health. Among the various antioxidants, astaxanthin has a long history of use as an antioxidant dietary supplement[6] and its antioxidant properties can be 10-fold greater compared with other carotenoids, such as lutein and β-carotene, and 100-fold greater compared with α-tocopherol.[7]

Collagen hydrolysate is also a popular nutraceutical, and collagen polypeptides have exhibited numerous bioactivities, including antioxidant activity, mineral binding capacity,

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antihypertensive activity, lipid-lowering effects, and immunomodulatory activity.[8–10] Collagen hydrolysate has also been shown to be involved in the synthesis of the extracellular matrix and is used for improving pathological conditions involving the joints, nails, and hair.[11] In the skin aging process, collagen hydrolysate might be beneficial for slowing chronological aging[11] and photoaging[8] in rats.

When used as nutraceuticals, antioxidants and collagen hydrolysate have different mechanisms of action, but might show additive or synergistic effects for preventing or reversing the skin aging process. Therefore, we investigated the effects of a dietary antioxidant, astaxanthin, combined with collagen hydrolysate on skin aging and UV-induced damage in human skin *in vivo*.

MATERIALS AND METHODS

We conducted a 12-week, randomized, double-blind placebo-controlled study to evaluate the effects of dietary astaxanthin and collagen hydrolysate supplementation on cutaneous aging. This study was approved by the Institutional Review Board of Seoul National University Hospital, and written informed consent was obtained from all subjects participating in the trial.

Study participants

A total of 44 healthy female volunteers, aged ≥40 years and who had wrinkles ≥grade 2,[12] were enrolled in the study. The subject exclusion criteria for the present trial were as follows: (1) received medical or cosmetic treatment that interferes with the general aging process within 3 months of the study; (2) reported taking functional foods within 1 month of the study; (3) history of acute or chronic disease such as severe liver or kidney disease or uncontrolled diabetes; (4) history of allergies against any component of trial foods; (5) any visible skin disease that might be confused with a skin reaction to the test procedure or materials used, or interfere with clinical measurements; and (6) abnormal blood test results (hemoglobin, hematocrit, aspartate aminotransferase [AST], alanine transferase [ALT], or fasting glucose).

Dietary supplement

Two types of oral dosage forms (capsules containing astaxanthin and tablets containing fish collagen) were prepared. The capsules were manufactured by Cerebos Pacific Limited (Singapore), and each capsule included 480 mg of medium chain triglycerides and 20 mg of dark red lipid extract of *Haematococcus pluvialis* microalgae, standardized with safflower oil to yield a minimum 5% content of astaxanthin (Cyanotech Corporation, Kailua-Kona, HI, USA; 1 mg of astaxanthin per capsule). The tablets were manufactured by Cerebos Pacific Limited (Singapore), and each tablet contained 0.75 g of enzymatic hydrolyzed fish collagen (Rousselot SAS, Puteaux, France). Identical placebo capsules with only medium chain triglycerides (500 mg per capsule) and tablets with hydrolyzed casein (0.75 g per tablet) instead of hydrolyzed collagen were prepared as controls. All 44 study participants were randomly assigned to either the placebo group or the supplement group. Each participant took two capsules of astaxanthin and four tablets of hydrolyzed collagen or the control capsules and tablets per day for 12 weeks.

Noninvasive assessment

Clinical assessments were performed at baseline and at 4 and 12 weeks of treatment. Skin elasticity on the cheek (at point 3 cm inferior to the lower eyelid) was measured using a Cutometer MPA580 (C+K Electronic, Cologne, Germany). Hydration of facial skin was evaluated on the cheek (at point 5 cm inferior to the outer corner of eye) measured using a Corneometer and a Tewameter (C+K Electronic). All measurements were performed in a room with a constant temperature of 20–25°C and a constant humidity of 45–55% at the Clinical Research Institute, Seoul National University Hospital.

Compliance and safety

Adverse events related to the medications and clinical protocol were evaluated at 4 and 12 weeks of treatment. The subjects were instructed to return any remaining capsules or tablets to the investigators, and subjects who failed to take ≥80% of the study medications were disqualified from further participation. Blood samples were drawn at baseline and 12 weeks after the start of treatment, and AST, ALT, glucose, hemoglobin levels, and hematocrits were measured.

UV irradiation and skin biopsy samples

Two 2-mm skin biopsy samples were obtained taken from the buttock area at 24 h following UV irradiation at baseline and after 12 weeks of supplementation in subjects who agreed to biopsies ($n = 6$/group). For these subjects, skin on the buttock area was irradiated with two minimal erythema doses at baseline and the same UV doses again at 12 weeks of study participation. The UV source was a Waldmann UV-800 (Waldmann, Villingen-Schwenningen, Germany; 285–350 nm, peak at 310–315 nm) phototherapy device fitted with Philips TL-20W/12 fluorescent lamps.

Specimens for immunohistochemical staining were immediately oriented in a low-temperature embedding medium (Tissue-Tek OCT compound; Miles, Naperville, IL, USA), frozen in liquid nitrogen, and stored at −70°C. Skin samples saved for RT-PCR analysis were frozen in liquid nitrogen.

Immunohistochemical staining

DNA damage induced by UV irradiation was evaluated by immunohistochemical staining for the presence of thymine dimers and 8-OHdG (8-hydroxy-2-deoxyguanosine), as previously described.[13]

Quantitative real-time RT-PCR

Expressions of procollagen type I, fibrillin-1, matrix metalloproteinase (MMP)-1, and MMP-12 were evaluated using quantitative real-time PCR. Total RNA was prepared from skin samples and converted to complementary DNA

Table 1. Baseline Demographics and Baseline Values of Key Parameters

	Control group (n = 22)	*Treatment group* (n = 22)	P *value*[a]
Age (years)	50.6 ± 5.3	51.5 ± 5.2	0.580
Weight (kg)	57.5 ± 6.5	55.3 ± 6.4	0.361
Epidermal hydration	44.5 ± 13.2	46.0 ± 14.0	0.573
TEWL (g/h/m^2)	9.2 ± 2.8	10.5 ± 3.8	0.162
Gross elasticity, R2	0.6446 ± 0.0675	0.6097 ± 0.0556	0.044
Net elasticity, R5	0.5244 ± 0.0953	0.4799 ± 0.1076	0.146
Biological elasticity, R7	0.3028 ± 0.0502	0.2813 ± 0.0501	0.136

[a]By Mann–Whitney *U* test.
TEWL, transepidermal water loss.

using the First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania). Quantitation of procollagen type I, MMP-1, and -12 cDNA, and endogenous reference 36B4 was performed using a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (Takara Bio, Inc., Shiga, Japan) using the primers for human genes listed in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/jmf). The comparative C_T method[14] was used to quantify relative changes in gene expression.

Statistical analyses

The Mann–Whitney U test was used to identify differences in values obtained by noninvasive measurements between two groups (R2, R5, and R7 values; Corneometer values; transepidermal water loss [TEWL] values). In addition, the treatment effects at weeks 4 and 12 were further compared using analysis of covariance (ANCOVA) to adjust for corresponding baseline values. The changes from baseline values for expression of mRNAs for procollagen type I, fibrillin-1, MMP-1, and MMP-12 were analyzed using the Mann–Whitney U test. The SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for all analyses. A P value $< .05$ was considered statistically significant.

RESULTS

Subject demographics

This study enrolled 44 Korean women between the ages of 41 and 60 years (mean 51.0 years, SD ± 5.2 years). Baseline values for most parameters were not significantly different between groups (Table 1). However, despite randomization, baseline values for gross elasticity (R2 by Cutometer) were significantly different between groups.

Skin elasticity as measured by Cutometer

An improvement in skin elasticity began to be noticeable in the supplement group at 4 weeks and was more prominent at 12 weeks. After 12 weeks of treatment, the mean improvement from baseline in elasticity was significantly greater for the supplement group vs. the placebo group (R2, 0.0252 vs. −0.0294, $P = .035$; R5, 0.0602 vs. −0.0195, $P = .020$; R7 0.0222 vs. −00185, $P = .012$, Fig. 1). No improvements in any parameters representing elasticity (R2, R5, R7) were noted in the placebo group. Additionally, the main parameters representing skin elasticity (R2, R5, R7) significantly improved in the supplement group compared with those in the placebo group when adjusted for the baseline corresponding values using ANCOVA (Supplementary Table S2).

Epidermal hydration by Corneometer and TEWL (barrier integrity) by Tewameter

Figure 2 shows the changes in epidermal hydration parameters as measured by Corneometer after 4 and 12 weeks

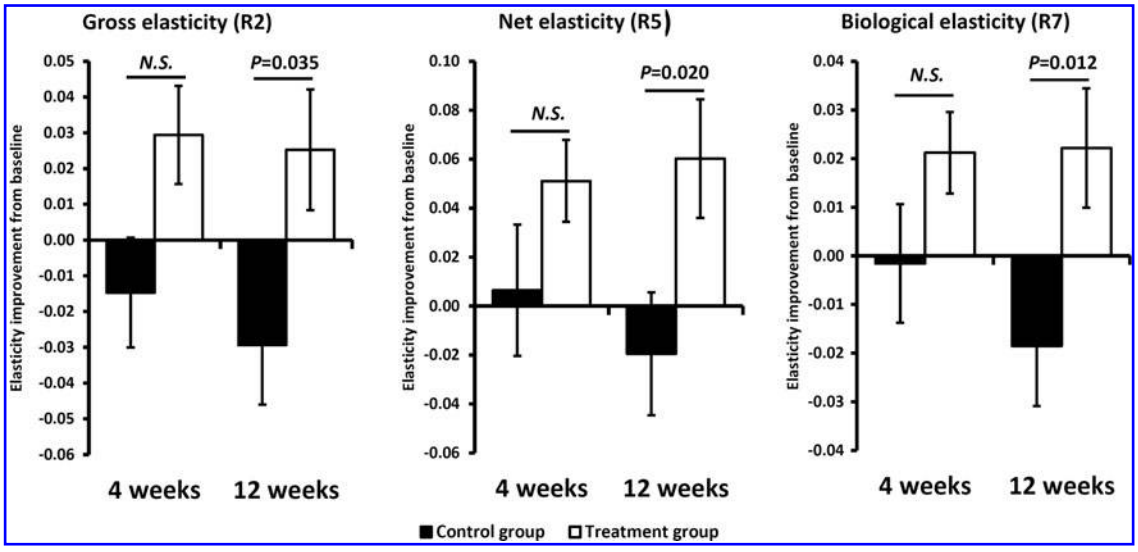

FIG. 1. Results of skin elasticity measurements using Cutometer. Subjects receiving supplements showed significant improvements in the three viscoelastic parameters compared with subjects in the control group. *P* values by Mann–Whitney U test.

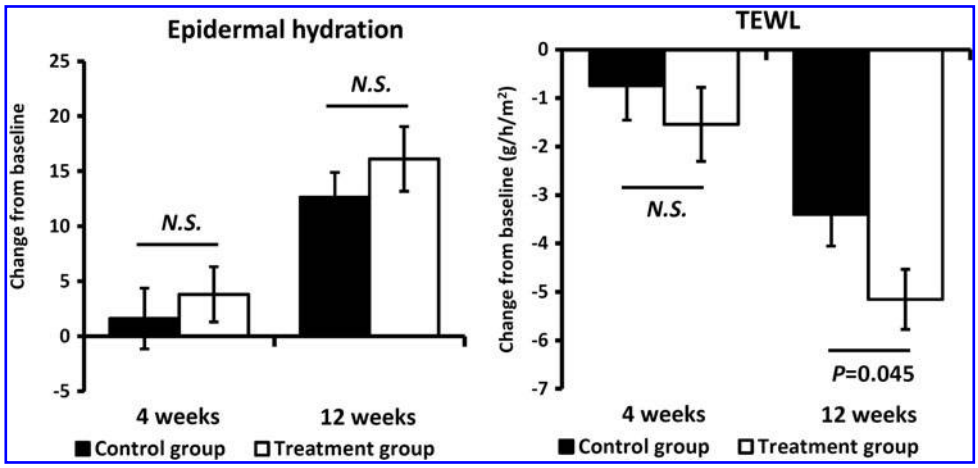

FIG. 2. Results of epidermal hydration measured using Corneometer, and skin barrier integrity measured with Tewameter. TEWL from facial skin in the supplement group was significantly lower than those in the placebo group at 12 weeks. *P* values by Mann–Whiney U test. TEWL, transepidermal water loss.

of treatment. Epidermal hydration was not significantly different between the two groups after 4 and 12 weeks. The stratum corneum barrier in both groups was significantly improved with TEWL decreasing from baseline values by 3.4 g/h/m^2 in the control group and 5.2 g/h/m^2 in the supplement group at 12 weeks. This difference in changes between the two groups after 12 weeks of treatment was statistically significant ($P = .045$ by Mann–Whitney U test, Fig. 2) and remained significant after controlling for the corresponding baseline TEWL values ($P = .048$ by ANCOVA, Supplementary Table S3).

Safety and compliance

Treatment was well tolerated and no subjective adverse events were reported during the 12-week trial period. Laboratory evaluations revealed no significant abnormalities after 12 weeks of treatment. One subject who took only 64.3% of the supplements was dropped from the study, according to the study protocol.

Real-time RT-PCR

Supplementing with astaxanthin combined with collagen hydrolysate induced a 3.4-fold increase in procollagen mRNA levels in UV-irradiated skin compared with those in the placebo group ($P = .038$ by Mann–Whitney *U* test). Induction of fibrillin-1 mRNA in the supplement group was greater than in the placebo group, but the difference was not statistically significant. After UV irradiation, expression of MMP-1 (collagenase) and MMP-12 (elastase) mRNA was suppressed by 68% ($P = .027$) and 77% ($P = .050$), respectively, in the supplement group compared with those expressions in the placebo group (Fig. 3).

UV-induced DNA damage

There were no differences between groups in immunohistochemical analyses of thymine dimers and 8-OHdG after UV irradiation (Fig. 4).

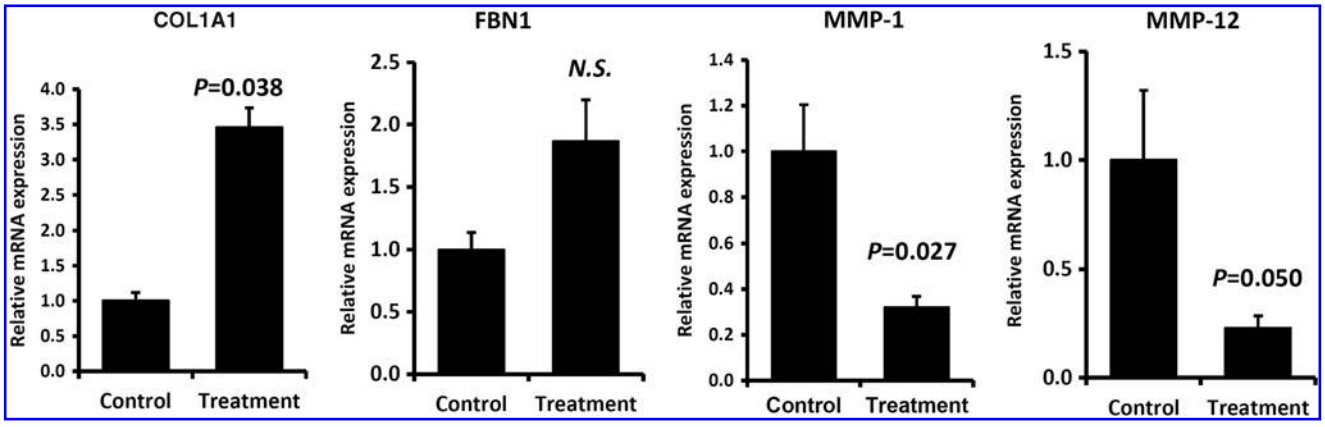

FIG. 3. Results of procollagen type I, fibrillin-1, and matrix metalloproteinase-1 (MMP-1) and -12 mRNA induction after UV radiation. While levels of procollagen type I mRNA in the supplement group increased, levels of MMP-1 and -12 mRNA in the supplement group decreased significantly compared with those in the placebo group. Expression of mRNA was measured by real-time RT-PCR ($n = 6$ for each group). *P* values by Mann–Whiney U test. UV, ultraviolet.

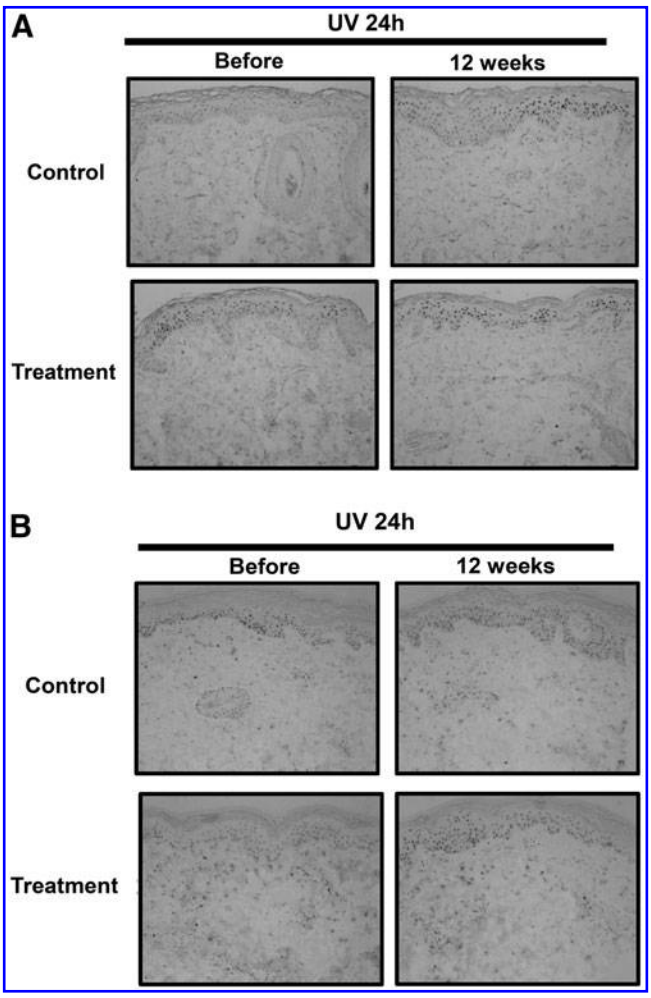

FIG. 4. **(A)** Thymine dimer immunostaining before and after supplementation. Nuclear staining of thymine dimer in UV-irradiated buttock skin 24 h after UV irradiation. The figures are representative images from six subjects (original magnification ×200). **(B)** 8-OHdG immunostaining before and after supplementation. Nuclear staining of 8-OHdG in UV-irradiated buttock skin 24 h after UV irradiation. Figures are representative images from six subjects (original magnification ×200). 8-OHdG, 8-hydroxy-2-deoxyguanosine.

DISCUSSION

The latest trend in antiaging strategies for skin is to use a combination of dietary and oral supplements to produce an appearance benefit. These methods of treatment are thought to work synergistically with topical agents to enhance efficacy.[4] In the antiaging market, there are many nutraceuticals, which are products derived from food sources and provide extra physiologic benefits in addition to their basic nutritional values.[15] Recently, astaxanthin and collagen hydrolysate have been described as beauty foods, which have the potential to prevent skin aging.[4,16]

We evaluated the effects of dietary supplementation with the antioxidant astaxanthin combined with collagen hydrolysate on facial skin elasticity, hydration, and dermal matrix homeostasis in photoaged skin. We found that 12 weeks of oral dosing with astaxanthin plus collagen hydrolysate improved elasticity and epidermal integrity in photoaged facial skin. Furthermore, the induction of procollagen type I mRNA was observed after 12 weeks of treatment, along with significant decreases in the expression of collagen-degrading enzyme, MMP-1 mRNA and elastin-degrading enzyme, MMP-12 mRNA after UV irradiation. The improvements in facial skin elasticity might be related to these molecular changes.

Astaxanthin (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) is one of the pigments that belongs to the xanthophyll subclass of carotenoids and is widely distributed in marine organisms.[6] Astaxanthin has been used as a food supplement ever since experimental studies revealed its antioxidant properties.[6] Further studies have suggested that astaxanthin has health-promoting benefits for the treatment and prevention of various diseases such as diabetes, cardiovascular diseases, and inflammatory diseases.[17,18] *In vitro*, astaxanthin effectively suppresses cell damage by free radicals and induction of MMP-1 in skin after UV irradiation.[19,20] Additionally, topical administration of astaxanthin prevented UV-induced skin damage in mice.[21] These studies suggest that astaxanthin may prevent tissue damage caused by UV irradiation.

Collagens are the most common family of proteins in the human body and have been used as dietary supplements for promoting articular function and for cosmetic purposes. Ingestion of collagen peptide induces increased fibroblast density and enhances formation of collagen fibrils in the dermis in a protein-specific manner.[22] Collagen hydrolysate has been demonstrated to have protective effects on chronological skin aging by its influence on collagen matrix homeostasis in rats.[11] The proposed mechanisms of action for collagen polypeptide mainly involve enhancing immunity, reducing the loss of moisture and lipids, promoting antioxidative activity, and repairing endogenous collagen and elastin protein fibers.[8]

Consistent with these previous reports, oral dosing with astaxanthin and collagen hydrolysate improved elasticity in photoaged facial skin and suppressed UV-induced MMP-1 and -12 expression in human skin *in vivo*. However, despite the known antioxidant properties of astaxanthin, DNA damage caused by endogenous or exogenous ROS, as measured by 8-OHdG staining, did not significantly differ between groups in this study. Nevertheless, the suppression of MMP expression in the supplement group suggests that oral supplementation with astaxanthin and collagen hydrolysate partially protects skin from UV-induced damage.

In addition to the improved viscoelastic property of skin, which is mainly due to the dermal extracellular matrix, we also found improved barrier integrity as represented by decreased TEWL. Because collagen hydrolysate itself has good moisture absorption and retention properties,[8] the improvement of skin hydration might be related to the effects of collagen hydrolysate. In addition, astaxanthin might contribute to the improvement in TEWL by protecting the keratinocyte differentiation and cornification from oxidative damages such as inflammation in epidermis.[23]

This study has some limitations. First, we did not prove the concentrations of individual ingredients in the skin.

Previous studies only measured the serum levels of collagen or astaxanthin. Ninety-five percent of enterally administered collagen hydrolysate is absorbed within the first 12 h as 2.5–15 kDa peptides.[24] Major collagen peptides in serum and plasma were identified as proline-hydroxyproline; this was identical to the abundance motif reported for collagen when healthy human volunteers ingested several food-derived collagen peptides.[25] In humans, the absorption of astaxanthin after 4 h ranges from 6% to 34%.[26] It should be elucidated whether the concentration of astaxanthin or collagen-derived peptide in skin is great enough to exhibit relevant biologic activity. Second, we did not examine changes in glycosaminoglycans, including hyaluronic acid in the skin. Ohara *et al.* reported that collagen-derived dipeptide, which was detected in circulation after collagen ingestion, stimulated proliferation and hyaluronic acid synthesis in cultured dermal fibroblasts.[27] Because hyaluronic acid is important for tissue elasticity and hydration,[28] the improvement of elasticity might be related to hyaluronic acid in the dermis.

Despite these limitations, the present study shows that a combination of astaxanthin and collagen hydrolysate improves elasticity and barrier integrity in human skin *in vivo*. Skin elasticity especially began to improve after 4 weeks of supplementation, and the effect was maintained with continued supplementation for 12 weeks. Further studies should focus on the underlying mechanism that produces an improvement in skin conditions after dietary supplementation with a combination of astaxanthin and collagen hydrolysate.

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AUTHOR DISCLOSURE STATEMENT

The authors declare they have no conflicts of interest.

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