

Effect of Mandarin Orange Yogurt on Allergic Conjunctivitis Induced by Conjunctival Allergen Challenge

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PURPOSE. To evaluate the effects of mandarin orange yogurt containing nobiletin and β -lactoglobulin on the allergic conjunctivitis induced by a conjunctival allergen challenge (CAC).

METHODS. Experiment 1 was performed on 26 asymptomatic patients (age, 25.3 ± 5.3 years) with proven seasonal allergic conjunctivitis due to cedar pollen. We compared the degree of conjunctivitis induced by CAC before and after ingesting mandarin orange yogurt for 2 weeks. Experiment 2 was a double-blind, placebo-controlled trial performed on 31 patients (age, 32.5 ± 12.2 years). A diet containing mandarin orange yogurt was compared to a diet containing yogurt lacking the mandarin orange on the conjunctivitis induced by CAC. The temperature of the inferior bulbar conjunctiva was measured before and 20 minutes after the CAC with an ocular surface thermographer (OST). The degree of conjunctival injection and chemosis was graded by slit-lamp biomicroscopy. The changes in the symptoms were evaluated by a questionnaire.

RESULTS. In experiment 1, the scores of redness (3.07 ± 3.03 vs. 1.05 ± 1.70), chemosis (2.84 ± 2.27 vs. 0.81 ± 1.11), itching (4.34 ± 3.05 vs. 1.39 ± 2.12), and temperature ($0.73 \pm 0.42^\circ\text{C}$ vs. $0.45 \pm 0.43^\circ\text{C}$) were significantly lower ($P < 0.001$) after a diet of mandarin orange yogurt for 2 weeks. In experiment 2, the scores of redness (1.03 ± 0.18 vs. 1.28 ± 0.52 ; $P = 0.0156$), itching (1.93 ± 1.92 vs. 2.82 ± 2.21 ; $P = 0.0133$), and surface temperature ($0.54 \pm 0.21^\circ\text{C}$ vs. $0.31 \pm 0.25^\circ\text{C}$; $P < 0.001$) were significantly lower in the mandarin orange yogurt group than in the control yogurt group.

CONCLUSIONS. Mandarin orange yogurt can be an effective nutritional intervention for allergic conjunctivitis.

Keywords: allergy, CAC test, functional food, nobiletin, β -LG

Allergic conjunctivitis is a common disease seen in outpatient clinics by allergists and ophthalmologists. The hallmark symptoms and signs of allergic conjunctivitis are ocular itching, conjunctival redness, ocular chemosis, mucous discharge from the eye, feeling of heat, and eyelid swelling.¹ The incidence of allergic conjunctivitis and allergic disorders has increased in the developed countries, and a variety of factors, for example, the environment, hygiene, diet, genetics, and other factors, are recognized to be the causes of the allergies.²⁻⁴ It is also known that complex immune cell interactions are involved in either the sensitization to the allergens or the help in alleviating the allergic manifestations.^{5,6}

Medications including antihistamines, steroids, and immunosuppressive drugs are used to treat the allergic symptoms and signs. However, there is concern on the unavoidable side effects of these drugs such as drowsiness for antihistamine drugs, and immunosuppressive effects and steroid-induced glaucoma for the glucocorticoids. Because of these side effects, antiallergic drugs have drawn concerns not only from medical providers but also from patients.

Numerous clinical trials have been performed that focused on nutritional interventions as a possible way to alleviate the antiallergic effects.⁷⁻¹⁰ Several studies have shown that

consumption of fermented foods, such as yogurt, can alleviate the allergic symptoms, and lactic acid bacteria contained in fermented foods can enhance the systemic production of type I and type II interferons.¹¹ It has also been demonstrated that the flavonoids can inhibit the degranulation of mast cells, and can reduce the release of histamine, tryptase, interleukin-6 (IL-6), and IL-8 from cultured mast cells.¹² In addition, it has been demonstrated that the flavonoids can inhibit the production of histamine, IL-4, and IL-13 by human basophils, suggesting that they may have potent anti-inflammatory and antiallergic activities.^{12,13}

We have been focusing on nobiletin and β -lactoglobulin as possible candidates for treating allergies due to their antiallergic effects. Nobiletin, a flavonoid containing six methoxy groups, is a major component of citrus fruits. Nobiletin has been reported to have anti-inflammatory, antitumorigenic, and antidiabetic activities,¹⁴ and it can suppress the degranulation of rat basophilic leukemia RBL-2H3 cells through the downregulation of the phosphorylation of Akt.¹⁵ Nobiletin can also reduce the release of cytokine and β -hexosaminidase from human intestinal mast cells stimulated by IgE.¹⁶

We have reported that the treatment of allergic conjunctivitis with nobiletin and β -lactoglobulin (LG) can suppress the



TABLE. The Contents of Nobiletin and β -LG in the Components of the Mandarin Orange Yogurt and Control Yogurt

Ingredients of Yogurt	Mandarin Orange Yogurt	Control Yogurt
β -LG	150.00 mg	93.00 mg
Nobiletin	0.53 mg	0.00 mg

Mandarin orange yogurt and control yogurt created by Shikoku Nyugyo Corporation.

increase in the degranulation of RBL-2H3 cells by the simultaneous inhibition of phosphatidylinositol 3-kinase (PI3K) and spleen tyrosine kinase (Syk) phosphorylation. In addition, the concurrent administration of nobiletin and β -LG strongly reduced the passive cutaneous anaphylaxis reaction in mice.¹⁷

Based on these *in vivo* and *in vitro* experiments, we prepared mandarin orange yogurt containing nobiletin and β -LG, and evaluated the effect of a diet containing mandarin orange yogurt on allergic conjunctivitis in humans. Although many clinical trials have been conducted worldwide of the effects of antiallergic drugs on allergic conjunctivitis, an objective verification of the effect has not been performed.¹⁸

We have shown that the temperature of the conjunctival surface is significantly higher in eyes with allergic conjunctivitis.¹⁹ The temperature was measured with an ocular-specific thermographer called the ocular surface thermographer (OST). The OST can measure the surface temperature of the eye noninvasively, and the validity and repeatability of the OST have been demonstrated in patients with glaucoma and dry eyes.^{20,21} We have also demonstrated that the OST can be used to evaluate the efficacy of topical antiallergic agents against allergic conjunctivitis objectively.¹⁹

The purpose of this study was to determine whether a diet containing mandarin orange yogurt supplemented with nobiletin and β -LG can alleviate the signs and symptoms of allergic conjunctivitis. To accomplish this, we induced conjunctivitis by a conjunctival allergen challenge (CAC), and graded the eyes by slit-lamp biomicroscopy and measured the surface temperature of the eye with the OST.

METHODS

The purpose of the study and the procedures to be used were presented to all of the subjects, and a signed informed consent was obtained from each. This study was approved by Institutional Review Board of Ehime University, and the procedures used conformed to the tenets of the Declaration of Helsinki.

Subjects

The subjects were healthy, asymptomatic volunteers with clinically diagnosed seasonal allergic conjunctivitis due to cedar pollen, and all subjects had symptoms of allergic conjunctivitis during the Japanese cedar pollen season. Subjects were excluded if they had any ocular disorders including inflammation of the conjunctiva, cornea, and iris. All of the subjects were not using topical or contraindicated systemic medications for more than 3 months prior to the beginning of the tests.

Mandarin Orange Yogurt and Control Yogurt

Mandarin orange yogurt and control yogurt were created by the Shikoku Nyugyo Corporation (Toon, Ehime, Japan). The

amount of β -LG in a 150-g bottle of mandarin orange yogurt was 150 mg, and that of nobiletin was 0.53 mg. In the control yogurt, the amount of β -LG was 93 mg and that of nobiletin was 0.0 mg (Table). The bacterial strains used in the yogurt were *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* (LA-5), and *Bifidobacterium lactis* BB-12. The subjects consumed one bottle of mandarin orange yogurt or control yogurt each day for 2 or 3 weeks before the CAC.

Conjunctival Allergen Challenge

The CAC was performed to induce type 1 allergic conjunctivitis. The allergen solution used for the CAC test was the Allergen Scratch Extract Torii Cedar Pollen for Diagnostic Use (Torii, Tokyo, Japan). The allergen solution and the control solution were diluted with phosphate-buffered saline by 100-fold, 50-fold, or 25-fold. To determine the optimal concentration of the antigen solution, 20 μ L of the lowest dilution of the allergen was dropped onto the conjunctiva of the right eye and the same volume at the same dilution of the control solution was dropped onto the left eye. If no reaction occurred in the right eye within 10 minutes, a higher concentration of the allergen was dropped on the conjunctiva. This was repeated every 10 minutes until a positive reaction was detected. Subjects who failed to respond to any dilution of the allergen in the right eye or responded to the control solution in the left eye were excluded from the study.

To score the slit-lamp findings and ocular symptoms, a visual analog scale (VAS) was created. The scale ranged from 0 = no reaction to 10 = most severe reaction.

The graders were masked to whether the subject had ingested the experimental or control yogurt.

Ocular Surface Thermographer

The ocular surface thermographer TG-1000 (TOMEY Corporation, Nagoya, Japan) can measure the ocular surface temperature by a noncontact method.^{20,21} The conjunctival temperature was measured with the OST in a standard clinical room maintained at a temperature of $26.5 \pm 1.5^\circ\text{C}$ and a humidity of $42.5 \pm 2.5\%$ as reported.²¹ The subjects were allowed to blink normally, and then they were asked to close both eyes for 5 seconds, and then open their eyes and look upward. The inferior bulbar conjunctival temperature was measured immediately after the eye was opened. The conjunctival vessels were used as markers for the alignment, and the average conjunctival temperature of the inferior bulbar conjunctiva in a circle of 4 mm was analyzed.

Experiment 1

Subjects. Twenty-six subjects were enrolled in the first experiment. There were 13 men and 13 women with a mean $\pm$ standard deviation age of 25.30 ± 5.31 years and a range of 20 to 44 years.

CAC-Induced Conjunctivitis Before and After Mandarin Orange Yogurt Ingestion (Fig. 1). The initial CAC measurements were made more than a week after determining the optimal antigen solution concentration. Before the CAC application, the subjects underwent slit-lamp examinations and measurements of the temperature of the lower conjunctival surface with the OST. These findings were used as the baseline values. Then, 20 μ L of the appropriate solution was dropped into the conjunctival sac of the right eye and the same volume at the same dilution of the control solution was dropped into the left eye. Twenty minutes after the instillation, the intensity of conjunctivitis was determined by slit-lamp biomicroscopy,

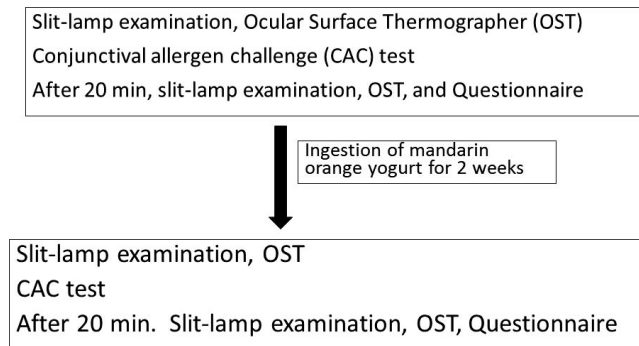


FIGURE 1. Protocol of experiment 1. The first experiment was conducted more than a week after the day on which the optimal antigen solution concentration was determined. Initially, each subject underwent a slit-lamp examination and measurements of the lower conjunctival surface temperature with the ocular surface thermographer (OST). Twenty minutes after the application of a conjunctival allergen challenge (CAC), the degree of conjunctivitis was determined by slit-lamp biomicroscopy and the temperature was measured by the OST. Subjects also reported the degree of ocular itching after the CAC. After ingesting mandarin orange yogurt for 2 weeks, subjects underwent the CAC, and the effect of ingesting mandarin orange yogurt was evaluated by slit-lamp biomicroscopy, OST, and the answers to a questionnaire.

and the temperature was measured by the OST. Subjects also reported the degree of ocular itching after the CAC.

After ingesting 150 g/day of mandarin orange yogurt for 2 weeks, subjects were stimulated with the CAC, and the effects of ingesting the mandarin orange yogurt were evaluated by slit-lamp biomicroscopy and the OST in same way. The findings at this time were compared to the baseline values.

Experiment 2

Thirty-one subjects were enrolled in the second clinical trial. There were 7 men and 24 women with a mean age of 32.5 ± 12.2 years and a range of 20 to 64 years. All of the subjects had a positive result for cedar pollen in the assay for antigen-specific IgE antibodies using the capsulated hydrophilic carrier polymer-radioallergosorbent test (CAP-RAST) method.

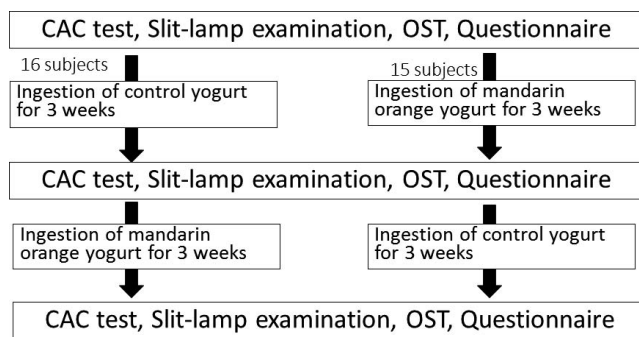


FIGURE 2. Protocol of experiment 2. The first test of experiment 2 was conducted more than a week after the day on which the optimal antigen solution concentration was determined. Slit-lamp examination and measurements of the conjunctival temperature were performed before and after the CAC application. Subjects were randomly assigned to ingest mandarin orange yogurt or control yogurt for 3 weeks. After ingesting the mandarin orange or control yogurt for 3 weeks, the CAC was applied and the measurements were repeated. Then, the subjects were directed to ingest mandarin orange yogurt or control yogurt, which were different from the first 3 weeks, for an additional 3 weeks. Then, the CAC was applied, and the measurements were repeated.

Comparison of Mandarin Orange Yogurt and Control Yogurt Ingestion (Fig. 2). The eyes were tested more than a week after the optimal antigen solution concentration was determined. Slit-lamp examinations and measurements of the conjunctival temperature were performed before and after the CAC as in experiment 1. Subjects were then randomly assigned to ingest mandarin orange yogurt or control yogurt for 3 weeks and then underwent the same testing protocol. The subjects were then directed to ingest mandarin orange yogurt or control yogurt, which was different from the first 3 weeks, for 3 weeks. Experiment 2 was a crossover and double-blind trial.

Statistical Analyses

All data are expressed as the means $\pm$ standard deviation of the means. In experiment 1, Wilcoxon matched pairs signed-rank tests were used to compare the clinical scores after each CAC test. Paired *t*-tests were used to determine the significance of the differences of the conjunctival temperature before and after the CAC test. In experiment 2, Friedman tests and Scheffe multiple contrast tests were used.

RESULTS

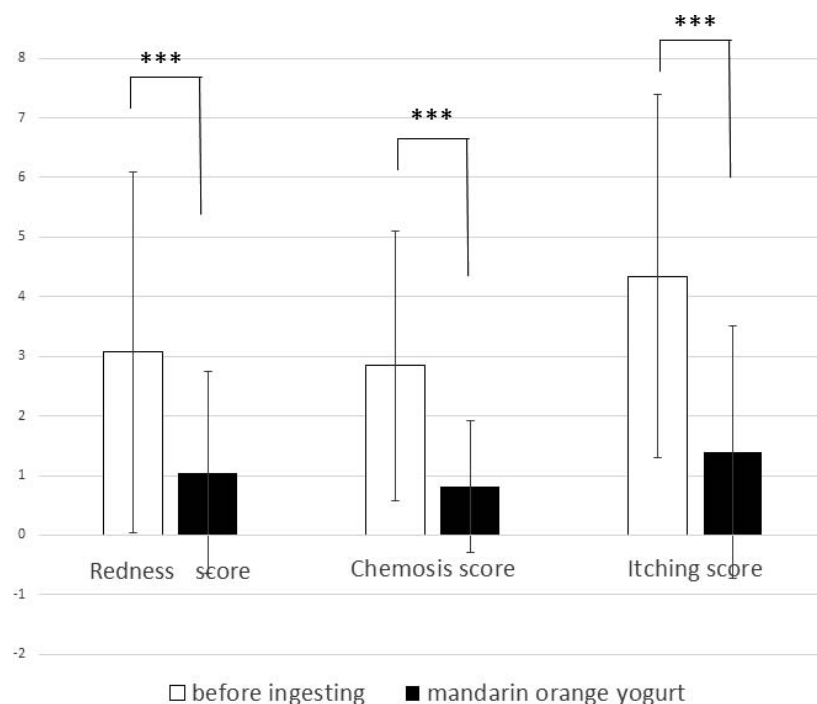
Experiment 1

Effect of Mandarin Orange Yogurt on Conjunctivitis Induced by CAC (Fig. 3). Before the CAC, none of the subjects had signs or symptoms of allergic conjunctivitis. The average score for redness of the bulbar conjunctiva was 3.07 ± 3.03 after the CAC and before ingesting mandarin orange yogurt. The average redness score was significantly decreased to 1.05 ± 1.70 after ingesting the mandarin orange yogurt for 2 weeks ($P < 0.001$; Wilcoxon signed-rank test). The average chemosis score of the bulbar conjunctiva was 2.84 ± 2.27 before, and it was significantly decreased to 0.81 ± 1.11 after, ingestion of the mandarin orange yogurt ($P < 0.001$; Wilcoxon signed-rank test). The mean itching score before was 4.34 ± 3.05 , and it was significantly decreased to 1.39 ± 2.12 after ingesting the mandarin orange yogurt ($P < 0.001$; Wilcoxon signed-rank test).

Effect of Mandarin Orange Yogurt on Conjunctival Surface Temperature After CAC (Fig. 4). The mean surface temperature of the inferior bulbar conjunctiva before the CAC was $34.65 \pm 0.46^\circ\text{C}$, and there was no difference between the right and left eyes. The surface temperature after the CAC was $35.26 \pm 0.37^\circ\text{C}$ before ingesting the mandarin orange yogurt, and it was $35.06 \pm 0.46^\circ\text{C}$ after ingesting the mandarin orange yogurt for 2 weeks. The increase in the temperature after CAC was significantly less after ingesting the mandarin orange yogurt ($0.45 \pm 0.43^\circ\text{C}$) than before ingesting the mandarin orange yogurt ($0.73 \pm 0.42^\circ\text{C}$; $P < 0.001$; paired *t*-test).

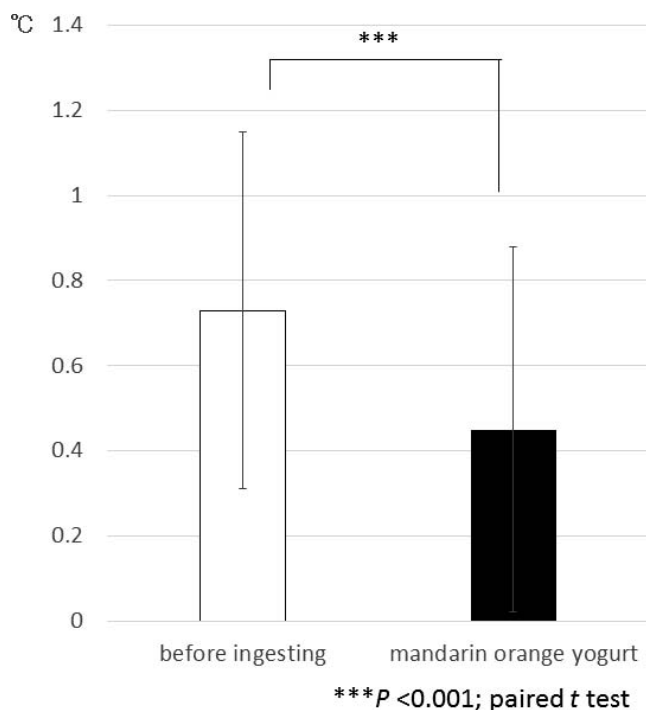
Experiment 2

Effect of Mandarin Orange Yogurt and Control Yogurt on Conjunctivitis Induced by CAC (Figs. 5, 6). Before ingesting the control yogurt or mandarin orange yogurt, the average score of redness was 1.90 ± 0.78 , the chemosis score was 2.62 ± 2.81 , and the itching score was 3.10 ± 2.85 . After ingesting the control yogurt, the average score of redness was 1.28 ± 0.52 , the chemosis score was 1.77 ± 1.94 , and the itching score was 2.82 ± 2.21 . The ingestion of the control yogurt reduced the redness score significantly ($P < 0.001$; Friedman test, Scheffe multiple contrasts test), but did not significantly reduce the scores of chemosis and itching. After ingesting the mandarin orange yogurt, the average score of redness was 1.03 ± 0.18 , the chemosis score was 1.34 ± 1.63 ,



*** $P < 0.001$; Wilcoxon signed-rank test

FIGURE 3. Effect of mandarin orange yogurt on clinical scores after CAC in experiment 1. All clinical scores are significantly lower after ingesting mandarin orange yogurt ($P < 0.001$; Wilcoxon signed-rank test).



*** $P < 0.001$; paired t test

FIGURE 4. Effect of mandarin orange yogurt on conjunctival temperature after the CAC for experiment 1. The change in conjunctival temperature was also significantly lower after ingesting mandarin orange yogurt ($P < 0.001$; paired t -test).

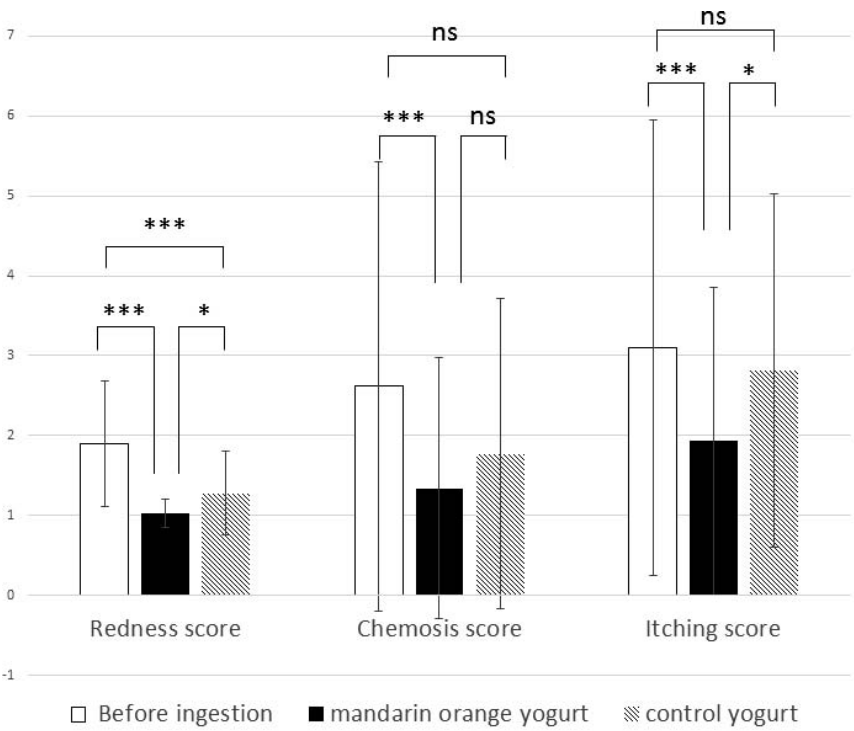
and the itching score was 1.93 ± 1.92 . The ingestion of the mandarin orange yogurt significantly decreased the redness score, the chemosis score, and the itching score ($P < 0.001$; Friedman test, Scheffe multiple contrasts test). In addition, the redness and itching scores in the mandarin orange yogurt group were significantly lower than those of the control yogurt group. There was no significant difference between the group ingesting mandarin orange yogurt in the first half and the group ingesting it in the second half.

Experiment 2

Effect of Mandarin Orange Yogurt and Control Yogurt on Conjunctival Surface Temperature After CAC (Figs. 6, 7). The mean surface temperature of the inferior bulbar conjunctiva before the CAC was $34.64 \pm 0.56^\circ\text{C}$, and it was $34.50 \pm 0.57^\circ\text{C}$ 3 weeks after ingesting the control yogurt and $34.64 \pm 0.68^\circ\text{C}$ after ingesting mandarin orange yogurt (all $P > 0.05$). The surface temperature after the CAC was $35.30 \pm 0.49^\circ\text{C}$, and it was $35.02 \pm 0.51^\circ\text{C}$ after ingesting the control yogurt and $34.64 \pm 0.66^\circ\text{C}$ after ingesting the mandarin orange yogurt. The increase in the surface temperature after CAC, control yogurt ($0.53 \pm 0.21^\circ\text{C}$; $P = 0.030$; Friedman test, Scheffe multiple contrasts test), and mandarin orange yogurt ($0.31 \pm 0.25^\circ\text{C}$; $P < 0.001$; Friedman test, Scheffe multiple contrasts test) was significantly lower than that before the ingestion ($0.67 \pm 0.30^\circ\text{C}$). In addition, the surface temperature of the mandarin orange yogurt group was significantly lower than that of the control yogurt group ($P = 0.0025$; Friedman test, Scheffe multiple contrasts test).

DISCUSSION

In two of our earlier studies, we demonstrated that both nobiletin and β -LG reduced allergic responses.^{22,23} A combi-



** $P < 0.05$ *** $P < 0.001$; Friedman test, Scheffe Multiple contrasts test

FIGURE 5. Effect of mandarin orange yogurt and control yogurt on clinical scores after CAC in experiment 2. The redness, chemosis, and itching scores were significantly lower after ingestion of the mandarin orange yogurt. In addition, the redness score and itching score of the subjects in the mandarin orange yogurt group were significantly lower than in the control yogurt group (** $P < 0.05$, *** $P < 0.001$; Friedman test, Scheffe multiple contrasts test).

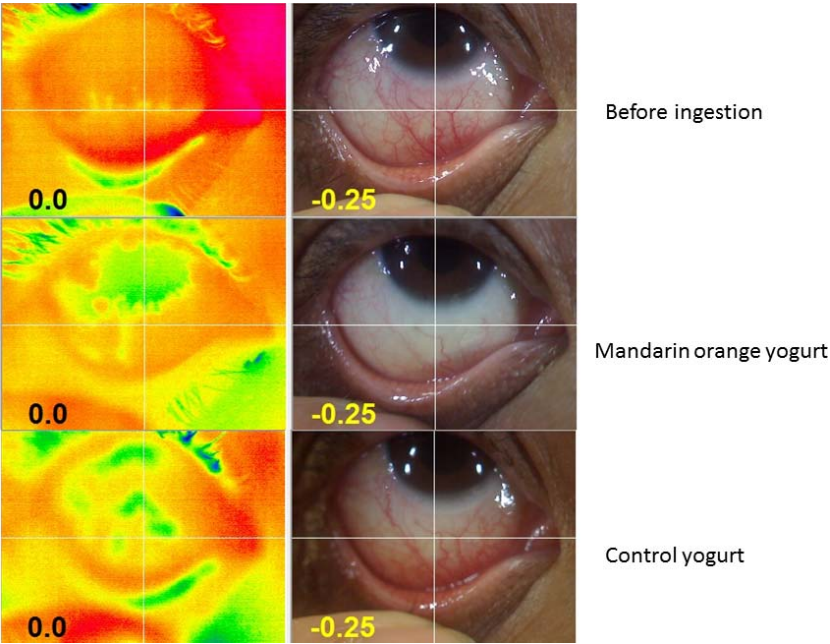
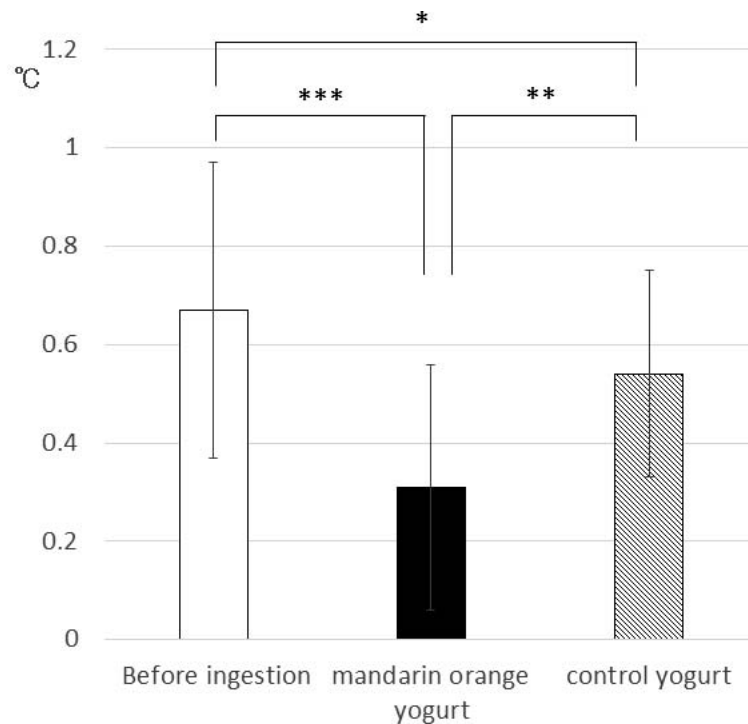


FIGURE 6. Effect of mandarin orange yogurt and control yogurt on conjunctivitis assessed by OST. Representative thermographic and photographic images after CAC by OST before ingestion (*upper*), after ingestion of mandarin orange yogurt (*middle*), and after ingestion of control yogurt (*lower*). The surface temperature of the inferior bulbar conjunctiva was 34.72°C (before ingestion), 34.37°C (mandarin orange yogurt), and 34.58°C (control yogurt).



* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$; Friedman test, Scheffe Multiple contrasts test

FIGURE 7. Effect of mandarin orange yogurt and control yogurt on conjunctival temperature after the CAC in experiment 2. The increase in temperature after the CAC was significantly less than that before the ingestion. In addition, the surface temperature of the mandarin orange yogurt group was significantly lower than in the control yogurt group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Friedman test, Scheffe multiple contrasts test).

nation of nobiletin and β -LG also reduced the degranulation of RBL-2H3 cells more strongly than either nobiletin or β -LG alone.¹⁷ The mechanism for these reactions was their ability to simultaneously inhibit PI3K and spleen tyrosine kinase (Syk) phosphorylation. PI3K plays an important role in the intracellular trafficking of actin polymerization and growth factor signaling.^{24,25} PI3K is also responsible for activating the signaling pathways when it is stimulated by an antigen.²⁶ The activity of PI3K is required for releasing the granular substances from mast cells. Syk is an enzyme that plays a critical role in the regulation of immune and inflammatory responses. It was recently established that Syk is involved in various signaling cascades including those originating from integrin and cytokine receptors.^{27,28}

In addition to the results of these *in vitro* studies, we found that the concurrent administration of nobiletin and β -LG strongly reduced the passive cutaneous anaphylaxis reaction in mice *in vivo*.¹⁷ In a similar fashion, the concurrent administration of freeze-dried citrus unshiu peel powder and yogurt whey containing nobiletin and β -LG also strongly reduces the passive cutaneous anaphylaxis reaction in a cedar pollinosis mouse model.¹⁷ An antiallergic effect was obtained in the cedar pollinosis mouse model by an oral ingestion of processed food products.

Based on these results on the effects of nobiletin and β -LG on the allergic conjunctivitis in humans, we prepared the mandarin orange yogurt containing nobiletin and β -LG in cooperation with Shikoku Nyugyo Corporation. In the first experiment, the effect of mandarin orange yogurt was evaluated by the slit-lamp findings of the ocular signs and the changes in the ocular surface temperature in eyes with allergic conjunctivitis induced by the CAC. CAC is an allergic examination that is used because of its reproducibility and

safety of the subjects.^{29,30} CAC is extensively used in Europe and the United States to assess the efficacy of ophthalmic agents against conjunctivitis. Generally, the grading scores have been used to evaluate the degree of conjunctival redness and edema after the CAC. However, there are some difficulties in comparing the efficacies because of differences in the subjective grading scores that often lead to interobserver variations, and differences in the symptoms scores arise due to intersubject variations. To overcome these difficulties, we used the ocular surface temperature to assess the severity of the allergic conjunctivitis. Our earlier results showed that the OST can detect changes of the conjunctival temperature with a high degree of reproducibility and sensitivity, and the temperature was significantly correlated with the severity of the conjunctival allergic reaction.¹⁹

Ingesting mandarin orange yogurt for 2 weeks led to a reduction in the conjunctival redness, the chemosis scores, and the subjective itching symptoms. Furthermore, it significantly suppressed the increase of the conjunctival surface temperature after CAC. These findings suggest that ingestion of mandarin orange yogurt can be effective in ameliorating allergic conjunctivitis.

The results of recent studies suggested that various kinds of fermented foods including yogurt have antiallergic effects.^{11,31–33} Therefore, the antiallergic effects observed in mandarin orange yogurt could have been due to the effect of yogurt and not the effect of nobiletin or β -LG.

To investigate the antiallergic effects of nobiletin and β -LG in mandarin orange yogurt, we carried out a randomized, double-blind, crossover, placebo-controlled formula challenge on allergic conjunctivitis induced by CAC. In this study, we did not measure the blood concentration of nobiletin because

there is no detailed report on the metabolism of nobiletin in humans. In rats, the maximum concentration of nobiletin in plasma was 1.78 µg/mL, which was detected at 1 hour after an oral dosing (50 mg/kg).³⁴ The results of that study indicated that the half-life of nobiletin in the plasma was less than 2 hours. This indicates that our use of a 3-week interval between the optimal antigen solution concentration determination and the experimental tests was appropriate to conduct our experiments. In the second clinical trial, control yogurt was prepared. The β-LG in the control yogurt was blended to one-half of the normal yogurt, and the control yogurt was mixed without nobiletin. For masking, the control yogurt was blended with an orange flavor that did not include the nobiletin. The results of experiment 2 showed that the ingesting of mandarin orange yogurt significantly reduced the conjunctival redness, chemosis, and itching as well as reducing the increase in the conjunctival surface temperature compared to that before the ingestion. In addition, conjunctival redness, itching symptoms, and the increase of the conjunctival surface temperature were also more reduced by the ingestion of mandarin orange yogurt than that of the control yogurt. Thus, ingestion of the control yogurt alone had antiallergic effect to some degree.

Various food groups, such as dairy products including yogurt, fruits, fish, and rice, have been reported to have antiallergic effects.^{11,31–33} Fermented milk products prepared with lactic acid bacteria have been reported to suppress pollinosis in a double-blind placebo-controlled clinical study.^{8,35} Therefore, the results from the control yogurt in experiment 2 are reasonable. In addition, the results also indicated that nobiletin and β-LG had additive antiallergic effects when they were added to the yogurt. Compared to the earlier reports, the results of our study further demonstrated objectively the antiallergic effect of mandarin orange yogurt by measuring the ocular surface temperature.

There are limitations to our experiments. Our findings showed the effect of mandarin orange yogurt using only the CAC, and we have not studied the antiallergic effects during the pollen scattering period. More extensive considerations may be required in the future.

In conclusion, the results of this study showed that the administration of mandarin orange yogurt containing nobiletin and β-LG reduced the conjunctival allergic reaction in a double-blind placebo-controlled clinical study. Although further studies are needed to clarify the mechanism of the allergy-suppressive effect of mandarin orange yogurt, mandarin orange yogurt can be a functional food item that can reduce the allergic symptoms. In addition, it might be able to reduce the dose of antiallergic medications.

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