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# Ingestion of Salmon Nasal Cartilage-Derived Proteoglycan Improves Skin Condition: A Randomized, Double-Blind, Controlled Study

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**Abstract:**

*Background:* Proteoglycan is one of the components of the extracellular matrix with various biological activities and has been used as the functional foods to improve knee joint health or skin condition.

*Objective:* To examine the effect of ingestion of salmon (*Oncorhynchus keta*, Salmonidae) nasal cartilage-derived proteoglycan (sPG) on skin condition, we performed a randomized, double-blind, controlled study in healthy adult volunteers.

*Methods:* Recruited subjects (n=156) were men and women, ages 21-62 years. From this population, we selected 19 subjects based on the exclusion criteria of the guidelines for evaluation of cosmetic functions of Japanese Cosmetic Science Society. Subjects were randomly divided into an sPG group (n=10; mean age, 39.1 years) and a placebo group (n=9; mean age, 39.6 years). The characteristics of the sPG used in this study were assessed by HPLC and electrophoresis analysis.

*Results:* The safety was confirmed by the monitoring of all volunteer subjects for the development of adverse reactions. We found no negative information on the safety of proteoglycan ingestion and no evidence for an interaction between proteoglycan and other functional foods/medicine in several different databases. Viscoelasticity and recovery after deformation as the skin elasticity increased significantly in the sPG group compared to the placebo group ($p<0.05$). Skin looseness significantly decreased in the sPG group ($p<0.05$). Moreover, the number of wrinkles, conspicuous or darkened facial pores, and blotches significantly decreased in the sPG group ($p<0.05$). Measurements of skin conductance showed that sPG improved skin moisture and micrographs of facial corneocytes showed that sPG improved rough skin.

*Conclusion:* Our results suggest the potential of sPG as a food ingredient to improve human skin condition, including skin elasticity, wrinkles, facial pores, blotches, moisture, and smoothness.

**Keywords:** Blotch, elasticity, facial corneocytes, facial pore, looseness, proteoglycan, salmon nasal cartilage, wrinkle.

## INTRODUCTION

Proteoglycan (PG) from salmon nasal cartilage is commonly ingested as a traditional food in Japan. The PG in salmon nasal cartilage is considered to be an excellent source of nutrition; however, it has been difficult to isolate with high purity. There are two methods for extraction of PG from the animal cartilage [1, 2]. One is the guanidine hydrochloride method, with which PG can be extracted from bovine tracheal cartilage or salmon nasal cartilage by guanidine hydrochloride denaturation [3]. However, with this method, maintenance of PG structure, complex formation of proteinglycan, and biological activity may be compromised. The other method is the acetic acid method. Recently, we established industrial methods for obtaining highly purified PG from salmon nasal cartilage using acetic acid [4]. This method improved the purification problem and decreased the cost. Using this salmon-derived PG (sPG) obtained with the acetic acid method, we are now able to perform a detailed analysis of its biological and functional effects.

Although PG is one of the major components of articular cartilage, brain, and blood vessels, it is present on the cell surface and in the extracellular matrix (ECM). The ECM consists of PG, collagen, fibronectin, laminin, and hyaluronan, and it regulates the extracellular niche *in vivo*. PGs are macromolecules with a core protein to which one or more glycosaminoglycan (GAG) side chains are covalently linked [5, 6]. Recently, the PG structure has been studied in detail; one or more GAG side chains are covalently linked to specific Ser residues within core protein *via* a common linkage tetrasaccharide (glucuronic acid-β1-3-galactose-β1-3-galactose-β1-4-xylose-β1) [7]. It has also been reported that sPG contains an EGF-like domain on the COOH-terminal side of the core protein and performs an EGF-like activity [8]. On the other hand, GAGs are strongly hydrophilic, so they retain many water molecules in the ECM.

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Although the structure-related function of PG is starting to become known, the effects of ingested PG remain unclear. In contrast, some studies of oral administration of glycosaminoglycan revealed its biological activities regarding cartilage aging and maintenance of joint health [9]. Very recently, Kawada *et al*. reported that ingestion of hyaluronans improved dry skin conditions [10]. These previous reports strongly suggested that ingested PG could possess functional activities. Thus, we focused on the effects of PG ingestion on skin condition.

Skin is the largest organ of the body and acts to regulate body temperature, water, and lipid stores. Furthermore, its functions are important for avoidance environmental stresses, including UV light exposure, heat, injury, and infection [11]. Reduced skin function is often observed in aged skin, which is regulated by hormones and somewhat similar to some chronic skin diseases, such as atopic dermatitis. In aged skin, retraction of the dermis and epidermis has been observed. The retraction is caused by the physiological changes of decreasing collagen and lipid content, loss of fibroblasts and mast cells, loosening of the collagen fiber matrix, and less supplementation of nutrition. To prevent and improve these changes, topical application of cosmetics is generally used. In addition, ingested nutrition and functional foods have been suggested to improve skin condition [12]. In fact, ingested small molecules, such as vitamin E, carotenoids, polyphenols, vitamin C, Se and Zn can distribute throughout all skin compartments by absorption through the gastrointestinal tract [13]. Recently, improvement of skin condition by ingestion of a particular nutrient or functional foods was confirmed in a randomized, double-blind, controlled study [10, 14, 15].

We performed a randomized, double-blind, controlled study to evaluate the effect of sPG ingestion on skin condition and showed its ability to improve skin condition, especially skin elasticity. This suggests an anti-aging effect of sPG. Thus, our results suggest the potential of sPG as a food ingredient. To investigate these interesting consequences, we have planned further research, including an assessment of the effects of metabolites after sPG ingestion on skin.

## MATERIALS AND METHODS

### Product

Salmon-derived proteoglycan (sPG) was extracted from salmon (*Oncorhynchus keta*, Salmonidae) nasal cartilage slices as previously reported [1]. Briefly, frozen (-20 $^{o}$C) salmon nasal slices were dissolved in a solution of 4% acetic acid, and sPG was extracted. We performed HPLC analysis (TSKgel G5000PWXL column, Tosoh Corporation, Tokyo, Japan) and differential refractive index detection by RID-10A (SHIMADZU, Kyoto, Japan) as quantitative and qualitative analyses, and the purity of sPG was determined as >99%. To compare sPG with other glycosaminoglycans, hyaluronic acid, chondroitin sulfate, heparan sulfate, and heparin, cellulose acetate electrophoresis analysis was performed with proteoglycan (WAKO, Tokyo, Japan) as a standard and the Acid Mucopolysaccharide kit (AMPS KIT, Seikagaku Corporation, Tokyo, Japan) as previously described [1].

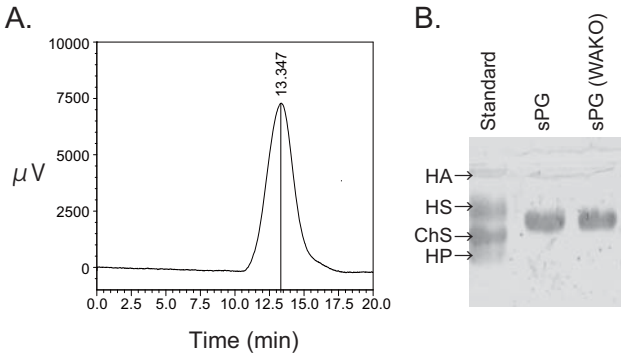


**Fig. (1). Characteristics of salmon nasal cartilage-derived proteoglycan (sPG).**
HPLC analysis for sPG (A). Comparison of sPG with other glycosaminoglycans, hyaluronic acid (HA), chondroitin sulfate (ChS), heparin sulfate (HS) and heparin (HP), and standard proteoglycan (WAKO) by cellulose acetate electrophoresis (B).

The characteristics of the PG used in this study are shown in Fig. (**1A**) for HPLC analysis and Fig. (**1B**) for electrophoretic analysis.

### Subjects

The subjects were selected from over 156 recruited candidates. We selected 19 healthy volunteers based on the exclusion criteria of the guidelines for evaluation of cosmetic functions (Japanese Cosmetic Science Society) [16] as follows:

1) Those with a past history of allergy to cosmetics

2) Those undergoing hormone replacement therapy

3) Women who are pregnant or breast-feeding

4) Those who have undergone medical skin treatments that could affect the testing area

5) Others whose participation is deemed inappropriate by a doctor.

Subjects were fully informed of the detailed procedures of the study before giving their written consent and entering the study, which was conducted in accordance with the principles of the Declaration of Helsinki and our institutional review board. Subjects were randomly divided in 2 groups: there were 10 subjects in the sPG group (27-53 years of age; mean age, 39.1 years) and 10 subjects in the placebo group (26-58 years; mean age, 39.6 years). One subject in the placebo group was excluded from this study due to conscious repeating lack of sleep. Then, 9 subjects in the placebo group were finally selected. This study was performed from Feb.1, 2010 to Feb. 14, 2010. Baseline characteristics are shown in Table **1**. The subjects in the sPG group ingested a capsule containing 5 mg sPG with dextrin powder, and the subjects in the placebo group ingested a capsule containing only dextrin powder each day for 2 weeks without dietary restriction During the 2 weeks, the use of other dietary supplements were prohibited.

Before measurements were made in this study, all subjects washed their faces and then stayed in the testing room,

**Table 1. Baseline characteristics of the study subjects.**

| Classification | sPG | Placebo |
|---|---|---|
| Number of subjects | 10 | 9 |
| Mean age (range), y | 39.1 (27 -53) | 39.6 (26 -58) |
| Sex (number) | Male (5) | Male (5) |
| | Female (5) | Female (4) |
| Skin elasticity | | |
| R8 | 0.89 ± 0.00 | 0.89 ± 0.01 |
| R6 | 0.78 ± 0.07 | 0.85 ± 0.07 |
| Wrinkle number | 8.5 ± 1.5 | 10.4 ± 0.75 |
| Facial pore number | | |
| Conspicuous | 1832.9 ± 188.4 | 1656.4 ± 125.0 |
| Darkened | 1341.8 ± 154.9 | 1275.6 ± 129.9 |
| Opened | 217.0 ± 40.6 | 217.8 ± 33.1 |
| Blotch number | 67.5 ± 8.3 | 79.6 ± 13.8 |
| Skin moisture | | |
| Conductance | 93.3 ± 25.9 | 90.8 ± 23.2 |
| Facial corneum | | |
| Score | 2.8 ± 0.1 | 3.1 ± 0.2 |

which was maintained at 19.7 °C ± 0.1 and 46.8% ± 1.5 humidity, for 20 min for acclimation.

### Skin Elasticity

Skin elasticity measurements based on the suction method were performed with the CUTOMETER SEM 575 (COURAGE + KHAZAKA Electronic GMbH Corp, Germany) as described previously [17]. Skin elasticity parameters R0 (firmness), R2 (gross elasticity), R5 (net elasticity), R6 (recovery after deformation), R7 (portion of elasticity), and R8 (skin viscoelasticity) were determined pre-ingestion and after two weeks of ingestion.

Skin loosening was assessed under our laboratory protocol. Subjects were stamped on their cheek with their face turned to the front and lying on their face at baseline. Ellipse axis length was measured. Images were analyzed using Image-Pro PLUS (NIPPON ROPER K.K., Japan).

### Wrinkles, Pore Condition, and Blotch Measurements

The number of wrinkles underneath the eye, and the numbers of conspicuous pores, darkened pores, and opened pores on the cheek were measured using a Robo Skin Analyzer (MM and Niic Co., Ltd., Japan) as previously reported [18]. The number of blotches was measured with VISIA Evolution (Canfield Scientific, NJ) as previously reported [19]. Computerized image analysis of these parameters was performed in all subjects before and after two weeks of ingestion of sPG and placebo.

### Corneum Microgram and Analysis

Corneum cells were collected from the face with a Kakushitu-checker (Asahi Biomed, Japan). These cells were stained with a mixture of 0.5% gentian violet and 1.0% brilliant green, and observed microscopically as previously described [20]. Corneum cells were collected from all subjects, stained, and micrographs were created and stored. The condition of the corneum was scored on a five-category scale based on the observations of well-practiced researchers as previously reported [21].

### Skin Moisture

Skin water content was measured before ingestion and after two weeks of ingestion by high-frequency surface electrical conductance with a Skicon-200 (IBS, Japan) as previously reported [22].

### Statistical Analysis

For all skin condition parameters in this study, statistically significant differences between the sPG group and the placebo group or between baseline and after 2 weeks of ingestion were examined. Thompson' outlier test was performed only for conductance. The conductance value of one subject in the placebo group was over the critical region. Data of all skin parameters are expressed as mean ± SE. Student's t-test was used for direct comparison of the sPG and placebo groups, or baseline and after two weeks of ingestion. The hazard ratio for all examinations was indicated by a p value, and $p<0.05$ was determined as a significant difference.

## RESULTS

### Safety

Proteoglycan has been used in functional foods to improve knee joint health or skin condition. Furthermore, for over one thousand years, PG-containing salmon nasal cartilage itself has been ingested, as well as bovine cartilage and other animal cartilage, as foods traditionally consumed in the world. We found no negative information on the safety of proteoglycan ingestion in several databases, including the Natural Medicine Data Base (NMDB), PubMed Advanced Search Builder, Information System on Safety and Effectiveness of Health Foods of the National Institute of Health and Nutrition Japan, and Information of Food Safety of the National Institute of Health Science Japan. Furthermore, we found no evidence for an interaction between proteoglycan and other functional foods/medicine in two different databases, including the Information System on Safety and Effectiveness of Health Foods of the National Institute of Health and Nutrition Japan, and Information of Food Safety of the National Institute of Health Science Japan.

Previously, the safety of sPG was confirmed by a 90-day repeated-dose toxicity study in rats [4].

In a preliminary study, we also confirmed that there were no adverse reactions specific for sPG ingestion over five years in normal human volunteers (data not shown). Furthermore, in this study, we monitored all volunteer subjects for the development of adverse reactions, erythema, itching, sensation, dryness of skin, intestinal sickness, and other up-

set. No subject monitored by the investigator experienced these adverse reactions during this study.

### Skin Elasticity

Next, we examined skin elasticity, which was measured and analyzed by CUTOMETER. We used two parameters, R8 (skin viscoelasticity) and R6 (recovery after deformation), to examine the effect of sPG ingestion. An increasing value of R8 represents skin elasticity improvement, and a decreasing value of R6 represents improvement in recovery. The average value of these parameters in the sPG group and the placebo group at baseline is shown in Table **1**.

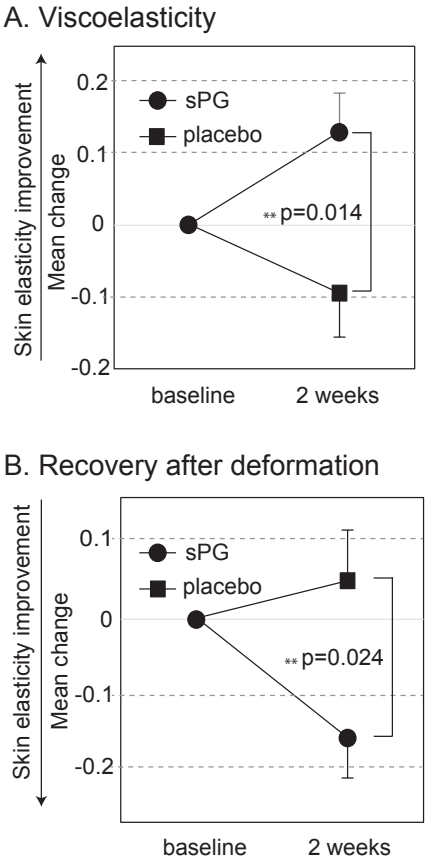


**Fig. (2). Effects of sPG on skin elasticity.**

Change in viscoelasticity (A) and recovery after deformation (B) can be regarded as skin elasticity, and was measured at baseline and after 2 weeks of daily ingestion of sPG or placebo. The circle represent the level for the sPG group and the square represent the level for the placebo group. An increasing value of viscoelasticity represents skin elasticity improvement (A). A decreasing value of recovery after deformation represents skin elasticity improvement (B). Data are expressed as the mean change ± SE (n=10 in the sPG group, n=9 in the placebo group).

Fig. (**2A**) shows the changes in values for the sPG and placebo groups. Ingestion of sPG significantly increased skin viscoelasticity (R8) from baseline (p=0.014). The average value of viscoelasticity changed from 0.89 ± 0.00 at baseline to 0.90 ± 0.01 after ingestion in the sPG group and from 0.89 ± 0.01 at baseline to 0.88 ± 0.01 after ingestion in the placebo group. In the placebo group, viscoelasticity was slightly decreased, but the change from baseline was not significant. The change from baseline for recovery after deformation (R6) is shown in Fig. (**2B**). Ingestion of sPG significantly decreased the value from baseline (p=0.024). Averages were changed from 0.78 ± 0.07 at baseline to 0.63 ± 0.09 after ingestion in the sPG group and from 0.85 ± 0.07 at baseline to 0.90 ± 0.10 after ingestion in the placebo group. Other parameters, R0 (firmness), R2 (gross elasticity), and R5 (net elasticity), were also tested and confirmed the improvement in skin condition after sPG ingestion (data not shown).

Analysis of skin looseness also confirmed the sPG-improved elasticity. A representative example from the sPG group is shown in Fig. (**3**). The subjects were stamped against their cheek with their face turned to the front (red circle) and with lying on their face (blue circle) before ingestion and after two weeks of ingestion. The smaller the gap between the red circle and the blue circle becomes, the greater the improvement in skin looseness. The proportion of ellipse axis length, which is shown as the blue circle in Fig. (**3B**), significantly approached 1 after two weeks of ingestion of sPG (p=0.031) (Fig. **3A**). In Fig. (**3B**), a representative subject in the sPG group is shown.

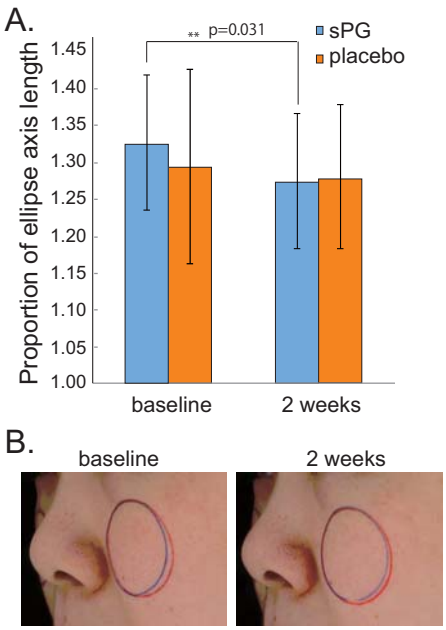


**Fig. (3). Effects of sPG on skin looseness.**

The skin looseness was measured at baseline and after 2 weeks of daily ingestion of sPG or placebo. The subjects were stamped on their cheek with their face turned to the front (red circle), and lying on their face (blue circle) at baseline and after 2 weeks of daily ingestion of sPG or placebo (B). The ratio of (length of red circle diameter) / (length of blue circle diameter) represents the proportion of ellipse axis length (A). The blue bars represent the level for the sPG group and the orange bars represent the level for the placebo group. Data are expressed as mean ± SE (n=10 in sPG group, n=9 in placebo group). A representative example of a subject in the sPG group is shown in B.

Together with these results, all of the parameters revealed improvement in skin elasticity with sPG ingestion.

### Facial Wrinkles, Pore Condition, and Blotches

The effects of sPG ingestion on wrinkles underneath the eye and on facial pore conditions on the cheek were exam-

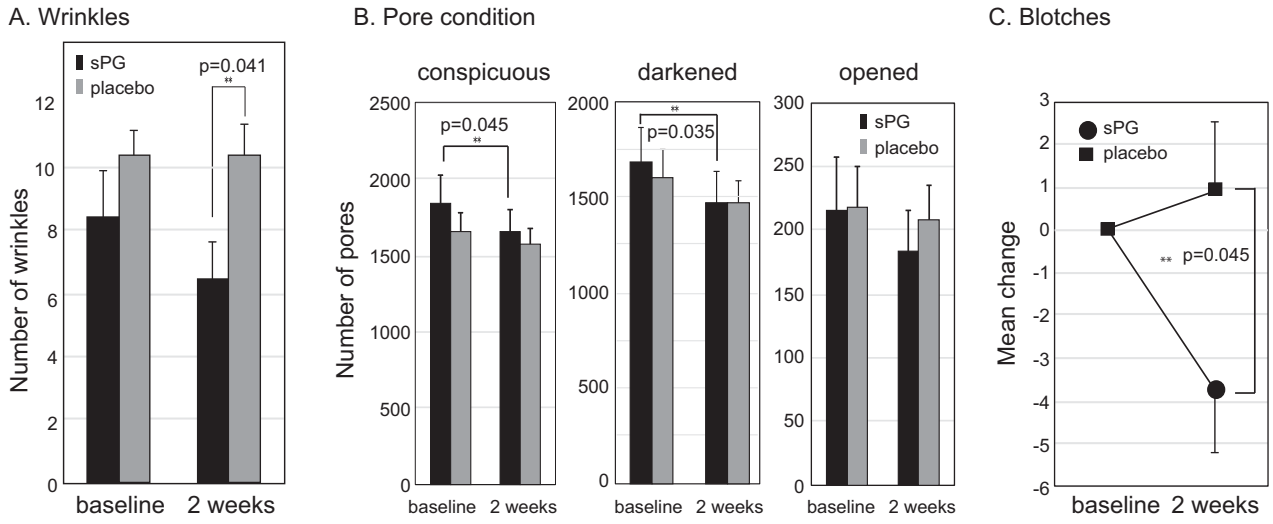


**Fig. (4). Effects of sPG on wrinkles, pore condition, and blotches.**

Number of wrinkles (A) and pore condition (B) which was determined as conspicuous (left), darkened (middle), and opened (right), were measured at baseline and after 2 weeks of daily ingestion of sPG or placebo. Changes in the number of blotches were measured at the same time (C). The black bars and circle represent the level for the sPG group, and the gray bars and square represent the level for the placebo group. Values are expressed as mean number ± SE for wrinkles and pores and mean change ± SE for blotches (n=10 in the sPG group, n=9 in the placebo group).

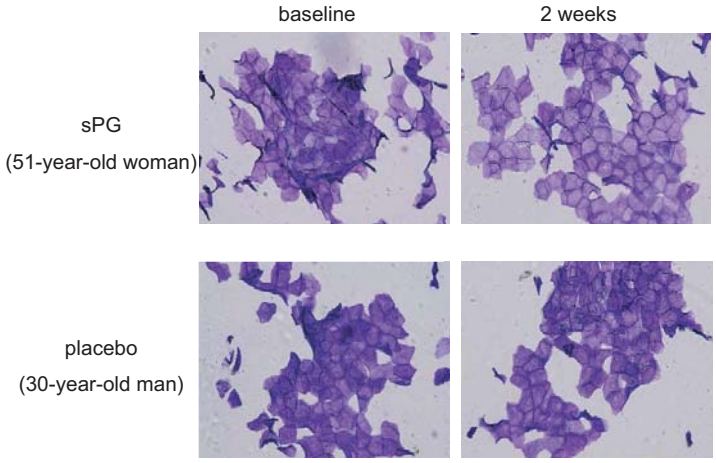


**Fig. (5). Effects of sPG on facial corneocytes.**

The corneocytes were collected and stained at baseline and after 2 weeks of daily ingestion of sPG or placebo. Representative micrographs of corneocytes of a 51-year-old woman in the sPG group and a 30-year-old man in the placebo group.

ined with a Robo Skin Analyzer. At the same time, blotches were also examined by VISIA Evolution. Analyses of all of these parameters were performed with computerized images. The average values of these parameters in the sPG group and the placebo group at baseline are shown in Table **1**.

The number of wrinkles underneath the eye was significantly reduced in the sPG group after ingestion compared with the placebo group (p=0.041), Fig. (**4A**).

The numbers of conspicuous pores and darkened pores were also significantly reduced from baseline after sPG ingestion (p=0.045 and p=0.035, respectively) (Fig. **4B**). The number of opened pores was slightly but not significantly reduced from baseline after sPG ingestion (p=0.073) (Fig. **4B**).

The change from baseline in number of blotches is shown in Fig. (**4C**). The change from baseline in number of blotches was significantly decreased after sPG ingestion (p=0.045). In the placebo group, no significant change from baseline was observed after ingestion (p=0.573). The average number of blotches changed from 67.5 ± 8.3 at baseline to 63.7 ± 8.0 after ingestion in the sPG group and from 79.6 ± 13.8 at baseline to 80.6 ± 13.7 after ingestion in the placebo group.

### Skin Moisture and Facial Corneocyte Condition

To evaluate the effect of sPG ingestion on skin moisture, skin conductance was measured. Average values increased from 93.25 ± 25.92 μs at baseline to 113.84 ±33 μs after ingestion in the sPG group and from 90.79±23.17 μs at baseline to 111.12 ±28.48 μs after ingestion in the placebo group. By statistical analysis, the hazard ratio for conductance between baseline and after two weeks of ingestion in the sPG

group was determined as p=0.078. This result suggests that sPG slightly improves skin moisture.

With regard to the condition of the facial corneum, micrograph observation and scoring and stripping of the corneum were performed. At baseline, corneum roll-up and partial disruption were observed in both groups. The average score increased significantly from 2.8 ± 0.1 at baseline to 3.1 ± 0.1 after sPG ingestion and decreased slightly from 3.1 ± 0.2 at baseline to 2.9 ± 0.2 after placebo ingestion. Furthermore, representative micrographs of corneocytes of a 51-year-old woman in the sPG group and a 30-year-old man in the placebo group are shown in Fig. (**5**). An increased number of good lamellar structures and less roll-up were observed in the sPG group after 2 weeks of ingestion. In the placebo group, a difference from baseline was not observed after ingestion. These corneum structural changes strongly suggest that ingestion of sPG led to improvement of rough skin.

## DISCUSSION

We evaluated the effects of sPG ingestion on skin condition in a randomized, double-blind, placebo-controlled study. We performed several tests to evaluate skin condition parameters, including skin elasticity, looseness, facial wrinkles, pore condition, blotches, facial corneocyte condition, and moisture, to confirm its effects in normal healthy subjects. The daily ingestion of 5 mg sPG significantly improved all of these skin condition parameters except for skin moisture. The dose was determined by a preliminary study. The dose used in our study may have been lower than those of other supplements evaluated for their effects on skin condition in randomized, double-blind, placebo-controlled trials. For example, oral consumption of hyaluronan was used at a range of 37.52 to 240 mg/day [10]. The specific bioactive collagen peptide (BCP) VERISOL® improved eye wrinkle formation and stimulated procollagen I, elastin and fibrillin biosynthesis at a dose of 2.5 g/day [15]. Ingestion of *Sparassis crispa* (Hanabiratake) decreased transepidermal water loss (TEWL) at a dose of 160 mg/day [23]. In the case of green tea, daily ingestion of green tea extract including 100 mg epicatechin, 980 mg epigallocatechin gallate (EGCG), and 238 mg epigallocatechin (ECG) was effective for improving skin condition [14]. In general, biogenics such as polysaccharides, peptides, and polyphenols are decomposed and absorbed in the small intestine, large intestine, or cecum after ingestion. Since PG consists of a highly complex structure of glycoprotein with a core protein and glycosaminoglycan chains [6], the stability of PG after ingestion in the gastrointestinal tract could be better than those of other biogenics. In rat models, orally administered sPG was detected in the rat colonic lumen, although its molecular weight was slightly decreased [24]. In the same condition, chondroitin sulfate was completely decomposed and undetected in the lumen. This sPG stability in the gastrointestinal tract may imply the efficacy of low-dose ingestion. Further study is needed to clarify this point.

In this study, we showed that ingestion of sPG improved skin elasticity clearly and skin moisture partially. As previously reported, skin elasticity is maintained by various factors, related to the structure of the ECM [25]. To improve skin condition, ingestion of nutrition or functional foods, which are components of animal ECM, is reportedly effective [10, 13-15]. However, mechanisms of orally administered functional foods on skin condition remain controversial.

We have proposed two hypotheses for the mechanism by which sPG ingestion improves skin condition. One hypothesis is that PG or partly digested PG reaches the skin by absorption from the gastrointestinal tract and then affects skin cell growth and function. Tsuchiya *et al*. reported that sPG was absorbed from the jejunum through clathrin-mediated endocytosis [26]. They also reported that a specific sequence within sPG seemed to be necessary for endocytosis. Since they used the everted intestinal sac method in the rat, the bioavailability of sPG in the body remained unclear, but it was suggested that the ingested sPG might reach the skin *via* the intestinal route. In our preliminary study, sPG directly enhanced human dermal keratinocyte proliferation *in vitro* (data not shown). If sPG can reach the skin region, its effect might be fully explained. However, this hypothesis has less evidence to our knowledge. Another hypothesis is that the indirect pathway, such as metabolites which was produced by the ingested sPG, can regulate skin condition *in vivo*. Recently, Asano *et al.* reported that oral administration of sPG could influence intestinal microbiota balances in mice [27]. It is well known that microbiota act not only to digest plant indigestable polysaccharides and to absorb nutrients but also to produce small metabolites, such as short-chain fatty acids (SCFAs), which can reach the skin [28]. Ota *et al.* reported that orally administered sPG significantly increased the concentration of total SCFAs and n-butyrate in rat colonic feces [24]. Together, these two reports can support the involvement of metabolites to improve skin condition *in vivo*. Interestingly, involvement of intestinal microbiota has been recently focused on the regulation of skin condition [12, 29]. To clarify the mechanisms of action of ingested sPG, specific metabolites, which are induced by the ingested sPG, should be determined in the future.

In the experimental animal models, daily ingested sPG reportedly prevented inflammatory reactions in collagen-induced arthritis, implying that sPG could be effective on systematic immune responses [30]. In skin biology, prevention of acute or chronic inflammation caused by UV exposure, obesity, and environmental stress is focused on the maintenance of healthy skin [12]. On the inflammatory region in skin, some of degradation enzymes, including matrix metalloproteinases, circulating proteases, leucocyte elastase, and mast cell chymase, can be reportedly induced and disrupt elastic fibers in the dermal tissue, which relates to regulate skin elasticity [31]. The previous report using hairless mice also revealed that oral administration of proteoglycan also suppressed the UVB-associated increases in epidermal and dermal thickness. And then, inflammatory cytokines in serum and dorsal skin was also reduced by the sPG administration [32]. As one of possible anti-inflammatory mechanism by the ingestion of sPG, FOXP3 Treg cells is considered to reduce the inflammatory cytokines production. Mitsui *et al.* reported that the increase of FOXP3 Treg cells and the reduction of inflammatory cytokines were detected after at least 7 days of the sPG administration in mouse experi-

mental colitis model [33]. We showed that sPG improved skin condition for 2 weeks in this study. If sPG can improve total skin condition through systemic immunological pathway, the periods can be permitted to understand to improve the total skin condition. Thus, we need to study more about the immunological effects of sPG to improve skin health.

## CONCLUSION

We performed a randomized, double-blind, controlled study for the effect of ingestion of salmon nasal cartilage-derived sPG on skin condition in healthy adult volunteers. This study demonstrates the ability of ingested sPG to improve skin condition, including skin elasticity, wrinkles, brightening (conspicuous or darkened facial pores, and blotches), moisture, and smoothness. Thus, our results suggest the potential of sPG as a food ingredient.

## CONFLICT OF INTEREST

T.T., J.M., K.W., Y.T.T., T.M., K.I., K.T-T., and M.T. are employees of ICHIMARU PHARCOS Co., Ltd.; and M.Y. is the employee of Kakuhiro Co. Ltd.. These companies produce proteoglycan. This research received no specific grant from any funding agency in the public. The authors have no conflict of interest directly relevant to the content of this article.

## ACKNOWLEDGEMENTS

Declared none.

## LIST OF ABBREVIATIONS

ECM = Extracellular matrix

GAG = Glycosaminoglycan

HPLC = High performance liquid chromatography

sPG = Salmon nasal cartilage-derived proteoglycan

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