Oral Intake of Soy Isoflavone Aglycone Improves the Aged Skin of Adult Women

Toru Izumi1, Makoto Saito1, Akio Obata1, Masayuki Arii2, Hideyo Yamaguchi2 and Asahi Matsuyama1

1 Research and Development Division, Kikkoman Corporation, 399 Noda, Noda 278–0037, Japan
2 Institute of Medical Mycology, Teikyo University, Tokyo 192–0395, Japan
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Summary A double-blind, placebo-controlled trial was conducted to clinically evaluate the effect of soy isoflavone aglycone on the aged skin of middle-aged women. Twenty-six women volunteers in their late 30s and early 40s were randomly assigned to receive either a test food (13 women, the test food group) or a placebo food (13 women, the control group). These groups were given the test food (40 mg of soy isoflavone aglycone per day) and placebo, respectively, for 12 wk. The extent of linear and fine wrinkles at the lateral angle of the eyes was selected as the major evaluation criterion to assess the effects of foods, and the wrinkles’ area ratio was used as the evaluation parameter. The extent of skin microrelief at the lateral angle of the eyes and that of malar skin elasticity were used as secondary evaluation criteria, and the skin microrelief’s area ratio and recovery of skin elasticity were used as the respective evaluation parameters. These parameters were assessed 4, 8, and 12 wk after the start of the test food or placebo intake. The test food group showed a statistically significant improvement of fine wrinkles at week 12 (p<0.05) and of malar skin elasticity at week 8 (p<0.05), compared with the control group. Although there was no significant difference between the test food group and the control group regarding effects on skin microrelief at lateral angle of the eyes, a significant intragroup improvement was observed at week 8 in the test food group (p<0.05). As for the test food safety, none of the subjects presented adverse symptoms during the study period or discontinued the intake of the test food. These data indicate that the oral intake of 40 mg soy isoflavone aglycones per day improves the aged skin of middle-aged women.

Key Words isoflavone, aglycone, skin, wrinkle, elasticity

Soy isoflavone has been reported to have an estrogen-like effect (1, 2). Such an effect suggests that soy isoflavones may contribute to prevent disorders such as osteoporosis (3), atherosclerosis (4), and menopausal syndrome (5) that are associated with a reduced secretion of estrogens in postmenopausal women. Numerous epidemiological studies on the mortality of hormone-dependent cancers such as breast cancer (6) and prostate cancer (7) have found a negative correlation between intake of soybean, especially soy isoflavones, and the incidence of these cancers.

Furthermore, soy isoflavones are considered to have beneficial effects on the skin via mechanisms such as prevention of lipid oxidation of the skin tissue (8), stimulation of fibroblast proliferation (9), reduction of collagen degradation (10), and inhibition of 5α-reductase (11), and have been widely used as ingredients of cosmetics (12). Moreover, cosmetics containing isoflavones are reported to retard skin aging (13). Soy isoflavones are chemically comprised of aglycone and glycosides, and all the beneficial effects on the human skin mentioned above have been confirmed to derive from aglycone (12, 14, 15). An isoflavone intake experiment in healthy humans demonstrated that isoflavone aglycones are absorbed faster and in larger amounts than their glycosides (16).

These results indicate that the oral intake of isoflavone aglycones could have an effect similar to its topical application on the changes of the skin associated with aging. However, the influence of dietary intervention with isoflavone aglycones has not been investigated yet.

Common changes of the skin due to aging are wrinkles, particularly linear and fine wrinkles, which appear in the malar skin at the lateral angle of the eyes or around the eyelids (17). Middle-aged Japanese women are deeply concerned about changes in the skin due to aging, especially wrinkles at the lateral angle of the eyes. These data prompted us to perform a clinical trial that aimed to determine whether the oral intake of soy isoflavone aglycones improved changes of the skin due to aging, including wrinkles at the lateral angle of the eyes, in middle-aged women.

MATERIALS AND METHODS

Selection and distribution of the subjects. The subjects enrolled in this study were 26 Japanese women in their
late 30 s and early 40s who had both linear and fine wrinkles at the lateral angle of the eyes or around the eyelids and were concerned about the sagging skin below the eyes. The exclusion criteria were as follows:

1) Those who routinely used medical products containing isoflavone as a major ingredient or took dietary supplements.

2) Those who had atopic dermatitis or other skin disorders.

3) Those who had severe diseases such as diabetes mellitus, liver disorders, renal disorders, or cardiovascular diseases.

4) Those who had a food allergy.

5) Those who were pregnant, breast-feeding or expected to become pregnant during the study period.

6) Those who were already enrolled in another study at the start of this study.

7) Those who were found to be unsuitable for this study by the investigators.

The wrinkles of each subject were replicated to measure linear and fine wrinkles before the intake. Based on the extent of the linear wrinkles on the replica, the subjects were randomly assigned to either group so that each group included subjects with the same extent of linear wrinkles, as evenly as possible. The mean (±SE) age of the test food group and the control group was 40.1±1.0 y (range, 35 to 48) and 40.5±0.95 y (range, 36 to 46), respectively; the difference was not statistically significant.

**Trial design.** This double-blind, placebo-controlled trial was carried out between November 2004 and March 2005. The effect of the test food and placebo on the wrinkles on the malar skin and other changes of the skin were evaluated after the oral intake of four tablets of the test food (10 mg isoflavone aglycones per tablet) or placebo daily for 12 wk.

**Evaluation of efficacy.** Skin wrinkles, so called “crow’s feet,” were classified into linear and fine wrinkles, according to Kligman et al. (17), and their area and area ratios were used as the principal evaluation criteria and parameters, respectively. The variables were calculated separately in the test food group and the control group and assessed before and at weeks 4, 8, and 12 during the study period. The intergroup differences were analyzed for statistical significance. In addition, the skin microrelief at the lateral angle of the eyes and malar skin elasticity were used as secondary evaluation criteria, and the intergroup differences in skin microrelief’s area ratio (%) and recovery of elasticity (%), were statistically analyzed. Intragroup differences were also examined for their statistical significance.

**Test food and administration.** Each tablet of 10 mg isoflavone aglycones consisted of 25 mg of fermented soybean extract containing 40% isoflavone aglycones (isoflavone aglycone 40(TM)) and 225 mg vehicle comprised of reduced maltose syrup, crystalline cellulose, cornstarch, and sucrose fatty acid ester. The color-matched placebo tablet was obtained by adding a small amount of caramel to 250 mg vehicle. Kikkoman Corporation provided both the test food and the placebo tablets. The test food group and the control group were asked to take four isoflavone aglycones tablets or four placebo tablets, respectively, per day with 100 mL water or tepid water for 12 wk.

**Measurement of malar skin parameters.** Parameters of the malar skin condition were measured with the instruments described below. Immediately before measurement, the subjects were asked to wash their face twice and to sit quietly for 20 min in a testing room kept at 21±1°C and a relative humidity between 50 and 60%. Two symmetrical regions of the face were evaluated and the mean value was calculated.

1) Analyses of skin wrinkles and skin microrelief using two-dimensional image analysis techniques: Two-dimensional image analysis was performed referring to the reports by of Corcuff et al. (18, 19) and Grove et al. (20). Wrinkles at the lateral angle of the eyes on both sides of the face were replicated, and a parallel light was applied to them from a single direction at an angle of 30 degrees to the horizontal plane to generate a shadow, which was imported using a microscope (KH-300V/HK3000YD type) and image processed using Photoshop (Adobe Version7.0). The shadow area and its length were measured via such processing. In the same manner, the shadow area and its length were calculated using a standard scale to correct the values obtained from the replica of wrinkles at the lateral angle of the eyes. Using the measured and corrected values, each area ratio of linear wrinkles, fine wrinkles, and skin microrelief were calculated.

2) Measurement of malar skin elasticity: Malar skin elasticity was measured by an aspiration method using Cutometer SEM575 (Courage+Khazaka Electronic GmbH, Koeln, Germany). As a local skin elasticity parameter, time to the recovery of elasticity (%) was measured after an abrupt release of a negative pressure that was constantly applied to both sides of the malar skin by aspiration of the skin with a Cutometer probe applied to the skin surface.

**Consideration for safety and its management.** If any subject presented subjective or objective adverse symptoms, it was reported to the physician in charge via the contract company (TTC Co.) so that he/she could make a decision, and the subject could withdraw from the study according to her decision or that of the physician in charge.

**Statistical analysis.** A paired t-test was used to assess intragroup differences between the values measured before intake (0 wk) and those measured at weeks 4, 8, 12. Intergroup differences at each time point were assessed using a two-sample t-test. A p value of less than 0.05 was considered to indicate statistical significance.

**RESULTS**

**Amounts of isoflavone aglycone ingested in the control group and the test food group**

Daily isoflavone aglycone consumption was calculated from the dietary record of individual subjects participating the present study. Based on the data, the
The mean consumption of isoflavone aglycone from routinely taken meals was estimated to be $7.3 \pm 0.972$ and $7.4 \pm 1.164$ mg per day in the control group and the test food group, respectively. Thus, the mean value of the total amount of isoflavone aglycone intake for the test food group was calculated to be 47.4 mg per day, which was approx. 6.5 times greater than that for the control group.

Effect of the test food on the wrinkles at the lateral angle of the eyes

Figure 1 illustrates the time-course changes of the mean area ratio of linear wrinkles in each group. As clearly shown in this figure, the difference between the mean area ratio of linear wrinkles at each time point and that at baseline was not statistically significant in either group, and the comparison of increment or decrement of the mean area ratio between the two groups revealed no statistically significant difference either.

The results of the same statistical analysis of time-course changes of the mean area ratio of fine wrinkles in each group and increment or decrement of the mean area ratio in the two groups is shown in Fig. 2. As seen in this figure, the baseline value for the test food group was appreciably higher than that for the control group due to the manner of subject grouping in which each subject was assigned primarily based on the linear wrinkles but not on the fine wrinkles. However, as there was no statistical difference in the value between the two groups ($p=0.110$), we considered that the comparison of increment or decrement of the value between the two groups would be possible. The mean area ratio of fine wrinkles did not change significantly during the study period in either group. In contrast, the significantly greater decrement of the mean area ratio of fine wrinkles was seen in the test food group at week 12 as compared with that in the control group ($p<0.05$).

These data revealed that the test food had a significant effect on fine wrinkles but not on linear wrinkles.

Effect of the test food on skin microrelief at the lateral angle of the eyes

As illustrated in Fig. 3, while the mean skin microrelief area ratio remained almost unchanged in the control group, it tended to increase with time, particularly after week 4 in the test food group, and a significant difference was noted in week 8 ($p<0.01$). The extent of variables in the mean area ratio of skin microrelief was higher in the test food group than in the control group at every time point, but the difference was not significant.
proved effective. Furthermore, there are studies demonstrateing that oral intake of those vitamins, carotenoids, and n-3 polyunsaturated fatty acids inhibits actinic skin aging in humans (30). These data are consistent with those from an epidemiological study (31) that explored the relationship between actinic skin aging, especially wrinkles, and various foods routinely ingested by the human population all over the world, which varies in terms of races and dietary habits; the epidemiological study showed that aging of the skin was significantly milder in a population taking a large variety of foods including vegetables, olive oil, and fish than in populations with other dietary habits. Furthermore, a significant retardation of aging of the skin was also observed in populations that ingested plenty of beans, but no study has evaluated the effect of isoflavone intake (31).

Since isoflavones have both an estrogen-like effect and an anti-oxidative effect, these substances are considered ideal candidates as anti-skin aging agents with the potential ability to retard the two skin aging processes mentioned above. Actually, it was demonstrated that external use of isoflavones inhibited skin aging or improved the skin conditions and that their effect on the skin was essentially due to isoflavone aglycones (10, 13). In addition to these findings, since isoflavone aglycones are effectively absorbed from the intestine after oral intake (16), the oral intake of the test food was expected to improve the skin as effectively as its external application. Thus, we conducted a clinical trial to prove this hypothesis with the use of soy isoflavone aglycones in a daily dose of 40 mg that was within the dose limit recommended by the National Authority.

In this trial, the wrinkles at the lateral angle of the eyes, a common sign of skin aging, were classified into linear wrinkles and fine wrinkles and evaluated separately. The wrinkle’s area ratio was used as a parameter to assess the effect of the test food. The reasons linear and fine wrinkles were chosen were as follows: Although linear wrinkles generally appear at about 30 y of age, their extent shows a low correlation with age (17); a significant improvement and delay of linear wrinkles can hardly be expected to occur during a study period as short as 12 wk; whereas, fine wrinkles are milder than linear wrinkles and thus are likely to be a reversible skin change. Moreover, to enhance the reliability of the evaluation methods, skin microrelief, which is considered an appropriate evaluation criterion to examine changes related to skin aging (18), and malar skin elasticity were used as secondary criteria. The present trial, in which 40 mg soy isoflavone aglycones per day was used as the test food, can be considered as a kind of intervention study, because the individual subjects in the control group and the test food group ingested isoflavone aglycones from meals in amounts of 7.3 and 7.4 mg per day, on average. However, such levels of isoflavone aglycone consumption was less than one-fifth of that of 40 mg per day supplemented soy isoflavone aglycones as the test food, leading us to the notion that the difference in the amount of isoflavone aglycone intake between the control group and the test food group was sufficient to study the effect

**Effect of the test food on malar skin elasticity**

Time-course changes in the recovery of elasticity (%) in each group are illustrated in Fig. 4. While the recovery of elasticity remained almost unchanged in the control group, it tended to increase in the test food group, and a significant difference was observed at week 8 with regard to its baseline value (p<0.05). The extent of recovery of elasticity of the skin was also significantly higher in the test food group than in the control group at week 8 (p<0.05).

**Safety**

None of the subjects who took the test food or placebo complained of subjective adverse symptoms during the study period or withdrew from the study.

**DISCUSSION**

Two kinds of processes contribute to aging of the skin in middle-aged women, particularly postmenopausal women. One process is intrinsic (estrogen-dependent) or chronological; a decrease of estrogens contributes to the development of skin wrinkles and a reduction of skin elasticity. Hormone replacement therapy (HRT), which retains the collagen level, elasticity, and thickness of the skin, has been shown to retard aging of the skin (21, 22). However, HRT can have adverse effects, such as an increased risk of developing breast cancer and cardiovascular diseases (23, 24), and thus the implementation of HRT for cosmetic reasons is medically questionable.

The other is extrinsic or actinic; reactive oxygen species generated by UV irradiation or other means can damage epidermal cells (25–27), leading to the aging of the skin. Antioxidants have been shown to delay this process, one of whose traits is to lodge in the skin tissue. In fact, topical use of a variety of antioxidants including nutrients such as vitamin C (28) and vitamin E (29) has proved effective. Furthermore, there are studies demonstrating that oral intake of those vitamins, carotenoids, and n-3 polyunsaturated fatty acids inhibits actinic skin aging in humans (30). These data are consistent with those from an epidemiological study (31) that explored the relationship between actinic skin aging, especially wrinkles, and various foods routinely ingested by the human population all over the world, which varies in terms of races and dietary habits; the epidemiological study showed that aging of the skin was significantly milder in a population taking a large variety of foods including vegetables, olive oil, and fish than in populations with other dietary habits. Furthermore, a significant retardation of aging of the skin was also observed in populations that ingested plenty of beans, but no study has evaluated the effect of isoflavone intake (31).

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The results of this study demonstrated that, of the two principal evaluation criteria, the fine wrinkle’s area ratio in the test food group (40 mg soy isoflavone aglycones per day) at week 12 showed a statistically significant reduction compared with that for the control group (placebo). No effect of the test food was observed on linear wrinkles. Thus the results indicated that under the conditions of this study, it was difficult to improve such age-independent, advanced skin changes. Malar skin elasticity was also significantly improved in the test food group compared with the control group. The extent of variables of the mean skin microrelief area ratio (or the skin microrelief at the lateral angle of the eyes) did not differ significantly between the two groups, and only in the test food group was there a significant improvement of the value at some given time point. A similar significant improvement was observed in the recovery of elasticity of the skin. Since no significant intragroup difference was observed in the control group, it may be concluded that the test food effectively improved skin microrelief at the lateral angle of the eyes even though this is based only on the data from the intragroup comparison. It has been reported that increase of the wrinkles at the lateral angle of the eyes and skin microrelief reflect changes of the elastic fiber network due to aging (19), and thus that conclusion is considered to be appropriate.

In other words, the results of the assessment of the principal and secondary evaluation criteria allow us to conclude that the intake of the test food (40 mg soy isoflavone aglycones per day) for 12 wk improved to some extent the aged skin. The effectiveness of the test food was observed only limited measurement times after intake in both the within and between group comparisons although there was a trend of improvement with time. One of the reasons for such results may be the small number of subjects (13 per group) evaluated in each group. Further studies involving a larger number of appropriately selected subjects are required to confirm the results obtained in this study.

If isoflavone aglycones were proved to effectively improve skin conditions in aged people, the next issue would be to elucidate its mechanism of action. Animal experiments underlined an enhancement effect on the generation of hyaluronic acid, which is a major component of the dermis, as is collagen (32). This possible effect is supported by the data that retinoic acid, possessing an enhancement effect on hyaluronic acid generation, has a similar improvement effect (33, 34) and that the hyaluronic acid content of the skin in women, especially women in their 40s or more, markedly decreases with aging (35). Isoflavone aglycones probably enhance the hyaluronic acid generation (14) via an estrogen-like effect (1, 2, 14). However, the possibility that an anti-oxidative effect of isoflavone aglycones contributes to the improvement of skin aging cannot be ruled out. Thus elucidation of the mechanism of action and determination of an optimum dose of isoflavone aglycones are key points for further study.

REFERENCES


