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Notes

Effects of a Simultaneous Intake of Soy Peptide and Collagen Peptide on the Skin Function of Healthy Adult Women

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Soy peptide is absorbed rapidly into the living body, where it manifests diverse physiological actions. We conducted an interventional study on healthy adult women to evaluate the effects on skin function of a test beverage composed of a combination of collagen peptide, which had previously been reported to increase skin elasticity and moisture, and soy peptide. A beverage composed of collagen peptide alone with a total peptide content equal to that of the test beverage served as the control beverage. Each subject was measured for the skin elasticity, skin moisture level and trans epidermal water loss (TEWL) before and after the 70-day beverage intake period. Although there was no statistically significant difference between the test and control groups in any parameter, the skin elasticity was significantly higher and TEWL was significantly lower than the pre-intake levels after intake of the test beverage. However, the change of either of these two parameters was statistically significant after intake of the control beverage. These findings suggest that the intake of collagen peptide combined with soy peptide might have improved the skin elasticity and skin barrier function.

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Keywords : soy peptide, collagen peptide, skin elasticity, transepidermal water loss (TEWL)

1. INTRODUCTION

The term "collagen" is used to refer to several types of structural proteins that constitute bone, cartilage, tendons, and skin. Collagen is made up of glycine (33%), proline (12-13%), hydroxyproline (9-10%), and alanine (12-13%) [1]. In dermal tissue, Type I collagen is the most abundant, and type III collagen is the second most abundant [2]. Type I collagen molecules are arranged in parallel with a deviation of about one-quarter of their length, forming fibrils. A collagen fiber is composed of numerous fibrils, and a collagen fiber bundle is formed by several collagen fibers, which function to keep skin elastic [3]. Recent studies have shown that oral collagen intake resulted in an increase of skin elasticity [4,5] and skin moisture [4,6,7]. Oral collagen intake has additionally been shown to increase collagen peptide levels in the blood [8,9]. Furthermore, collagen peptides stimulate the proliferation of skin fibroblasts, which are involved in collagen synthesis, and the formation of hyaluronic acid by the cells [10]. These findings suggest that oral collagen intake increases skin elasticity and moisture content.

Soy protein contains not only essential amino acids, which are not synthesized in the living body, but also the major amino acids that make up collagen (glycine, proline, and alanine) in a well-balanced manner [11]. Soy peptide made from the enzymatic degradation of soy protein has been reported to manifest diverse physiological actions, such as the stimulation of skeletal muscle growth and the suppression of its decomposition [12-14], the alleviation of abnormal lipid metabolism [15,16], and the stimulation of growth hormone secretion [17]. It has also been

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shown that soy peptide is absorbed more rapidly than soy protein and amino acids of the same composition [18]. The treatment of cultured human fibroblasts with soy peptides resulted in the stimulation of the synthesis of type I collagen, which is a major component of dermis [19]. These reports suggest that the oral intake of soy peptide may improve skin elasticity.

Because of these findings, we recently conducted an interventional study with the expectation that the simultaneous oral intake of soy peptide, which has a variety of physiological actions, and collagen peptide may affect skin conditions more favorably than collagen peptide alone following the intake. In addition to the skin's elastic force and water content, which have been previously reported to improve following oral collagen intake and transepidermal water loss (TEWL), were measured as indicators of skin condition.

2. EXPERIMENTAL METHODS

(1) Selection of subjects

This study was carried out after approval was obtained from the Ethics Committee on Studies of Human Subjects of Sagami Women's University and Sagami Women's Junior College. Information on the objectives and the methods of the study were provided when the subjects for this study were recruited. Adult women free of skin disease and intake of supplement such as collagen, soy peptide and viamin C were eligible for the study. Informed consent was obtained for all candidate subjects, and those candidates that provided consent in writing were enrolled in the study. The subjects were divided into 2 groups, the test beverage intake group (Group T) and the placebo beverage intake group (Group P), in a manner designed to prevent a significant inter-group difference in the data from pre-intervention measurement. Each subject was instructed to avoid changing eating habits and the cosmetics that were used during the study period. Subjects who did change eating habits and the cosmetics were excluded from analysis. The mean ± SE age of the subjects was 52.3 ± 2 .8 years in Group T (n = 16) and 56.7 ± 3.8 years in Group P (n = 17).

(2) Beverages

The test beverage contained soy peptide (Fuji Oil Co., Ltd. , Osaka, Japan) and fish-derived collagen peptide (Nippi, Inc. , Tokyo, Japan). The placebo beverage contained collagen peptide so that the amount of peptide contained would be equal to that contained in the test beverage. Minor ingredients were the same for both the test beverage and the placebo beverage. Table 1 shows the amounts of soy peptide, collagen peptide, energy, proteins, fats, and carbohydrates contained in each beverage. Each beverage was made up to a volume of 100 mL each time.

(3) Intervention schedule

Pre-intervention measurements were performed in May 2010. One week after the pre-intervention measurements, the subjects were asked to take once daily either the test beverage or the placebo beverage for 70 consecutive days. Subjects were blinded to the type of beverage they took. Each day, the beverage was taken between supper and bedtime. No restrictions were imposed on the daily living of the subjects, except for beverage intake. Each subject was instructed to maintain her pre-intervention lifestyle during the study as much as possible. Post-intervention measurements were performed 71 days after the start of the intervention. This study is a placebo-controlled, double-blind study.

(4) Parameters and methods of measurement

All parameters were measured on the elevated part of the pharynx. A piece of cleansing sheet (Kao Corporation, Tokyo, Japan) was applied to the skin at the site to be measured for 30 seconds, and the skin was gently wiped with it. The skin site was subsequently wiped with defatted cotton pre-immersed in distilled water for injection (Otsuka Pharmaceutical Co., Ltd. , Tokyo, Japan). The subjects remained quiet in the measuring

Table 1. Composition of beverages

Contents	Test beverage	Placebo beverage
Soy peptide	4500 mg	0 mg
Collagen peptide	4300 mg	8600 mg
Energy	60 kcal	59 kcal
Protain	8.0 g	8.0 g
Fat	0.0 g	0.0 g
Carbohydrate	12.0 g	11.9 g

/100 mL

room for 20 min (room temperature, 20℃; relative humidity, 50%) before measurement was started. Three parameters were measured, including skin elasticity (by the aspiration method), transepidermal water loss (TEWL) and skin moisture (by the electrostatic measurement of volume) with a skin meter MPA580 (Courage+Khazaka electronic GmbH, Kern, Germany). For the measurement of skin elasticity, the skin was aspirated once at a certain negative pressure, and the negative pressure was then left. The resultant pattern of skin height within the probe was analyzed to yield indices of skin elasticity. The indices used were Result5 (R5) and Result7 (R7). R5 was obtained by dividing Ur by Ue, as shown in Figure 1. R7 was obtained by dividing Ur by Uf, as shown in Figure 1. Both parameters are 1 at maximum, and the values of both parameters that are closer to 1 indicate higher skin elasticity. At this site, skin elasticity and skin moisture were measured 5 times, and TEWL was measured 3 times. When the measurement of the same parameter was repeated, the site of measurement was slightly moved in order to avoid overlapping of the center of the measured site.

(5) Statistical analysis

For each of the parameters that were measured 5 times, the maximum and minimum values were excluded and the mean of the 3 remaining measurements was accepted as the value. For TEWL, the mean of the 3 measurements was accepted as the value. A paired t-test was employed for the comparison of the post-intervention data with the pre-intervention data within the same group. A Student's t-test was employed for statistical evaluation of the results between Group T and Group P. The values were expressed as means ± SE. P values < 0.05 were regarded as statistically significant. PASW Statistics 18 (SPSS, an IBM Company, Tokyo, Japan) was used for the statistical analysis.

3. RESULTS

(1) Increase in skin elasticity following simultaneous soy peptide and collagen peptide intake.

In Group P, R5 (an index of skin elasticity) increased from 0.42 ± 0.03 before the intervention to 0.46 ± 0.02 after the intervention, although this change was not statistically significant. In Group T, R5 increased significantly from 0.43 ± 0.02 before the intervention to 0.48 ± 0.02 after the intervention ($p < 0.01$). R7 also increased only slightly in Group P, from 0.30 ± 0.02 to 0.31 ± 0.02, but it increased significantly ($p < 0.05$) in Group T, from 0.31 ± 0.02 to 0.34 ± 0.02 (Figure 2).

Although skin elasticity improved in Group T, difference was not statistically observed between Group T and Group P (Table 2).

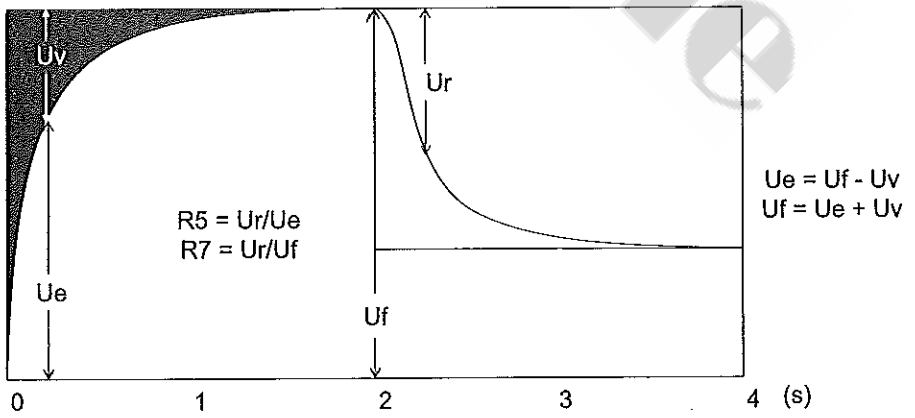

Fig. 1. Method of calculating R5 and R7
The shape of waves shows the height of the skin in the probe. After the skin was aspirated once at sudden negative pressure, the pressure was canceled. Ue shows the height of the skin that gone up almost vertically by rapid negative pressure. Uf shows the width of the maximum suck. Uv is a difference between Uf and Ue. Ur shows height where the skin returned 0.1 seconds releasing later.
The source from the manual of skin mater (Courage + Khazaka electronic GmbH)

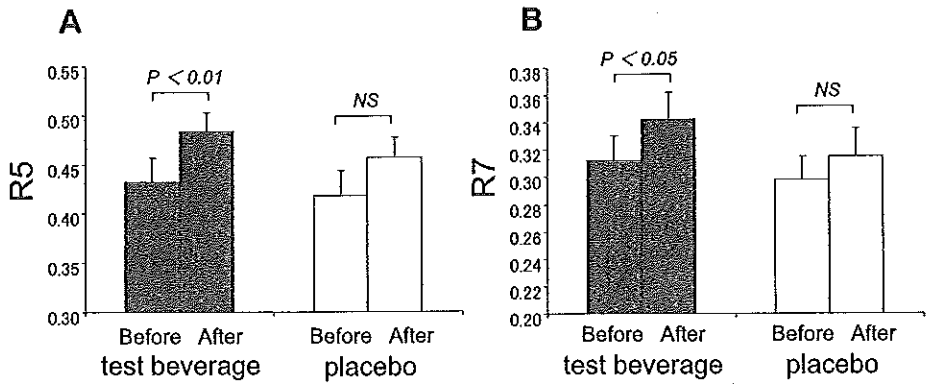

Fig. 2. Change in R5 (A) and R7 (B) owing to in take beverages.
Bar graph illustrating R5 and R7, indices of skin elasticity, before and after the intervention period of beverage consumption for the groups taking the Test Beverage and Placebo Beverage. Error bars indicate SE. *P*-values were obtained with a paired *t*-tests.
R5 and R7 was measured by using Cutometer

Table 2. Comparison of differences before and after intake of beverage between the groups

		Differences	*p*-values
R5	test beverage	0.05 ± 0.01	0.62
	placebo	0.04 ± 0.02	
R7	test beverage	0.03 ± 0.01	0.48
	placebo	0.02 ± 0.02	
TEWL	test beverage	−3.15 ± 1.33	0.25
	placebo	−0.96 ± 1.32	
Moisture value	test beverage	−0.74 ± 2.98	0.85
	placebo	0.22 ± 3.87	

The values were expressed as mean ± SE . *P*- values were obtained with Student's t-tests. R5 and R7 were measured by the aspiration method with a skin meter MPA580. (Courage+Khazaka electronic GmbH, Kern, Germany). TEWL and skin moisture were measured by the electrostatic measurement of volume with MPA580.

(2) Decrease in TEWL and no change in skin moisture following simultaneous soy peptide and collagen peptide intake

In Group P, TEWL decreased from 8.65 ± 0.84 before the intervention to 7.69 ± 1.54 after the intervention, although this change was not statistically significant. In Group T, TEWL decreased significantly ($p < 0.05$) from 9.64 ± 1.40 before the intervention to 6.49 ± 1.19 after the intervention (Figure 3). However, statistical difference was not observed between Group T and Group P (Table 2). Skin moisture after the intervention did not differ from that before the intervention in either Group P or Group T (Figure 4).

4. DISCUSSION

In the present study, simultaneous soy peptide and collagen peptide intake resulted in a significant elevation in R5 and R7 from their pre-intervention levels. The intake of collagen alone resulted in a tendency for an elevation in R5 and R7, but the change in both these indices was not statistically significant. These results indicate that simultaneous soy peptide and collagen peptide intake increased skin elasticity.

A previous analysis of blood amino acid levels in healthy individuals revealed that soy protein-derived oligopeptide is absorbed more rapidly than soy protein

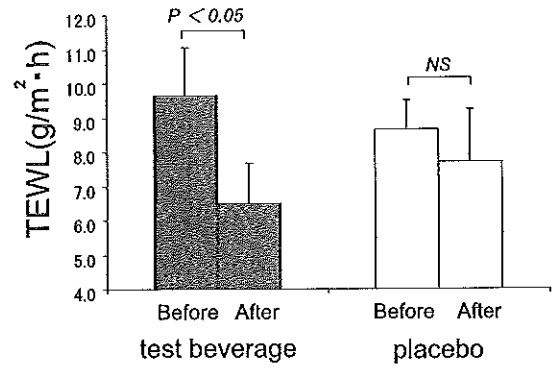

Fig. 3. Change in TEWL owing to in take beverages.
Bar graph illustrating transepidermal water loss (TEWL) before and after the intervention period of beverage consumption for the groups taking the Test Beverage and Placebo Beverage. Error bars indicate SE. *P*-values were obtained with a paired *t*-tests.
TEWL was measured by using Tewameter

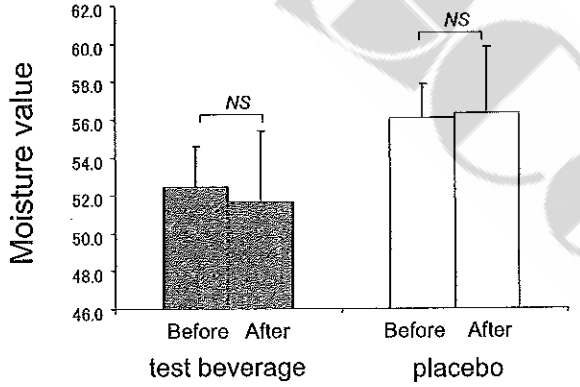

Fig. 4. Change in Moisture value owing to in take beverages.
Bar graph illustrating Moisture Value before and after the intervention period of beverage consumption for the groups taking the Test Beverage and Placebo Beverage. Error bars indicate SE. *P*-values were obtained with a paired *t*-tests.
Moisture was measured by using Corneometer

itself during the period immediately after intake [17]. It has also been shown that blood amino acid levels were higher following the intake of soy peptide than following the intake of a mixed amino acid solution of the same composition [17]. Because the physiological activity of this kind of substance in the human body may depend on its blood levels, these results suggest that soy peptide is promising as a method for the full utilization of the physiological activity of soy. In a previous study involving healthy male volunteers loaded with exercise, post-exercise muscle injury was suppressed in the soy peptide intake group than in the soy protein intake group [14].

Soy peptide was shown *in vitro* to increase the formation of type I collagen by skin fibroblasts. When human dermic fibroblasts were treated with soy peptide, type I collagen formation increased about 2-fold [18]. When incubated with a medium supplemented with magnesium ascorbyl phosphate (a vitamin C derivative indispensable for collagen formation), the amount of collagen formed by human dermic fibroblasts increased in a manner dependent on the soy peptide concentration [18]. Perrier *et al.* reported that collagen biosynthesis increased by as much as 60% after the addition of 1.25% soy peptide to the medium containing the human fibroblasts compared to human fibroblasts not treated with soy peptide [19]. A significant stimulation of biosynthesis following treatment with soy peptide has also been reported for hyaluronic acid, chondroitin, and elastin, which are synthesized by skin fibroblasts [19]. Glycine, which is also contained in soy, has been shown to induce elastin promoter activity and to stimulate elastin synthesis *in vitro*. Glycine has been additionally shown to reduce elastase activity and to suppress the degradation of elastin by elastase. When soy extracts were applied to the skin of pigs and mice, elastic fibers increased in density and the levels of elastin and desmosine (a collagen-containing amino acid) increased [20]. The elastic force of skin is determined by collagen fibers, which are made of collagen, and elastic fibers, which are made of elastin. The results from the present study suggest that the intake of soy peptide increased the skin' s elastic force by increasing the levels of collagen and elastin through action on skin fibroblasts. Furthermore, the skin's elastic force was increased more markedly following the intake of soy peptide and collagen peptide together compared to the intake of collagen alone, suggesting that soy peptide either reinforces the activity of collagen peptide or manifests another activity that differs from that of collagen peptide.

TEWL decreased slightly following the intake of collagen peptide, while it decreased significantly following the simultaneous soy peptide and collagen peptide intake. It has been reported that the skin fat ceramide level in rats increased following the intake of soy protein or soy peptide, thus indicating the effectiveness of soy peptide [21]. Histidine, which is contained relatively abundantly in soy peptide, reduced TEWL in dogs when

it was ingested together with pantothenic acid, choline, nicotinamide, and inositol [22]. The results from the present study endorse the effectiveness of soy peptide demonstrated in these previous reports. Skin moisture, on the other hand, showed no increase after intervention in the present study. The present study was carried out in May through July. It seems probable that mild dermatitis arising from sunburn resulted in the lack of an increase in skin moisture. In the present study, the neck was selected as the site of measurement because the skin condition in the neck resembles the condition on the face and the neck skin is less susceptible to the influence of ultraviolet rays. However, the influence of ultraviolet rays could not be completely ruled out. If the body trunk or some other site had been selected as the site of measurement instead of the neck that may possibly be markedly affected by ultraviolet rays, the improvement in skin moisture following the intervention might have been greater. In addition, the effect of exogenous factors, such as relative humidity and ambient temperature, cannot be ruled out. If the present study had been executed in the mild season, skin moisture might have increased following intake of beverages.

In past studies, an increase in the skin's elastic force and moisture in the stratum corneum was shown also following the intake of collagen peptide alone [4,6,7,9]. *In vitro*, the treatment of collagen peptide resulted in the proliferation of skin fibroblasts and hyaluronic acid formation by cells, suggesting that some efficacy is expected also from the intake of collagen peptide alone. The results from the present study indicate that the efficacy continues if about half of the amount of collagen peptide is replaced with soy peptide. The results suggest that the long-term intake of a combination of soy peptide and collagen peptide enables better utilization of the advantages of these 2 substances, resulting in a more marked improvement in the skin's elastic force and barrier function. The efficacy of the intake of this combination deserves further assessment.

As illustrated above, the present study suggested the efficacy of the intake of a combination of peptides from 2 different sources. Many reports have been published concerning the efficacy of collagen peptides on the skin, and studies on the mechanisms of this activity have been carried out. In addition, reports on the effects of soy peptide on fibroblasts have been published, suggesting that soy peptide is expected to have favorable effects on skin. In the future, we plan to conduct a further evaluation of the effect of each peptide taken separately and the additive or synergistic effects of their combined use.

5. ACKNOWLEDGMENTS

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健常成人女性の皮膚機能に対する大豆ペプチドならびにコラーゲンペプチドの同時摂取効果

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大豆から抽出したタンパク質を酵素処理して得られた大豆ペプチドは吸収が速く，多くの生理活性を有することが期待されている．本研究では，肌弾性力や肌水分量を増加させると報告されているコラーゲンペプチドに大豆ペプチドを混合した試験飲料を用いて，両ペプチドの併用が皮膚機能に与える効果についての介入試験を実施した．被験者は健康な成人女性とし，試験飲料は大豆ペプチドとコラーゲンペプチドの混合とし，対照飲料は試験飲料と総ペプチド含有量がほぼ同量のコラーゲンペプチド単独とした．70 日間の摂取前後で，肌弾性力，水分量，経皮的水分蒸散量（TEWL）を測定した．試験飲料と対照飲料との間に 3 項目とも統計学的な差はみられなかったものの，大豆ペプチドとコラーゲンペプチドの同時摂取群では，摂取後の肌弾性力が摂取前に比べて有意に増加した．また，同群では摂取後の TEWL が摂取前に比べて有意に減少した．一方，対照群では摂取前後で肌弾性力と TEWL に有意差を認めなかった．大豆ペプチドをコラーゲンペプチドに加えると摂取後に肌弾性力と肌のバリア機能が改善する可能性が示唆された．

キーワード：大豆ペプチド，コラーゲンペプチド，肌弾性，経皮的水分蒸散量（TEWL）