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Co-ingestion of soy isoflavones and lactobionic acid improves facial stratum corneum hydration: A randomized, placebo-controlled trial

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ABSTRACT

Background: Low-dose soy isoflavones (<30 mg/day) alone often show limited effects on skin hydration; co-ingestion with lactobionic acid may enhance absorption and equol bioconversion, potentially improving corneum hydration.

Objective: To evaluate whether co-ingesting soy isoflavones (25 mg aglycone equivalents) with lactobionic acid (250 mg) improves stratum corneum water content compared with isoflavones alone or placebo.

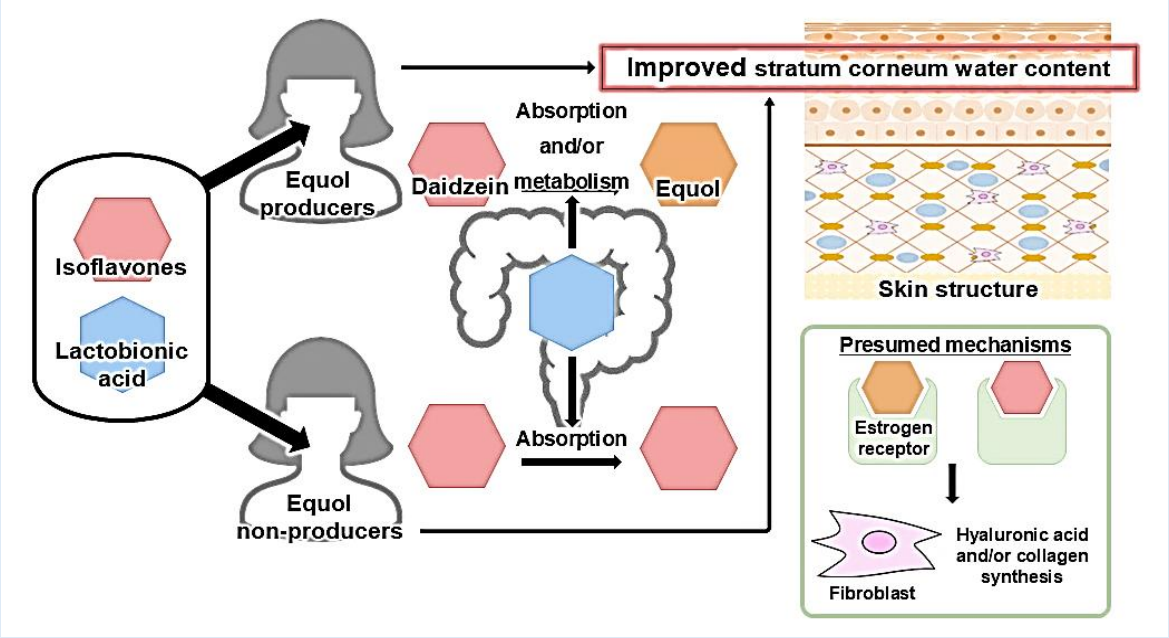
Methods: This randomized, placebo-controlled, parallel-group trial aimed to determine the effects of 12-week consumption of soy isoflavones alone (Iso, 25 mg soy isoflavone aglycone equivalents), soy isoflavones and lactobionic acid (Iso+LAB, 25 mg soy isoflavone (aglycone equivalents) and 250 mg lactobionic acid), or placebo (no soy isoflavone or lactobionic acid) on the stratum corneum water content and urinary isoflavone concentration in 33 healthy adult women.

Results: The stratum corneum water content of the left cheek significantly increased in the Iso+LAB group at 12 weeks, whereas no significant improvement was observed in the Iso group compared with the placebo group. Among equol producers, the Iso+LAB group exhibited significantly higher urine equol levels at 4 weeks compared with the Iso group. Among equol non-producers, the urinary daidzein and genistein concentrations were consistently higher in the Iso+LAB group, although dietary isoflavone intake did not differ among groups.

Conclusions: Co-ingestion of soy isoflavones and lactobionic acid enhanced the stratum corneum water content, likely through improved absorption and metabolism of soy isoflavones, including equol production.

Novelty: This study isolates the specific contribution of lactobionic acid by directly comparing low-dose soy isoflavones alone, soy isoflavones combined with LBA, and placebo, linking skin hydration changes to urinary isoflavone and equol kinetics.

Keywords: soy isoflavone, lactobionic acid, stratum corneum water content, equol, skin, functional food.



Graphical Abstract: Intake of soy isoflavones and lactobionic acid improved the stratum corneum water content in both equol producers and non-producers.

INTRODUCTION

The skin comprises the epidermis, dermis, and subcutaneous tissue. The epidermis, which is the outermost layer of the skin, plays an important role in defending the human body against external environmental factors such as water evaporation, foreign body invasion, and ultraviolet light [1]. The dermis is situated beneath the epidermis. The collagen fibers, which are the main components, form a network structure, whereas components such as hyaluronic acid retain moisture. These ingredients maintain moisture in the skin and provide elasticity to the skin [2]. Additionally, hyaluronic acid, which fills the gaps in the network, supplies moisture to the epidermis, keeping the skin hydrated and maintaining the barrier function of the stratum corneum [3, 4].

Soy isoflavones are flavonoids present in soybeans, and most exist as glycosides in soybean seeds [5, 6]. Upon ingestion of soy isoflavones, glycosides are converted to aglycone forms by the intestinal bacterial β-glucosidase and absorbed in the intestinal tract. The absorbed isoflavones are conjugated to glucuronic acid and sulfate

and then translocated into the blood for intestinal circulation [7]. Among isoflavone aglycones, daidzein is further metabolized by intestinal microbiota into equol and O-desmethylangolensin [8, 9]. Soy isoflavones and equol have weaker estrogenic effects compared with estradiol [10], and equol has stronger estrogenic activity compared with genistein [11]. However, only 40-50% of individuals in Asian populations and approximately 30% in Western populations are capable of producing equol [12, 13], with younger individuals in Japan even lower rates [14]. Once bound to estrogen receptors, estrogen stimulates epidermal cell proliferation and enhances fibroblast-mediated synthesis of collagen and hyaluronic acid [15, 16]. Previous human studies reported increased stratum corneum water content [17-19], reduced facial wrinkle depth [20], improved skin-condition visual analog scale (VAS) scores [21], and maintenance of skin elasticity [22] following high-dose soy isoflavone or soy-based processed products intake. However, low-dose soy isoflavone intake (<30 mg/ day) has generally not demonstrated significant skin benefits.

Lactobionic acid, an indigestible oligosaccharide produced from lactose by the *Acetobacter* [23, 24], promotes growth of *Bifidobacterium* and *Lactobacillus* [25], enhances calcium absorption [26], and increases equol production in animal studies [26]. Because equol exhibit higher estrogen receptor affinity than daidzein, the combined intake of soy isoflavones and lactobionic acid may synergistically enhance stratum corneum water content and reduce transepidermal water loss (TEWL) by facilitating greater isoflavone absorption and metabolism. Indeed, a prior study reported that co-ingestion of low-dose soy isoflavones (25 mg/ day) and lactobionic acid (250 mg/ day) for 12 weeks improved hydration, barrier parameters, and viscoelasticity compared with placebo [27]. However, because that study lacked a soy isoflavone only group, the independent contribution of lactobionic acid could not be clearly determined. This present preliminary study was therefore designed to evaluate the study by Akagi et al. [27]. It established a group receiving only soy isoflavones, alongside the placebo group and another receiving a combination of soy isoflavones and lactobionic acid. Subsequently, the impact of these treatments on skin condition was assessed. Additionally, urinary concentrations of isoflavones and equol were measured to determine whether lactobionic acid enhances isoflavone absorption or bioconversion.

METHODS

Study design: This single-blind, placebo-controlled, parallel-group study was conducted at Fujicco Co., Ltd. (Kobe City). The study period ranged from September 17 to December 11, 2019 (12 weeks). This study was performed in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Review Board of Fujicco Co. Ltd (No. 6001, on May 15, 2019). Written informed consent was obtained from all participants. Assignment of test food to the study participants was performed by the person in charge of the allocation and was strictly controlled until the end of the study. This exploratory study was not prospectively registered in any public clinical trials registry.

Participants: Healthy women aged ≥30 years who were employees of Fujicco Co., Ltd. and provided written informed consent to were enrolled. There was no predefined upper age limit; enrolled participants were 31-57 years old at consent (mean ages per group shown in Table 3). Exclusion criteria were; (1) known allergy to soy or milk; (2) current use of medicines; (3) pregnancy at screening or during the study; (4) participation in other clinical studies (including clinical trials),; or (5) any condition deemed by the researcher to interfere with study conduct.

Criteria for participant discontinuation/ dropout: Discontinuation criteria: The discontinuation criteria

were as follows: (1) the study participant declined research participation or withdrew their consent, (2) the inclusion criteria were not met after enrollment, (3) it was difficult to continue the study owing to the occurrence of adverse events, (4) the investigator considered it appropriate to discontinue the study for other reasons, and (5) the entire study was discontinued.

Dropout criterion: The refusal of the study participants to continue consuming the test meals was set as the dropout criterion.

Intervention: Two test foods and a placebo were prepared as previously described by Akagi *et al*. [27]. The soy isoflavone product (Iso) provided 25 mg/day of soy isoflavones (as aglycone equivalents), and the combination product (Iso+LAB) provided 25 mg/day of soy isoflavone (as aglycone equivalents) plus 250 mg/day of lactobionic acid. The placebo was prepared by adding a coloring agent to the same amount of maltodextrin to produce the same shape and weight. Fujiflavone® K25 (Fujicco Co., Ltd., Japan) extracted and purified from soybean hypocotyls and standardized by adding β-cyclodextrin, was used as the isoflavone source. Lactobionic acid (Daicel Corporation, Japan) was produced from lactose using the acetic acid bacterium. All products were prepared into an indistinguishable tablet; participants consumed three tablets once daily after dinner with water or lukewarm water for 12 weeks. The formulation of each food is shown in Table 1. The nutritional content of each food item consumed per day is shown in Table 2. Participants were instructed not to initiate new supplements or cosmetic treatments and to avoid topical medications at measurement sites. They were advised to maintain their usual cosmetic skincare routine without any changes. No other dietary restrictions were imposed, including those related to soy products.

Table 1. Formulation of test meals.

	Placebo	Iso	Iso + LAB
Fujiflavone®K25 (mg)	0	36.6	36.6
Lactobionic acid powder (mg)	0	0	164.6
Maltodextrin (mg)	201.2	164.6	0
Crystalline cellulose (mg)	79.1	89.8	89.8
Fine silicon dioxide (mg)	3	3	3
Calcium stearate (mg)	6	6	6
Safflower pigment (mg)	8	0	0
Purple cabbage pigment (mg)	2.7	0	0
Total (mg/a grain)	300	300	300

The formulation of each test meal is shown below. Subjects consumed three tablets once daily with water or lukewarm water after dinner for 12 weeks.

Table 2. Nutritional composition of test foods.

	Placebo	Iso	Iso + LAB
Energy (kcal)	2.5	2.2	2.2
Protein (g)	0.0004	0.007	0.007
Lipids (g)	0.02	0.02	0.02
Carbohydrates (g)	0.6	0.6	0.5
Sodium chloride equivalent (g)	0.0002	0.0006	0.0007

The nutritional composition per daily intake of each test food is shown.

Outcomes: The primary outcome was the stratum corneum water content of the left cheek and left upper arm, whereas the secondary outcomes included TEWL and subjective symptoms related to the patient's quality of life (QOL) and skin condition assessed using a questionnaire. Additionally, urinary isoflavone (daidzein, genistein, and glycitein) and equol concentrations were measured as the secondary outcomes. Dietary intake of soy products was also evaluated using questionnaire, and the amount of soy isoflavone (aglycone equivalents) was calculated.

The stratum corneum water content was measured by assessing the capacitance of the skin surface at a depth of 10–20μm, using 1-s readings under constant spring pressure with a Corneometer® CM825 (Courage+Khazaka electronic GmbH) [19, 28]. The amount of TEWL was determined using Tewameter® TM300 (Courage+Khazaka electronic GmbH) [29, 30], which detects temperature and humidity gradients between paired high-sensitivity sensors located at the tips of the probes. The amount (g/hm^2) was calculated based on these gradients. Measurements were obtained at two positions on the left cheek and left upper arm and repeated eight times per site, averaged after excluding the maximum and minimum values. The cheek was rinsed with water following the application of decorated sheets, whereas the left upper arm was swabbed with a water-containing sheet. All participants were acclimated for 20 minutes in a controlled laboratory environment (20°C ± 2°C, 50% ± 10% relative humidity) before measurements.

Urinary isoflavone levels were measured using high-performance liquid chromatography and normalized to creatinine concentration. The urinary equol levels were measured using ELISA (Healthcare Systems Inc.). Urine samples or standards, and HRP-conjugated anti-equol antibodies were added to equol-coated microplates. After washing, 3,3',5,5'-Tetramethylbenzidine reagent was added, and measurements were performed at 450 nm (reference 570 nm). The equol concentrations were calculated from a standard curve and creatinine-corrected.

The patients' subjective symptoms in the last 4 weeks were evaluated using a questionnaire. The QOL-related questionnaire included the following items: eye fatigue, blurring of vision, eye pain, shoulder stiffness, muscular pain/stiffness, palpitations, shortness of breath, weight gain, weight loss, lethargy, feelings of thirst, anorexia, early satiety, epigastralgia, increased susceptibility to colds, coughing and sputum, diarrhea, constipation, hair loss, gray hair, headache, dizziness, tinnitus, lumbago, arthralgia, edematous, increased sweating, frequent urination, hot flashes, cold skin, irritability, easily angered, shallow sleep, difficulty in falling asleep, inability to sleep due to anxiety, memory lapse, loss of motivation, inability to concentrate, pessimism, depression, a sense of tension, and feelings of anxiety for special reason. The skin-related questionnaire included the following items: skin pores, skin smoothness, coarse skin, presence of spots or freckles, skin complexion, clarity of the skin, dullness or dryness of the skin, presence or absence of crows on feet, presence or absence of eye bags, smoothness of the skin, skin elasticity, whether makeup products run easily on the skin, whether makeup products are difficult to apply smoothly on the skin, skin condition, oiliness of the face,

presence of acne, and itchy skin. The participants were asked to answer questions related to the subjective symptoms they experienced using a five-point scale: (1) they did not think at all, (2) they did not feel very much, (3) they could say neither, (4) they felt slightly, and (5) they felt strongly.

Dietary intake of soy products was assessed using an open-ended questionnaire. The respondents were asked to indicate the types of soy products they consumed during the last four weeks, the frequency with which they consumed them per week, and the amount per serving. The amount of soy isoflavones (aglycone equivalents) was estimated using reference values from Toda *et al.* [31].

Randomization: All 33 participants who provided informed consent met eligibility criteria and no exclusion criteria. To evaluate baseline equol-producing status, participants consumed a supplement containing 25 mg of soy isoflavone alone (aglycone equivalents) as a pre-test for 2 days, and morning urine samples were collected on day 3. Participants with urinary equol levels ≥1 nmol/mg Cre were classified as equol producers, while others were classified as non-producers, according to Akagi *et al*. [27]. With age, urinary equol level, and menopausal status as allocation factors, the participants were randomly assigned to the placebo group (n=11), Iso group (n=11), or Iso+LAB group (n=11).

Blinding: After allocation, the allocations were concealed only from the participants until the end of the study.

Statistical analysis: All data were expressed as mean ± standard deviation. Stratum corneum water content and TEWL were analyzed using two-way repeated-measures analysis of variance was performed, followed by Dunnett's test, for between-group comparisons (placebo as reference) and within-group comparisons (pre-ingestion baseline). To assess the specific effect of lactobionic acid, Tukey-Kramer's HSD test was performed for all pairwise comparisons when significant group differences were detected. Tukey's honestly significant difference test of urinary isoflavone and equol levels was performed to investigate the effects of lactobionic acid. Steel's multiple comparison test was conducted using questionnaires. Subgroup analyses of the equol producers and non-producers were performed, and same statistical methods were used for inter- and intragroup comparisons in the subgroup analyses. JMP (SAS Institute Inc.) was used to perform all statistical analyses. The level of statistical significance was set at 5% (two-sided).

RESULTS

Flow of participants: Figure 1 shows a flowchart of the participant selection procedure and the process from the allocation of the test food to the analysis. Thirty-three participants who provided consent were randomly assigned to treatment groups (placebo-treated group: 11, Iso group: 11, and Iso+LAB group: 11). One participant in the Iso+LAB group discontinued at week 8 due to an adverse event judged unrelated to the intervention. Data through week 4 from all 33 patients (mean age, 42.1 ± 7.26 years) and data from 32 participants thereafter were included in the analyses of primary and secondary outcomes.

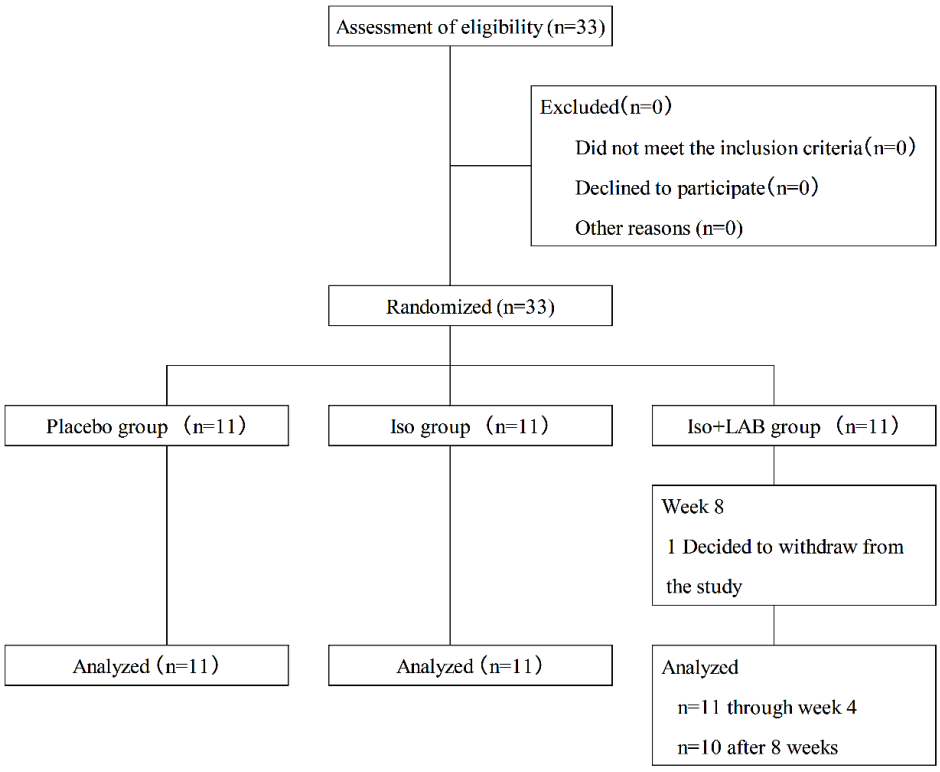


Fig. 1. Flowchart of the participant selection process. Based on the results of a screening conducted on 33 candidates who agreed to participate in the study, 33 participants were selected and assigned to the placebo, isoflavone, or isoflavone + lactobionic acid groups. One patient in the isoflavone + lactobionic acid group dropped out of the study of her own volition at week 8. The analysis was performed on 11 participants in the placebo group, 11 in the isoflavone group, and 11 in the isoflavone plus lactobionic acid group up to week 4 and 10 after week 8. Iso, isoflavones; LAB, lactobionic acid.

Baseline data: The characteristics of the enrolled participants are presented in Table 3. There were no significant between-group differences in age, urinary equol levels (after the isoflavone challenge), or the number of postmenopausal women at allocation. At enrollment, ages ranged from 31-56 years in the Iso group (mean age, 41.2 ± 7.2 years), 32-57 years in the Iso+LAB group (mean age, 42.6 ± 7.9 years), and 32-54 years in the placebo group (mean age, 41.6 ± 7.4 years). The body fat percentage was significantly lower in the Iso and Iso+LAB groups than in the placebo group, whereas height, weight, systolic blood pressure, and diastolic blood pressure did not differ significantly among groups.

In order to perform subgroup analysis by equol-producing phenotype, participants ingested 25 mg of soybean isoflavones (aglycone equivalents) during a pre-test, and urinary equol level was measured. Individuals with urinary equol levels ≥1 nmol/mg Cre were classified as equol producers, and those with <1 nmol/mg Cre were classified as equol non-producers; then, the efficacy of consuming the two test diets was examined in each group.

At baseline, there were five equol producers in the placebo group, six in the Iso group, and six in the Iso+LAB group. The stratum corneum water content in the left upper arm was significantly higher in the Iso group than in the placebo group (Table S1 [Supplemental Online Material]). For other items, no differences were observed in the initial values between the groups. There were six equol non-producers in the placebo group, five in the Iso group, and five in the Iso+LAB group. The body fat percentage was significantly lower in the Iso and Iso+LAB groups than in the placebo group (Table S2 [Supplemental Online Material]). For other items, no differences were found in the initial values between the groups.

Table 3. Baseline data of all participants.

Number of subjects	Placebo group	Intervention group		p value (one-way ANOVA)
		Iso group	Iso+LAB group	
	11	11	11	
Assignment items				
- Age (years)	41.5 ± 7.35	42.2 ± 7.21	42.5 ± 7.88	N.S.
- Equol (nmol/mg Cre) urine	5.27 ± 13.2	9.02 ± 16.9	4.12 ± 6.64	N.S.
- Number of menopausal women	2	1	2	
Stratum corneum water content (a.u.)				
- Left cheek	67.6 ± 7.23	66.0 ± 12.0	71.1 ± 6.78	N.S.
- Left upper arm	32.6 ± 4.99	37.6 ± 5.53	35.3 ± 6.92	N.S.
Urinary isoflavones (nmol/mg Cre)				
- Daidzein	19.9 ± 19.3	15.9 ± 21.6	16.7 ± 14.4	N.S.
- Genistein	13.0 ± 11.4	11.1 ± 17.2	16.5 ± 21.6	N.S.
- Glycitein	2.46 ± 1.89	2.68 ± 2.71	2.36 ± 1.78	N.S.
Physical Measurements				
- Height (cm)	160.5 ± 6.49	159.5 ± 4.16	159.8 ± 5.60	N.S.
- Body weight (kg)	60.6 ± 11.7	53.6 ± 4.81	53.6 ± 6.48	N.S.
- Percent of body fat (%)	31.8 ± 4.86	27.6 ± 2.57	26.9 ± 4.01	0.0127
- Systolic blood pressure (mmHg)	116.9 ± 14.6	118.5 ± 10.4	114.5 ± 15.3	N.S.
- Diastolic blood pressure (mmHg)	68.5 ± 8.29	69.5 ± 10.8	69.4 ± 17.0	N.S.

Stratum corneum water content and TEWL: The pre-intake values of the stratum corneum water content (primary outcome) did not differ among groups at either the left cheek or left upper arm. At 12 weeks, the Iso+LAB group showed significantly higher values in the left cheek compared with the placebo group (Fig. 2a). No significant between-group differences were observed for the left arm at any time point (Fig. 2b). Within each group, neither site showed significant change versus pre-ingestion.

For TEWL (a secondary outcome), pre-ingestion values did not differ among groups at either site. The Iso group showed significantly higher values in the left cheek compared with the placebo group at 12 weeks, whereas the Iso+LAB group showed significantly lower values in the left cheek at 12 weeks (Fig. 2c). In the placebo group, the values in the left upper arm at 8 and 12 weeks were significantly lower; in the Iso group, the values in the left upper arm at 12 weeks were significantly lower (Fig. 2d).

Among equol producers, the stratum corneum water content in the left cheek was not significantly different between the groups at the pre-ingestion period (Fig. 3a), but was significantly higher in the left upper arm of the Iso group compared with that of the placebo group (Fig. 3b). No significant differences were found in the duration of ingestion in either the left cheek or the left upper arm between the Iso or Iso+LAB groups and the placebo group. Within-group comparisons of the pre-ingestion values were not significantly different at any time point in the left cheek and left upper arm of the different groups (Fig. 3a and b). The TEWL of equol producers did not differ significantly between the groups based on the pre-ingestion values of either the left cheek or left upper arm. No significant differences were also found in the duration of ingestion in the left cheek or left upper arm of the Iso and the Iso+LAB groups compared

with that in the placebo group (Fig. 3c and d). However, within-group comparisons of the pre-ingestion values showed significantly lower values at 12 weeks in the left upper arm of the Iso group (Fig. 3d).

Among equol non-producers, the stratum corneum water content was not significantly different in each group based on the pre-ingestion values of the left cheek and left upper arm (Fig. 4a and b). The Iso+LAB group showed significantly higher values in the left cheek at 12 weeks compared with the placebo group (Fig. 4a). No significant differences were found at any time point in the left upper arm of the Iso and the Iso+LAB group compared with the placebo group (Fig. 4b). Within-group comparisons with pre-ingestion values showed significantly lower values at 12 weeks in the left cheek of the placebo group (Fig. 4a). No significant differences were found in the left upper arm between groups at any time point (Fig. 4b). The TEWL of equol non-producers did not differ significantly between the groups based on the pre-ingestion levels in the left cheek and left upper arm (Fig. 4c and d). The Iso+LAB group showed significantly higher values in the left cheek at 4 and 12 weeks compared with the placebo group (Fig. 4c). No significant differences were observed in the left upper arm of the Iso and the Iso+LAB group at any time point (Fig. 4d). Within-group comparisons of pre-ingestion values showed significantly lower values at 12 weeks in the left cheek of the placebo group, significantly lower values at 12 weeks in the left cheek of the Iso+LAB group (Fig. 4c), and significantly lower values at all time points in the left upper arm of the placebo group (Fig. 4d).

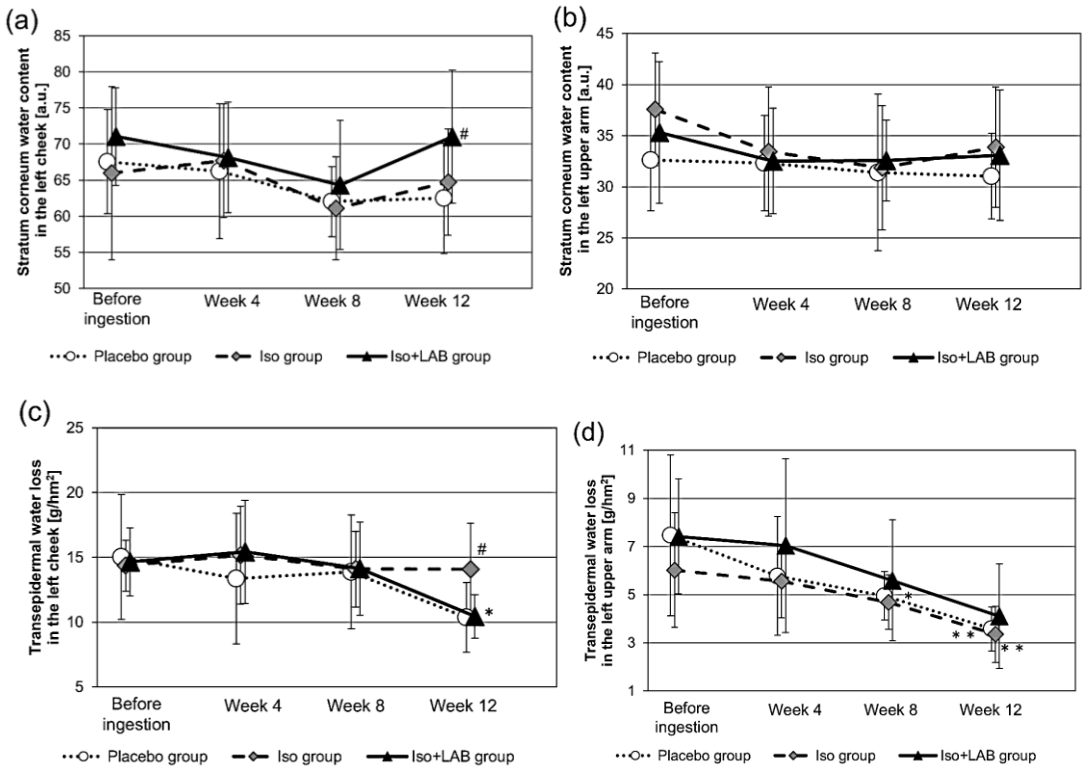


Fig. 2. Transition of stratum corneum water content and TEWL. Stratum corneum water content of (a) the left cheek or (b) the left upper arm, and TEWL of (c) the left cheek or (d) the left upper arm is shown. Each value is expressed as mean ± standard deviation.

($p<0.05$) vs. placebo group; * ($p<0.05$) vs. before intake; ** ($p<0.01$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

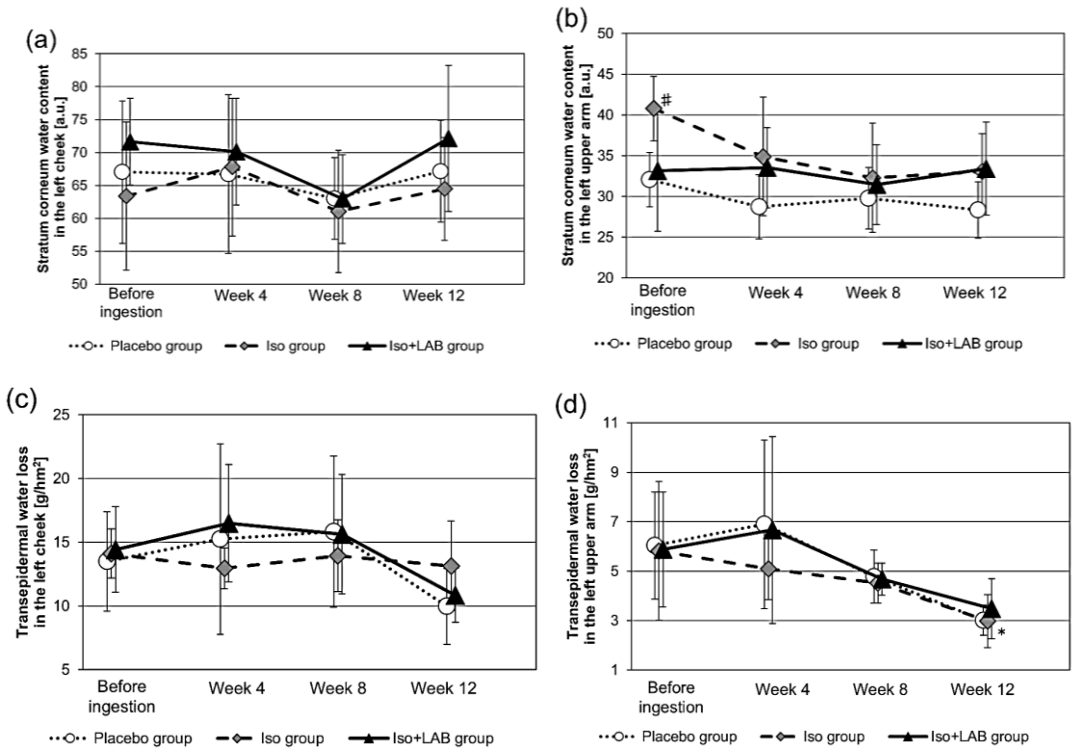


Fig. 3. Transition of stratum corneum water content and TEWL of equol producers. Stratum corneum water content of (a) the left cheek or (b) the left upper arm and TEWL of (c) the left cheek or (d) the left upper arm of equol producers is shown. Each value is expressed as mean ± standard deviation. # ($p<0.05$) vs. placebo group; * ($p<0.05$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

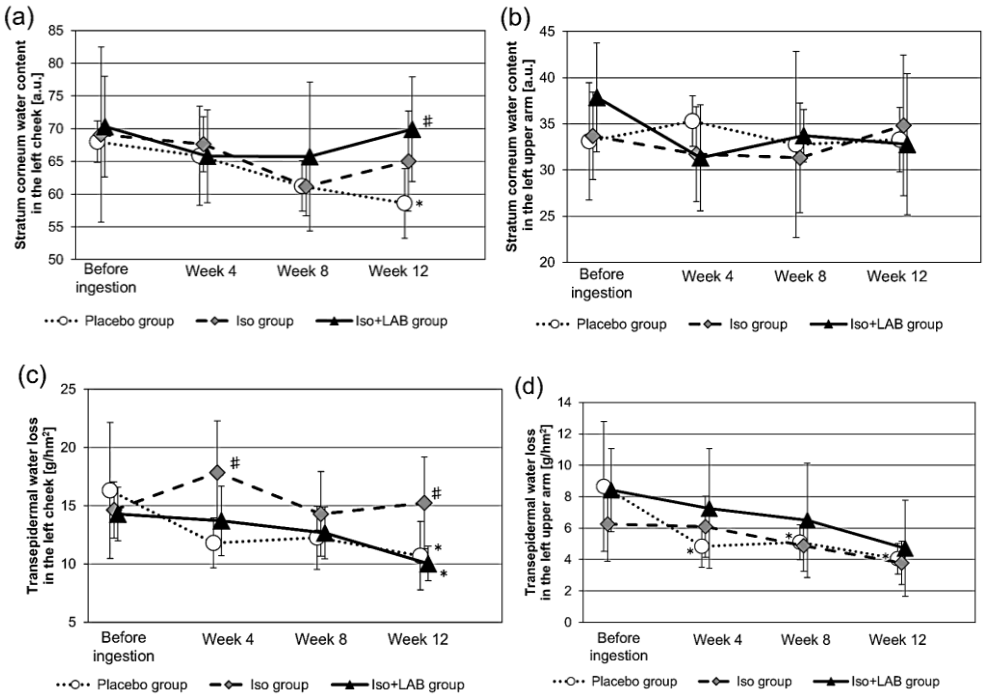


Fig. 4. Transition of stratum corneum water content and TEWL of equol non-producers. Stratum corneum water content of (a) the left cheek or (b) the left upper arm and TEWL of (c) the left cheek or (d) the left upper arm of equol non-producers is shown. Each value is expressed as mean ± standard deviation; #($p<0.05$) vs. placebo group; *($p<0.05$) vs. before intake; **($p<0.01$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

Subjective symptoms: The QOL and skin-related questionnaires are presented in Table S3 (Supplemental Online Material). Prior to ingestion, the "Early satiety" score in the Iso group was significantly lower than that in the placebo group, the "Dryness of the skin" score in the Iso+LAB group was significantly lower than that in the placebo group, and the "Makeup products are difficult to apply smoothly on the skin" score in the Iso+LAB group was significantly higher than that in the placebo group. When the groups were compared based on the duration of ingestion, the Iso group obtained a significantly lower score on the "Edematous" item (improved) at 4 weeks, the Iso+LAB group obtained a significantly higher score on the "A sense of tension" item (worsened) at 4 weeks, and the Iso+LAB group obtained a significantly higher score on the "Skin pores" item (worsened) at 12 weeks compared with the placebo group. When within-group comparisons were performed of pre-ingestion values, the Iso group obtained significantly lower scores on the "Eye fatigue" (improved), "Lethargy" (improved), and "Skin condition" (improved) items at 12 weeks, 12 weeks, and 8 and 12 weeks, respectively. No significant differences were found at any time point in any of the parameters in the Iso+LAB group.

Based on the ratings to the questionnaire items related to the QOL and skin condition of equol producers, prior to ingestion, the Iso and Iso+LAB groups obtained higher scores on the "Muscular pain/stiffness" item compared with the placebo group, the Iso+LAB group obtained higher scores on the "Palpitations" item compared with the placebo group, and the Iso+LAB group obtained lower scores on the "Dryness of the skin" item compared with the placebo group. When the groups were compared based on the duration of consumption, the Iso group obtained significantly higher scores on the "Increased susceptibility to colds" item (worsened) compared with the placebo group at 4 weeks, the Iso group obtained significantly lower scores (improved) on the "Edematous" item compared with the placebo group at 4 weeks, and the Iso group obtained significantly higher scores (worsened) on the "Irritability" item compared with the placebo group at 4 weeks. Within-group comparisons of pre-ingestion values were not significantly different at any time point in any of the parameters in the Iso and Iso+LAB groups (data not shown).

With regard to the questionnaire items related to the QOL and skin condition of the equol non-producers, the Iso+LAB group had significantly higher scores on the "Whether makeup products run easily on the skin" item compared with the placebo group and "Whether makeup products are difficult to apply smoothly on the skin" in the pre-ingestion period. However, the values of the groups were compared in the ingestion period; the Iso+LAB group showed significantly lower scores (improved) on the "Weight gain" item at 4 weeks, the Iso group obtained a significantly higher score (worsened) on the "Anorexia" item at week 8, the Iso group obtained a significantly higher score (worsened) on the "Hot flashes" item at 12 weeks, and the Iso group showed a significantly higher value (worsened) on the "Cold skin" item at 4 weeks compared with the placebo group. Based on the pre-ingestion values, no significant difference was found between the Iso and Iso+LAB groups at any time point in any of the parameters (data not shown).

Urinary isoflavone and equol concentrations: Fig. 5 shows changes in urinary daidzein, genistein, glycitein, and equol levels. Before ingestion, no significant differences were observed between the groups (Table 3).

When the changes in the duration of ingestion were compared between the groups, the daidzein levels were significantly higher in the Iso and Iso+LAB groups than in the placebo group at 4 and 12 weeks (Fig. 5a). The genistein levels were significantly higher in the Iso+LAB group than in the placebo group at 4 weeks (Fig. 5b). The glycitein levels were significantly higher in the Iso and Iso+LAB groups than in the placebo group at 4, 8, and 12 weeks (Fig. 5c). The equol levels were significantly higher in the Iso+LAB group than in the placebo and Iso groups (Fig. 5d).

Within-group comparisons of pre-ingestion values showed that the daidzein levels of the Iso group were significantly higher at 4, 8, and 12 weeks, whereas those of the Iso+LAB group were significantly higher at 4 weeks only (Fig. 5a). In terms of genistein levels, no significant differences were found between the groups at any time point (Fig. 5b). With regard to the glycitein levels, the Iso group showed significantly higher values at 4, 8, and 12 weeks, whereas the Iso+LAB group showed significantly higher values at 4 and 8 weeks only (Fig. 5c). In terms of equol levels, no significant differences were found at any time point between the groups (Fig. 5d).

The urinary daidzein, genistein, glycitein, and equol levels of the equol producers did not differ significantly between the groups (Table S1 [Supplemental Online Material]). When the changes in the duration of ingestion were compared between the groups, the daidzein levels of the Iso and Iso+LAB groups showed significantly higher values compared with the placebo group at 4 weeks (Fig. 6a). In terms of genistein, no significant between-group differences were observed at any time point (Fig. 6b). The glycitein levels of the Iso and Iso+LAB groups showed significantly higher values compared with the placebo group at 4 weeks, whereas those of the Iso group showed significantly higher values compared with the placebo group at 8 weeks (Fig. 6c). The equol levels of the Iso+LAB group showed significantly higher values compared with the placebo and Iso groups (Fig. 6d). Within-group comparisons of pre-ingestion showed that the daidzein levels of the Iso group had a significantly higher value at 8 weeks (Fig. 6a). In terms of genistein levels, the Iso+LAB group showed significantly higher values at 4 weeks (Fig. 6b). The Iso group showed significantly higher glycitein levels at 4 and 8 weeks (Fig. 6c). For the equol levels, the Iso+LAB group showed significantly higher values at 4 weeks (Fig. 6d).

The urinary daidzein, genistein, glycitein, and equol levels in the equol non-producers did not differ significantly between the groups (Table S2 [Supplemental Online Material]). When the changes in duration of ingestion were compared between the groups, the daidzein levels of the Iso+LAB group showed significantly higher values compared with the placebo group at 12 weeks (Fig. 7a). No significant differences were observed in the genistein levels between the groups at any time point. At 4 weeks, the glycitein of the Iso and Iso+LAB groups showed significantly higher values compared with the placebo group. At 12 weeks, the glycitein of the Iso group showed significantly higher values compared with the placebo group (Fig. 7c). In terms of equol levels, no significant differences were observed between the groups at any time point (Fig. 7d). Within-group comparisons of pre-ingestion values showed that the daidzein levels were significantly higher in the Iso group at 4 and 8 weeks (Fig. 7a). The genistein levels in the Iso group were significantly higher at 4 weeks (Fig. 7b). The glycitein levels were significantly higher in the Iso group at 4, 8, and 12 weeks (Fig. 7c). No significant differences were observed in the equol levels between the groups at any time point (Fig. 7d).

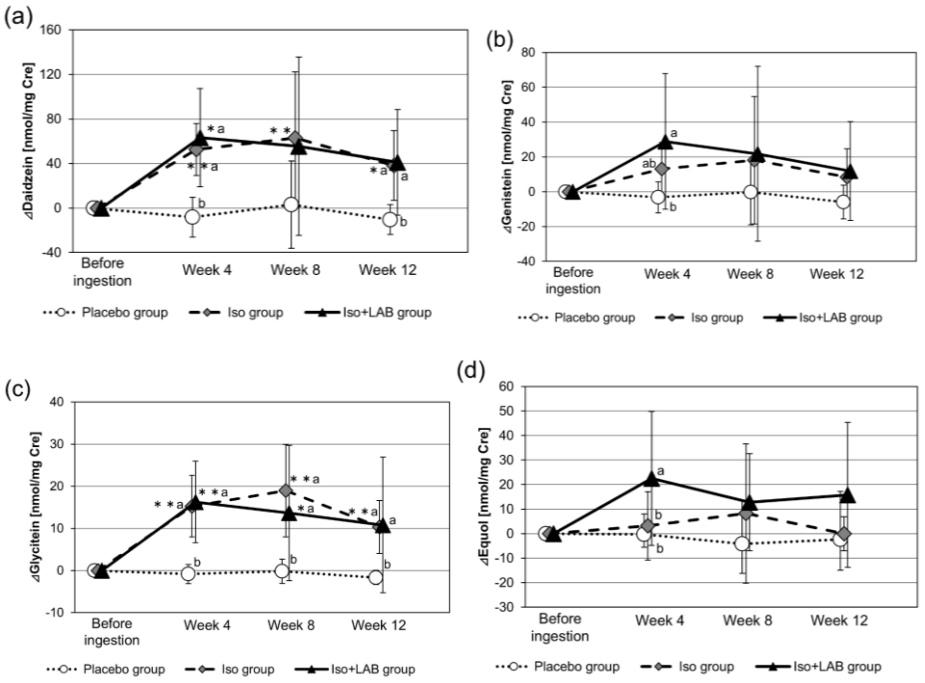


Fig. 5. Changes in the urinary levels of soy isoflavones.

Changes in the urinary levels of (a) daidzein, (b) genistein, (c) glycitein, and (d) equol during the intake period compared to before consumption of the tested foods are shown. Each value is expressed as mean ± standard deviation. Alphabets that differed at each time point were significantly different between the study groups ($p<0.05$). *($p<0.05$), **($p<0.01$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

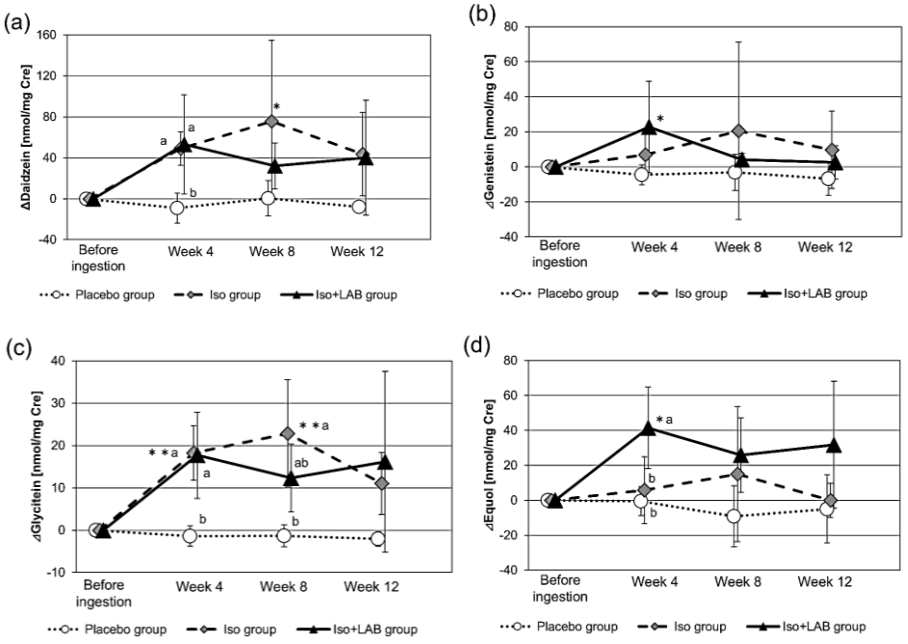


Fig. 6. Changes in the urinary levels of soy isoflavones of equol producers. Changes in the urinary levels of (a) daidzein, (b) genistein, (c) glycitein, and (d) equol of equol producers during the intake period compared to before consumption of the tested foods are shown. Each value is expressed as mean ± standard deviation. Alphabets that differed at each time point were significantly different between the study groups ($p<0.05$); *($p<0.05$), **($p<0.01$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

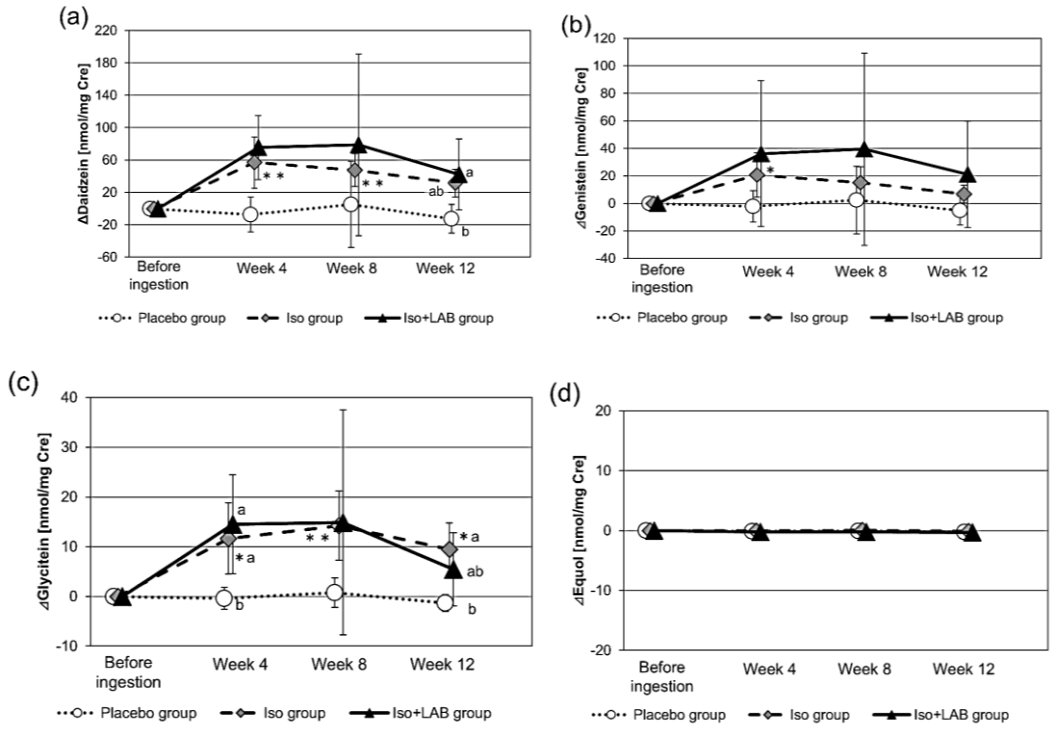


Fig. 7. Changes in the urinary levels of soy isoflavones of equol non-producers. Changes in the urinary levels of (a) daidzein, (b) genistein, (c) glycitein, and (d) equol of equol non-producers during the intake period compared to before consumption of the tested foods are shown. Each value is expressed as mean ± standard deviation. Alphabets that differed at each time point were significantly different between the study groups ($p<0.05$); *($p<0.05$), **($p<0.01$) vs. before intake; Iso, isoflavones; LAB, lactobionic acid.

Dietary survey: Weekly dietary intake of soy isoflavones (as aglycone equivalents) is shown in Table S4 (Supplemental Online Material). There were no significant differences between groups before intake or during the study period. There were also no significant differences in within-group comparisons with pre-intake values at all time points in each group.

Safety: One participant in the Iso+LAB group experienced an adverse event during the interventional phase, which was judged by the physician to be unrelated to the study diet. Therefore, it was concluded that the soy isoflavone- and lactobionic acid-containing foods had no safety-related problems under the conditions of this study.

DISCUSSION

Compared with the placebo group, the Iso+LAB group showed significantly improved stratum corneum water content in the left cheek at 12 weeks (Fig. 2a). By contrast, the Iso group did not show significant improvements in the left cheek and left upper arm compared with the placebo group (Fig. 2a and b). These results indicate that the effect of soy isoflavone ingestion at a low daily dose of 25 mg (in terms of aglycones) on the stratum corneum water content can be achieved by the simultaneous ingestion of lactobionic acid. However, no significant differences were observed between the Iso, Iso+LAB, and placebo groups in terms of the stratum corneum water content in the left upper arm at any time point (Fig. 2b). In vivo confocal Raman spectroscopy has shown that the cheek possesses a thinner stratum corneum than the volar forearm and exhibits distinct depth profiles of water and water-binding constituents; moreover, deeper hydration displays relatively low seasonal variability at the cheek compared with the forearm [32]. Therefore, these features make hydration changes more readily detectable at the cheek than at the upper arm. In addition, seasonal and environmental

factors may also help explain the site-specific differences observed in this study, which was conducted from early autumn through early winter. It has shown that the volar forearm exhibits greater seasonal fluctuations in hydration-related depth profiles than the cheek, where deeper stratum corneum hydration tends to remain comparatively stable [32]. Such variability at the forearm may obscure modest intervention-related changes during drier months. On the other hand, TEWL, a measure of skin barrier function, was not significantly improved in either the Iso or Iso+LAB groups compared with that in the placebo group (Fig. 2c and d). In the study by Akagi *et al*. [27], the mean left cheek stratum corneum water content at baseline was 48.1 [a.u.]; in the present study, the mean left cheek stratum corneum water content at baseline was relatively high (68.2 [a.u.]), and many of the participants had less severe skin dryness. This may have caused the lack of improvement in TEWL.

Among the equol producers, the urinary equol levels were significantly higher in the Iso+LAB group than in the placebo and Iso groups at 4 weeks. Moreover, the urinary equol levels in the Iso+LAB group were consistently higher than those in the placebo and Iso groups at 8 and 12 weeks, although the difference was not significant (Fig. 6d). This finding suggests that co-ingestion of soy isoflavones and lactobionic acids promoted equol metabolism from soy isoflavones in equol producers. In a study conducted by Akagi *et al*. [27], unlike the findings of this study, urinary equol levels were significantly decreased when the food containing soy isoflavone and lactobionic acid was consumed for 8 weeks compared with the urinary equol levels in the food loaded with soy isoflavones alone during the pre-test. However, Akagi *et al*. [27] conducted a study involving the Iso+LAB group and the placebo group but did not establish a group that would continue to consume soy isoflavones alone. Therefore, it was difficult to accurately assess the effect of lactobionic acid on equol production. Additionally, 24-hour urine samples were used in the study by Akagi *et al*. [27], whereas early morning urine samples were used in this study, which may have influenced the differences in the results. Therefore, further validation of findings is necessary by conducting new human studies. In the Iso+LAB group of equol non-producers, the urinary equol levels remained largely unchanged during ingestion compared with those during pre-ingestion (Fig. 7d), and none of the participants was able to produce equol even when soy isoflavones and lactobionic acid were simultaneously consumed continuously for 12 weeks. The continued consumption of soybean foods and soybean isoflavones does not readily alter the equol-producing phenotype [33]. When soy isoflavones and lactobionic acid are simultaneously consumed, it is possible that the improved equol production ability associated with lactobionic acid intake was only observed among individuals who already had a certain level of equol production ability. It has been reported that indigestible oligosaccharides such as difructose anhydride III and fructooligosaccharides increase serum equol levels in rats when ingested concurrently with soy isoflavones [34, 35]. Lactobionic acid, an indigestible oligosaccharide, may also increase the equol levels, as observed with these saccharides; however, the mechanism by which these saccharides are involved in isoflavone metabolism has not been clarified.

For the urinary isoflavones, the daidzein levels were 1.3–1.6 times higher in the Iso+LAB group of equol non-producers than in the Iso group (Fig. 7a), whereas the urinary genistein levels were 1.8–2.6 times higher (Fig. 7b), suggesting that lactobionic acid enhances the uptake of daidzein and genistein. By contrast, the Iso+LAB group of equol producers did not exhibit higher urinary isoflavone levels compared to the Iso group (Fig. 6a-c). Equol is produced by the intestinal bacteria from daidzein in equol producers. The urinary equol level was significantly higher at 4 weeks in the Iso+LAB group than in the Iso group (Fig. 6d). Therefore, in equol producers, daidzein may be converted to equol more than it is

absorbed. The amount of soy isoflavones ingested from the diet did not differ between groups at each time point, and there were no significant differences between the groups in comparison to before ingestion (Table S4 [Supplemental Online Material]). Therefore, the behavior of urinary isoflavones was considered to be poorly related to dietary soy isoflavone intake. In a study by Akagi *et al*. [27], the urinary isoflavone levels were significantly increased at 12 weeks in equol producers when consuming foods containing soy isoflavone and lactobionic acid compared with the pre-tested urinary equol levels. Taken together, for equol producers, the co-ingestion of soy isoflavones and lactobionic acid may promote the absorption of isoflavones and the production of equol, although further validation is required. With regard to the mechanism underlying the promotion of isoflavone absorption, the effective prebiotic action of the lactobionic acid is considered. In a previous study examining the effects of genistein or genistin (glycoside of genistein), genistein was administered orally to rats. The blood levels of genistein increased within 2 h after administration, and soy isoflavone aglycones were absorbed more rapidly than glycosides [36]. Fructooligosaccharides are indigestible oligosaccharides that activate the intestinal bacterial β-glucosidases, induce the conversion of isoflavone glycosides to aglycones through glycolysis [35], and promote the colonic absorption of genistein [37] in rats. Stachyose, an indigestible oligosaccharide, inhibits the degradation of genistein by intestinal bacteria, thus contributing to the increased bioavailability of soy isoflavones [38]. However, because it has a prebiotic function, only a few studies have investigated the effect of lactobionic acid on soy isoflavone absorption [25]. Given that soy isoflavones used in this study were mostly glycosides, the absorption of soy isoflavones was improved through a mechanism similar to that of indigestible sugars.

As described above, the co-ingestion of soy isoflavones and lactobionic acid improved the absorption of daidzein and genistein by equol non-producers, resulting in an improvement in the stratum corneum water content. In equol producers, no significant improvement was observed in the stratum corneum water content despite the increase in urinary equol levels; however, at 12 weeks, it was higher than the values in the placebo and Iso groups, resulting in a significant improvement in stratum corneum water content in all participants. Although the estrogen receptor-mediated biomodulatory function of soy isoflavones is more effective among equol producers [39-41], lactobionic acid promotes the uptake of soy isoflavones; therefore, the ingestion of soy isoflavones at low doses (25 mg) in combination with lactobionic acid could enhance the efficacy of soy isoflavones regardless of the equol producing phenotype.

CONCLUSIONS

In conclusion, consecutive co-ingestion of soy isoflavones (25 mg [aglycone equivalents]) and lactobionic acid (250 mg) for 12 weeks improved the stratum corneum water content. The mechanism suggested that lactobionic acid may promote equol production in equol-producers and enhance isoflavone absorption in non-producers, thereby providing skin-hydration benefits regardless of equol-producing phenotype.

List of Abbreviations: QOL: quality of life, TEWL: transepidermal water loss

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Authors' contributions: K.H. performed a part of the

experiments and wrote the manuscript. A.K. designed the study and performed most of the experiments. S.Y., F.N., K.O., Y.U., and K.M. designed the study. T.M. edited the manuscript. T.S. conceived, designed, and supervised the study.

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Data availability: The data underlying this article will be shared at a reasonable request to the corresponding author.

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