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Original article

Oral intake of porcine placental extract improves skin hydration and wrinkles in a double-blind placebo-controlled clinical study

Eun Young Jung[1], *Yang Hee Hong*[2,*]

[1]Department of Home Economic Education, Jeonju University, Jeonju, Republic of Korea; [2]Department of Beauty Art, Suwon Women's University, Suwon, Republic of Korea

Abstract. Placenta extract is used as an agent for the promotion of skin wound healing and as an ingredient in cosmetics. However, there have been few clinical studies on skin improvement through oral intake. Here, we aimed to investigate the effects of the oral intake of porcine placenta extract (PPE) on skin hydration, transepidermal water loss, and wrinkles through a clinical trial. Seventy-eight subjects participated in this study that was conducted through a 12-week period and were randomly assigned to two groups: the placebo (40 subjects) and PPE (38 subjects) groups. The PPE group took 200 mg of PPE orally daily. In this clinical trial, skin moisture retention, transepidermal water loss, and wrinkle analyses were performed with the help of a skin replica. The change in skin hydration after the experiment was significantly different between the PPE and placebo groups ($p < 0.05$, respectively). The delta value of transepidermal water loss after the experiment was also different between the PPE and placebo groups. At week 4, the reduction in transepidermal water loss from baseline values in the PPE group was significantly greater than that in the placebo group (-1.93 versus -0.52 g/(hm^2), $p < 0.01$). These results suggest that PPE can be used as a nutraceutical for the promotion of skin hydration and wrinkle improvement.

Key words: porcine placental extract, skin hydration, transepidermal water loss, wrinkle

Introduction

The skin is the organ that surrounds the body and protects it from external stimuli and bacterial invasion through processes, such as temperature regulation, sensory function, and waste discharge. The skin, similar to other organs of the body, undergoes the aging process; it ages due to a decrease in human bodily functions and changes in physiological functions, such as hormone production, which occur with age or due to the effects of various environmental factors, including exposure to ultraviolet (UV) rays (1). Improper nutritional habits and physical inactivity has proved to be an independent risk factor for many diseases (2). The healthy nutritional and lifestyle habits are important not only for good skin but also for health of human organism in general (3).

Moisture loss occurs in the stratum corneum as a result of aging, and when the moisture level decreases to less than 10%, the amount of moisture loss increases further because of the damage to the skin barrier, which results in rough and dry skin. Therefore, sufficient skin hydration is important for maintaining healthy skin (4). In order for the skin to be moisturised, both the epidermis and dermis should function normally (5). Therefore, there is a continuous increase in the development of health functional foods and functional cosmetics with natural ingredients that are expected to play similar functional roles within the skin (6, 7).

Placenta extracts contain a variety of physiological components, such as proteins, lipids, hormones, glycosaminoglycans, nucleic acids, vitamins, and minerals, and have been used since ancient times for their

antioxidant, anti-inflammatory, immune enhancement, and other effects (8, 9). In particular, these extracts are effective in improving the appearance of wrinkles and in moisturising the skin; further, they have also been used to treat skin inflammation, dry skin, and wounds (10, 11). However, it is difficult to use the human placenta due to the risk of infection and ethical issues; therefore, animal placentas are used. Placenta-based treatment is known to improve skin wound healing (11), increase hair growth due to the increased protein expression of insulin-like growth factor-1 (IGF-1) in rats (12), and inhibit liver fibrosis through the regeneration of damaged hepatocytes (13). In addition, based on the results of *in vivo* experiments involving the oral administration of porcine placenta extract (PPE) in mice, it was determined that the anti-wrinkle effect is produced by a decrease in the expression levels of matrix metalloproteinases (MMP-2 and -9) and a suppression of the decrease in the expression of the tissue inhibitors of matrix metalloproteinase (TIMP-1 and -2) in the dermal layer. In addition, through a toxicity evaluation, it was confirmed that the extract does not produce liver toxicity or skin irritation following oral administration in hairless mice and guinea pigs (14).

Several studies on PPE have been conducted to investigate its photoprotective effect on skin cells and its effect on skin improvement produced by oral ingestion in hairless mice. Although there are preclinical results indicating that PPE administration aids in improving the skin, there have been few clinical studies on skin improvement through PPE intake. Therefore, in this study, we aimed to investigate the possibility that PPE supplements could aid in skin improvement by measuring skin hydration levels, transepidermal water loss (TEWL), and wrinkle formation as skin-related parameters in healthy adults who consumed PPE for 12 weeks.

Materials and methods

Preparation of PPE

Porcine placenta extract was prepared according to an experimental method described in a previous paper (13, 14). Briefly, the porcine placenta was washed with sodium hydroxide (NaOH) for the removal of impurities, following which it was dried. Water equivalent to 10 times the weight of the dried placenta was added, and it was subjected to the extraction process for 3 h at 37.5 MPa and 200°C using a subcritical extraction device (DIONEXASE 100, Dionex Corporation, Sunnyvale, CA, USA). The supernatant obtained by centrifuging the extract was concentrated and lyophilised in order to produce the supplement to be used in the experiment.

Subjects

The subjects included in this study were healthy Korean males and females in their 40s and 60s. Eighty-four individuals who volunteered for participation in the study were selected after being provided a full explanation regarding the purpose and methodology of the study. Among these volunteers, 78 completed the experiment through the entire 12-week period after the exclusion of 6 participants who dropped out. The exclusion criteria that were used were based on the standards stipulated by the Korean Food and Drug Administration, and all the individuals who met these criteria were excluded from the experiment.

Study protocol

This clinical trial was conducted in accordance with the ethical regulations outlined in the Helsinki Declaration and was approved by the ethics committee of Jeonju University (1041042-141023-02). The participants were informed that all of them had the same probability of being assigned to the placebo or experimental group; subsequently, they were randomly assigned to receive one of the two food supplements: the vehicle (dextrin) or PPE (dextrin and PPE). The research subjects were allocated to the groups based on a randomisation table generated by a statistician. In this randomisation table, blocks of a certain size were used to allocate the participants to the intervention groups, and food supplements 1 and 2 were in one block. The participants were assigned to receive the supplements twice daily.; further, because of the differences in the skin function in each age group of the study subjects, a stratified block randomisation method was used to stratify the participants of each age group. Based on whether they would receive the placebo (500 mg dextrin/capsule) or PPE

(400 mg dextrin and 100 mg PPE/capsule), the participants were divided into two groups of 40 and 38, respectively. For 12 weeks, the subjects took the capsule (500 mg) twice daily, 30 min before breakfast and dinner with drinking water, thereby consuming a total of 1 g per day (Figure 1).

Skin assessments

Skin hydration and TEWL were measured four times during the 12-week period at 4-week intervals (0, 4, 8, 12 weeks, Figure 1). Further, skin hydration and TEWL were measured three times at a distance

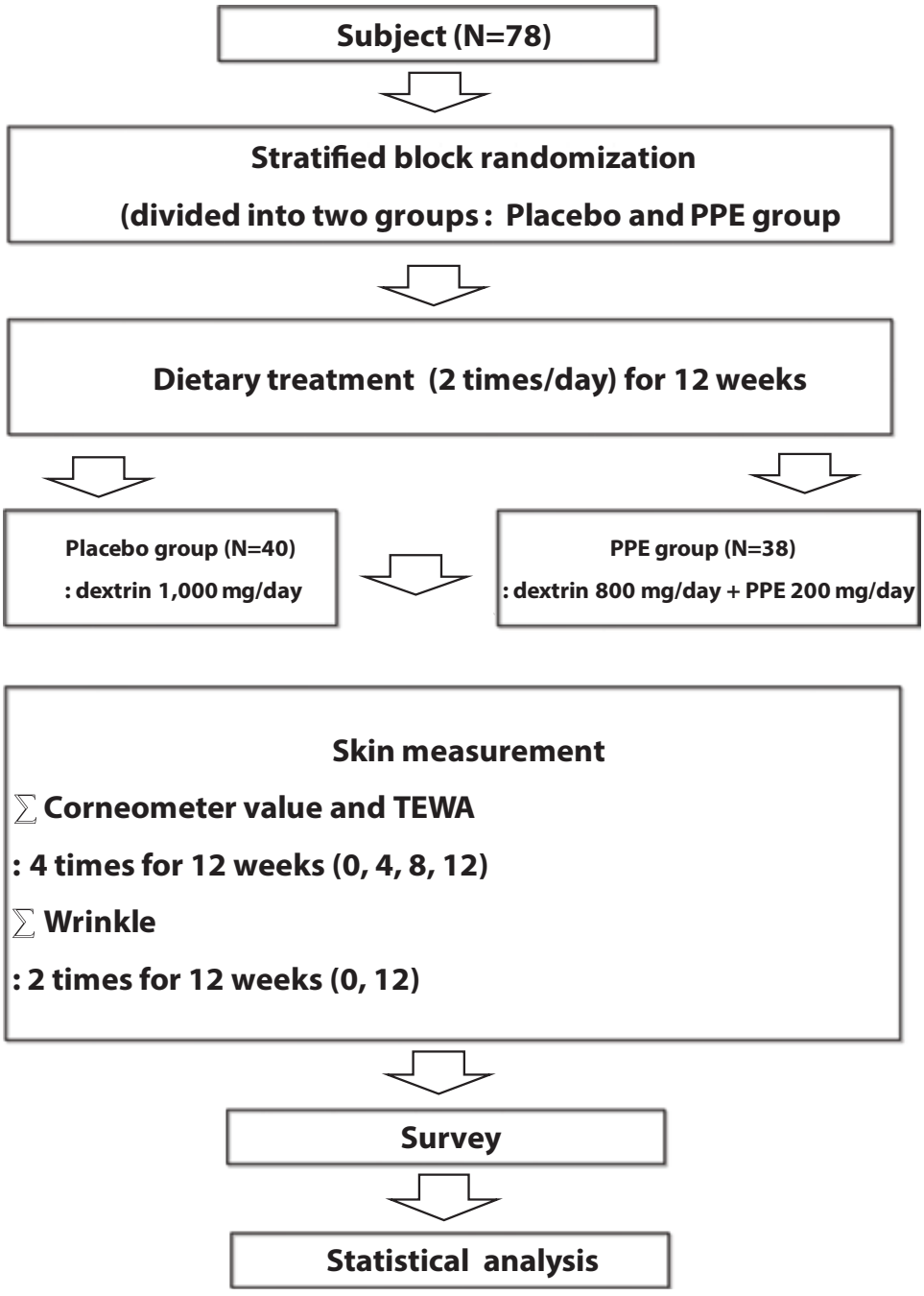

Figure 1. Flowchart of the methods. Abbreviations: PPE, porcine placental extract.

of 1 cm from the crow's feet, except when obtaining the skin surface replica. These parameters were measured using the Corneometer CM825 (Courage and Khazaka electronic GmbH, Cologne, Germany) and Tewameter TM300 (Courage and Khazaka electronic GmbH, Cologne, Germany), respectively. The Corneometer displays hydration measurements in system-specific arbitrary units (AU). The Tewameter measurements are based on diffusion in an open chamber and are recorded in g/h/m^2. Wrinkles were measured twice, once at the beginning of the study and once at the end (0 and 12 weeks, Figure 1). For the wrinkle analysis, a replica was obtained using a silicone polymer (SILFLO impression material, Flexico, England). With the help of this replica, four items including total wrinkle area, percentage of wrinkle area, wrinkle depth, and number of wrinkles were measured using Visioline® VL650 (Courage and Khazaka electronic GmbH, Cologne, Germany).

Statistical analysis

The statistical analysis was performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA), and all the data were expressed as mean and standard error of the means (SEM). Differences between the two groups (placebo group vs. PPE group) were statistically assessed using t-tests. The differences in the changes in parameters from baseline to week 12 within each group were evaluated using a repeated measure analysis of variance (ANOVA) followed by a Bonferroni-adjusted pairwise comparison, and all the data were tested for significance at $p < 0.05$.

Results

Changes in skin hydration and TEWL after the intake of PPE

Table 1 presents the changes in skin hydration and TEWL in the placebo and PPE groups throughout the 12-week period. In the placebo group, skin hydration tended to increase after 4 weeks (73.62 AU), 8 weeks (75.46 AU), and 12 weeks (75.94 AU) compared with at week 0 (73.06 AU); however, there was no significant difference. On the other hand, in the PPE group, skin hydration increased significantly from 70.04 AU at week 0 to 74.40 AU at week 4 ($p < 0.01$), 75.68 AU at week 8 ($p < 0.01$), and 77.61 AU at week 12 ($p < 0.001$).

In the PPE group, TEWL tended to decrease after 4 weeks (17.14 g/h/m^2, $p < 0.05$), 8 weeks (17.14 g/h/m^2, $p < 0.05$), and 12 weeks (17.54 g/h/m^2, $p < 0.01$) compared with at week 0 (19.37 g/h/m^2); there was a significant difference after 4 weeks. On the other hand, in the placebo group, there was no significant change in TEWL from weeks 0 to 12.

Figure 2 presents the changes in the differences in skin hydration and TEWL (Δvalue) within each group. There was a significant increase in skin hydration at 4 weeks ($p < 0.05$) and 12 weeks ($p < 0.05$) in the PPE group compared with in the placebo group (Figure 2A). Figure 2B presents the changes in the differences in TEWL (Δvalue) within each group. The Δvalue of TEWL revealed a significant difference after 4 weeks in the PPE group compared with in the placebo group ($p < 0.01$); however, there were no significant differences thereafter (Figure 2B).

Table 1. Changes in Corneometer and TEWL measurements in the placebo and PPE (oral intake for 12 weeks) groups.

	Placebo group (N = 40)				PPE group (N = 38)			
Variable	**0 week**	**4 week**	**8 week**	**12 week**	**0 week**	**4 week**	**8 week**	**12 week**
Corneometer value (skin hydration)	73.06 ± 1.45	73.62 ± 1.17	75.46 ± 1.40	75.94 ± 1.52	70.04 ± 1.74	74.40 ± 1.80**	75.68 ± 1.85**	77.61 ± 1.83***
TEWL (g/h/m^2)	17.84 ± 1.12	18.36 ± 0.90	17.37 ± 0.82	17.17 ± 0.78	19.37 ± 0.86	17.14 ± 0.71*	17.14 ± 0.90*	17.54 ± 0.76**

Asterisks indicate a significant difference ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) between the values at baseline and at each week calculated by a repeated measure analysis of variance (ANOVA), followed by Bonferroni-adjusted pairwise comparisons within the groups. Values are expressed as mean ± SEM. **Abbreviations:** PPE, porcine placenta extract; TEWA, transepidermal water loss; SEM, standard error of the means.

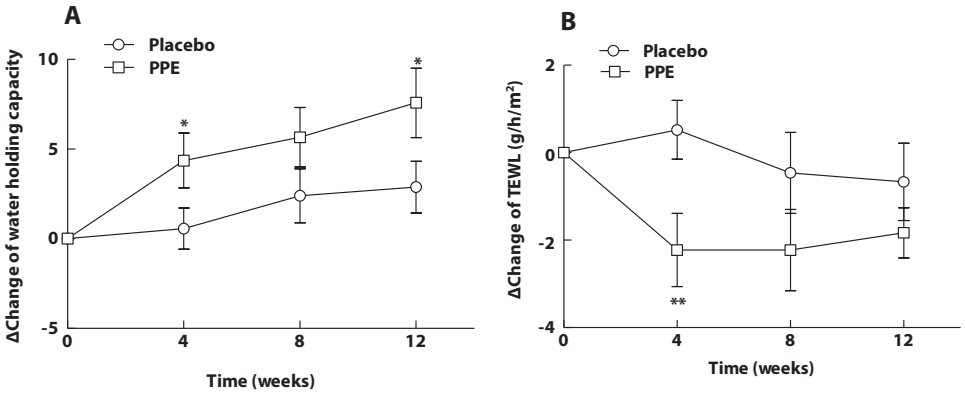

Figure 2. Effects of PPE on the skin's water holding capacity and TEWL in healthy adults. Data are represented as mean ± SEM, and different symbols indicate significance at $^{*}p < 0.05$ and $^{**}p < 0.01$ in the comparison of the PPE group vs. the placebo group. Abbreviations: PPE, porcine placental extract; TEWL, transepidermal water loss; SEM, standard error of the means.

Table 2. Changes in wrinkle parameters in the placebo and PPE (oral intake for 12 weeks) groups.

	Placebo group (N = 40)		PPE group (N = 38)	
Variable	**0 week**	**12 week**	**0 week**	**12 week**
Total wrinkle area (mm^2)	15.04 ± 0.99	16.11 ± 1.00	17.42 ± 0.78	13.46 ± 1.12**
Percentage of wrinkle area (%)	56.31 ± 3.70	60.32 ± 3.74	63.73 ± 3.19	52.00 ± 5.35*
Wrinkle depth (μm)	5092.85 ± 774.19	4969.71 ± 485.77	4361.84 ± 482.17	4002.65 ± 476.76
Number of wrinkles	244.15 ± 35.66	205.43 ± 25.06	250.05 ± 30.72	193.74 ± 22.29

Values are expressed as mean ± SEM.
Abbreviations: PPE, porcine placental extract; SEM, standard error of the means.

Changes in the appearance of wrinkles after the intake of PPE analysed using the replica

In order to evaluate the changes in facial wrinkles, a replica was used to analyse the following four indicators: total wrinkle width, percentage of wrinkle area, wrinkle depth, and number of wrinkles (Table 2 and Figure 3). Table 2 presents the changes in the wrinkles in the PPE and placebo groups. On analysing the total wrinkle area and percentage of wrinkle area at 12 weeks compared with at the beginning of the study, it was found that the wrinkles in the placebo group tended to increase, but those in the PPE group decreased significantly ($p < 0.01$ and $p < 0.05$, respectively). In both groups, the depth of wrinkles and number of wrinkles decreased compared with the baseline values; however, there were no significant differences. When comparing the Δvalue, which is the difference in the wrinkle-related parameters between the baseline and end of the experiment, a significant difference was found in the total wrinkle area and wrinkle area percentage in the PPE group compared with in the placebo group ($p < 0.01$ and $p < 0.05$, respectively) (Figure 3A, 3B). Figure 4 depicts the change in wrinkles, which was analysed with the help of the replicas after 12 weeks of taking PPE. As shown in Figure 4, there was a difference in wrinkle formation compared with the baseline values.

Discussion

Extracts obtained from the placenta are used as agents that can be expected to produce skin

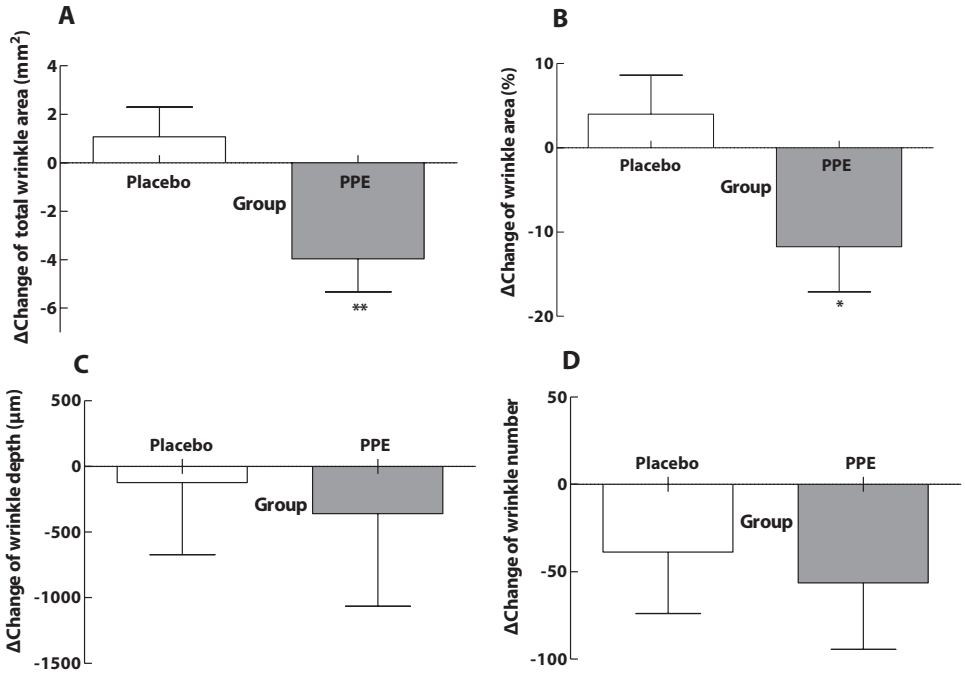

Figure 3. Effects of PPE on wrinkle formation in healthy adults. Data are represented as mean ± SEM, and different symbols indicate significance at $^{*}p < 0.05$ and $^{**}p < 0.01$ in the comparison of the PPE group vs. the placebo group. Abbreviations: PPE, porcine placental extract; SEM, standard error of the means.

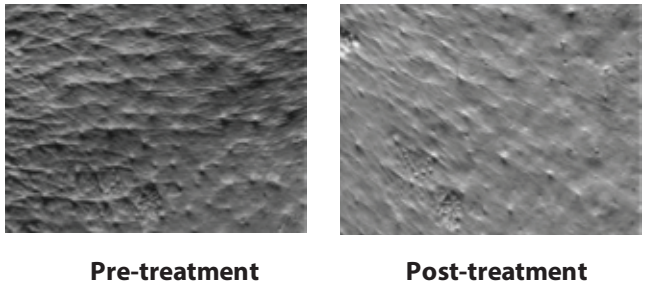

Figure 4. Replica photographs of crow's feet in healthy adults who consumed PPE for 12 weeks. Abbreviations: PPE, porcine placental extract.

improvement-related effects, such as an improvement in the appearance of wrinkles, enhancement of skin hydration, and increase in skin elasticity; the predominantly used placenta extracts are from sheep and pigs (9, 10). The anti-wrinkle effect of PPE has been found to be excellent, and excellent results were generated in a stability test of a lotion formulation containing PPE; therefore, it can be used as an ingredient in cosmetics. PPE was also confirmed to be a very safe agent that does not produce skin irritation when administered orally or applied topically in hairless mice (14).

The normal function of the skin deteriorates due to the aging process. Further, the progress of skin deterioration is accelerated by external factors, such as UV rays and environmental pollution, and by internal factors, such as increasing age and mental stress. The skin hydration levels decrease due to skin barrier damage along with wrinkle formation, elasticity reduction,

and skin pigmentation. To maintain healthy skin, it is very important to maintain the skin hydration level, which is the skin's water-holding capacity. Skin hydration and TEWL play important roles in regulating the skin barrier function. As a result of the oral administration of PPE in the experimental group of this study, improvements were observed both in skin hydration and TEWL at the end of the experiment in comparison with the baseline values (Table 1). In addition, skin hydration was significantly different between the PPE group and placebo group at the end of the experiment (Figure 2A). Treatment with placenta extract results in high expression levels of ceramide synthase in human fibroblasts (15). The increase in the expression level of ceramide synthase strengthens the lamella structure of the stratum corneum, which helps in maintaining water retention and skin barrier function (16). It has been suggested that the effect of maintaining the skin barrier function through the ingestion of PPE is related to the changes in the expression level of ceramide synthase.

Porcine placenta extract has been developed into a supplement that helps in improving the appearance of wrinkles and is currently being used in clinical practice. The oral intake of PPE was found to improve the appearance of fine lines under the eyes and to reduce the width of wrinkles (17). As shown in Figure 3, wrinkle formation was reduced in the PPE group. Fibroblasts, which are involved in the expression of extracellular matrix proteins, including collagen, play a major role in skin regeneration. PPE affects fibroblast regeneration and differentiation, which plays an important role in skin regeneration and collagen synthesis and increases fibroblast collagen production (17). Collagen is a component of the extracellular matrix, which contributes to the mechanical strength of the skin, and is closely related to the formation of wrinkles (18). Changes in the expression levels of MMP and TIMP are involved in collagen degradation and synthesis, and thereby affect the formation of wrinkles. The increase in the level of MMP expression caused by UV rays affects collagen degradation and synthesis, leading to wrinkle formation (19). PPE treatment decreases the expression levels of MMP-2 and MMP-9 induced by UV irradiation and also exerts a suppressive effect on the decrease in the expression levels of TIMP-1 and TIMP-2 (20). In addition, it was reported that the inhibition of collagen degradation due to the reduction in MMP-9 protein expression in human fibroblasts contributes to wrinkle reduction (17). Therefore, the anti-wrinkle effect of the oral administration of PPE appears to be a result of the suppression of the increase in MMP expression and decrease in TIMP expression. Skin hydration affects skin elasticity due to the structural changes caused by elastin hydration, which is involved in skin elasticity (21). Decreased moisture retention or dry skin can also cause fine wrinkles (22, 23). As such, the increase in collagen production resulting from the controlling effect of the ingestion of PPE on the expression levels of MMP and TIMP, which are involved in the decomposition and synthesis of collagen, appears to have an influence on the improvement in wrinkle appearance and increase in skin hydration.

Collagen not only improves wrinkles but also affects skin elasticity. The structure of collagen consists of repeated glycine (G)-X-Y tripeptides, and X and Y are mainly composed of proline and hydroxyproline, respectively. Human placenta extract contains the collagen peptide, G-X-Y tripeptide (8), and G-X-Y tripeptide increases collagen and hyaluronic acid content in fibroblasts (24). Since PPE also contains proline and hydroxyl proline, it appears to improve the skin through a collagen peptide. The exact mechanism behind the skin hydration or TEWL improvement effects resulting from the oral intake of PPE is not clear; nevertheless, the peptides (Gly-Leu, Leu-Gly, and Leu-Leu) contained in PPE induce an improvement in skin hydration and skin elasticity (25).

Porcine placenta extract intake produces skin hydration and wrinkle improvement effects. The wrinkle improvement effect is due to the regulation of the expression levels of MMP and TIMP, which are involved in collagen degradation and synthesis. In addition, it also produces an improvement in skin hydration through the inhibition of the degradation of collagen, a major protein in the skin. These effects are believed to have been influenced by the proteolytic products or collagen peptides contained in PPE; however, further research should be conducted to confirm this. PPE can be used as a skin improvement supplement as it exerts skin improvement effects which have been confirmed through fibroblasts tests and human clinical tests.

Conflicts of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Correspondence:
Yang Hee Hong,
Department of Beauty Art, Suwon Women's University,
Suwon, Republic of Korea
Tel: +82-32-290-8378
Email: hongyh@swc.ac.kr
https://orcid.org/0000-0001-8259-0056