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Effects of repeated oral intake of a quercetin-containing supplement on allergic reaction: a randomized, placebo-controlled, double-blind parallel-group study

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Abstract. – OBJECTIVE: The present study aimed to investigate the effects of a 4-week repeated oral intake of a quercetin-containing supplement on allergen-induced reactions and relative subjective symptoms in Japanese adults who complained of discomfort in the eyes and nose.

SUBJECTS AND METHODS: A randomized, placebo-controlled, double-blind parallel-group study was conducted on 66 subjects (22-78 years old) with allergic symptoms of pollinosis. The subjects were given the test product (200 mg quercetin) or the control product (vehicle) daily for 4 weeks. The Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) scores and other tests were examined in each subject before and after starting the product intake. A new food-grade bioavailable formulation of quercetin, Quercetin phytosome®, was used.

RESULTS: At 1:4 weeks after the start of the supplement intake, several scores of JRQLQ, including allergic symptoms, such as eye itching, sneezing, nasal discharge, and sleep disorder, were significantly improved in the quercetin-containing supplement group compared with the placebo group. Furthermore, the quality of life of these subjects significantly improved based on the original questionnaire and visual analog scale. Minor notable adverse effects were noted throughout the study.

CONCLUSIONS: The results indicated that oral intake of quercetin-containing supplements might effectively reduce some allergy symptoms derived from pollinosis.

Key Words:
Quercetin, Healthy subject, Allergy symptoms, Nasal discharge, Sleep disorder.

Introduction

The prevalence of allergic diseases, such as asthma, rhinitis, eczema, and food allergy, has been rising in developed and developing countries[1]. This upward trend is particularly marked in the prevalence of seasonal allergy (pollinosis) in Japan, which is now called "a national disease" afflicting many people. According to the survey conducted in 2016 by the Tokyo Metropolitan Government[2], the prevalence of Japanese cedar (Sugi) pollinosis in Tokyo was estimated at 48.8% of the population. It has been theorized that changes in environmental factors (indoor/outdoor-sensitizing materials, such as allergens, air pollution, and various infections) are responsible for the high prevalence of such illnesses[3]. It has also been suggested that the recent change in the diet in Japan, involving changes in the type and quantity of nutrients ingested, has been increasing and aggravating allergic symptoms[4]. Using synthetic drugs or herb medicines to treat such illnesses can occasionally induce various adverse reactions. Phytochemicals, such as flavonoids and polysaccharides, are contained in many plant-derived foods and have been reported to have immunomodulating and anti-inflammatory actions[5].

Quercetin is a kind of flavonoid distributed widely in the vegetable kingdom. It is contained in the Family Brassicaceae, plant-derived foods (onion, Japanese tea, wine, fruits, etc.), and herbs[6]. In Japan, quercetin is now used as an existing food additive and a component of food supplements. Numerous reports[7] are available concerning the physiological activity of querce-

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tin, which ranges widely from anti-inflammatory action to hypotensive action, anti-allergic action, a means of nutrition for athletes, and so forth. Regarding the physiological activity of quercetin against allergy, an *in vitro* study[8] demonstrated its effects in modulating the immune function by inhibiting mast cell activity and histamine release, suppressing eosinophilic inflammation, and other effects. This and some other *in vitro* and *in vivo* studies[8] suggested that quercetin is effective against allergy. To date, however, the efficacy of quercetin in humans has not been evaluated sufficiently. The lack of sufficient evaluation of its effectiveness in humans is probably attributable to the low bioavailability of quercetin in humans after oral ingestion.

Under such circumstances, the present study was undertaken to evaluate the efficacy of quercetin in healthy volunteers and mildly sick individuals with allergic symptoms, such as rhinitis and ocular itching sensation, caused by antigens (pollen or house dust). Quercetin with high solubility and trans-oral bioavailability by using a new delivery system involving phytosome® (Indena S.p.A, Milan, Italy) was used for this study[9,10].

The present paper was originally published in Japanese in the *Japanese Pharmacology & Therapeutics* journal[11].

Subject and Methods

This study was designed as a placebo-controlled randomized, double-blind parallel-group study. Before the start of the study, its protocol was approved on 19 November 2019 by the Medical Corporation Seishinkai, Takara Clinic (Approval No. 1911-1911-IJ01-01-TC) and registered with UMIC-CTR (UMIN000038765) on 3 December 2019. It was carried out under sufficient consideration of individual participants' human rights, safety, and well-being following the Declaration of Helsinki, the Clinical Study Act, and the Clinical Study Act Enforcement Regulations.

Management of study participants was assigned to the clinical research organization ORTHOMEDICO Inc. Study participants were recruited by holding a preliminary orientation meeting about the study. Individuals who intended to participate in the study were informed about the study design. Written consent was obtained from the study participants later at each study site. The study lasted from 3 December 2019 to 4 April 2020, with no changes to the study protocol during this period.

Subjects

Of the 90 Japanese adults complaining of eye and/or nose discomfort and managed at the Medical Corporation Seishinkai, Takara Clinic, 66 were enrolled in this study. A major inclusion criterion was relative high nose/eye symptoms scores with the Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) during the screening and pre-medication evaluation. Individuals satisfying all inclusion criteria and falling under the exclusion criteria were eligible for the study. The percentage of subjects with JRQLQ nose/eye symptom scores in the normal range was ≥50% of the total number of subjects (subjects with normal range scores + subjects with mildly abnormal scores). Written informed consent was obtained from all subjects before enrollment in the study.

Inclusion Criteria

Inclusion criteria were: (1) Individuals judged by the Principal Investigator to be eligible for the study without any problem and (2) individuals whose JRQLQ nose/eye symptom score was relatively high in the screening and pre-medication evaluation. Definition of terms: "Subjects with normal range of scores" indicate the individuals having allergic reactions in the nose and/or eye (including those having shown such responses before) but taking no anti-allergy drug before and during the study period. "Subjects with mildly abnormal scores" indicate the individuals having allergic reactions of the nose and/or eye (including those having shown such responses before) and occasionally (not routinely) taking the anti-allergy drug(s) before and during the study period. "Occasionally" means intake of medication as needed. "Routinely" means periodical drug intake regardless of the presence/absence of symptoms.

Exclusion Criteria

The exclusion criteria were the following:

1. Patients currently receiving or having a history of receiving treatment for malignant tumors, heart failure, or myocardial infarction.
2. Patients using a pacemaker or an implanted defibrillator.
3. Patients currently receiving treatment for arrhythmia, liver dysfunction, kidney dysfunc-

tion, cerebrovascular disorders, rheumatoid arthritis, diabetes mellitus, dyslipidemia, hypertension, or other chronic illness.
4. Individuals routinely ingesting foods for specified health uses, foods for function claims, or other foods/ beverages for potential functional claims.
5. Patients requiring continuous use of drugs possibly affecting the nose/eye allergic symptoms (anti-allergy drugs, antihistamines, steroids, vasoconstrictors, antihypertensive agents, etc.) during the study period.
6. Individuals routinely taking drugs (including herb medicines) and/or supplements.
7. Individuals allergic to any medicine or test food-related food.
8. Individuals unable to take the test food by the method and at the dose level outlined in the protocol.
9. Individuals planning to make an overseas trip during the study period.
10. Individuals habitually practicing nose gurgling.
11. Pregnant or lactating women or women who intended to become pregnant during the study period.
12. Individuals having participated in any other clinical study during the 3-month period before acquiring consent to this study or planning to participate in any further clinical study during the study period.
13. Other individuals judged by the Principal Investigator to be inappropriate for this study.

Randomization and Blinding

Of the 90 individuals satisfying all of the inclusion criteria, 24 were excluded as they fell into any of the exclusion criteria, and the remaining 66 were enrolled in the study. For these 66 subjects, the allocation sequence was determined with Statlight #11 Ver.2.10 (Yukms Co., Ltd., Tokyo, Japan). Individual subjects were randomly allocated, in a stratified ratio of 1:1, to the placebo food group or the test food group. The allocation table was sealed and stored until the completion of the study. It was unsealed after the completion of the study.

Test Food

The test food was given for 4 weeks, from 24 February to 4 April 2020. The test food contains quercetin as a major active ingredient. The solubility and trans-oral bioavailability of quercetin in the test food was improved by a new delivery system involving lecithin[9,10]. This test food and the placebo food were supplied from Indena S.p.A (Milan, Italy). The safety of the test food had been confirmed in previous studies[9,10] with triathlon athletes, i.e., a study involving 2-week ingestion of the test food 500 mg/day (containing 200 mg quercetin) and another study involving a single dose of the test food 250-500 mg/day. The subjects of the present study ingested the test food or the placebo food (containing 50 mg quercetin or the same quantity of vehicle per tablet) at a daily dose level of four tablets divided into two doses (breakfast and supper; two tablets each time) together with water. It was confirmed in advance that distinction between test and placebo foods through taste and external appearance was not possible.

Evaluation Method

Test and evaluation were conducted before starting the study and 2 and 4 weeks after starting food ingestion. Subjects who withdrew their consent to the study or ceased ingesting the test or placebo food were discontinued from the study. Each complication or adverse event reported by subjects was recorded. Each subject was instructed to keep their lifestyle unchanged during the test/placebo food ingestion period.

Tests

The general test included height, body weight, BMI, body-fat ratio, blood pressure, and heart rate. Blood tests included measurement of white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, differential leukocyte count (percentage and count of neutrophils, lymphocytes, monocytes, eosinophils, and basophils), aspartate aminotransferase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, leukocyte alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase (CK), calcium, serum amylase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glycoalbumin, serum iron (Fe), sodium (Na), potassium (K), chlorine (Cl), inorganic phosphorus, glucose, and hemoglobin A1c (HbA1c). Urinalysis covered protein (qualitative), glucose (qualitative), urobilinogen (qualitative), bilirubin (qualitative), occult blood reaction (qualitative), ketone body (qualitative), specific gravity, and

pH. Virological and immunoserological tests covered hemoglobin antigen, hepatitis C virus antibody III, HIV antigen/antibody, and syphilis (qualitative). Physical examinations, blood tests, urinalysis, and allergic rhinitis-related tests were conducted at baseline and 2 and 4 weeks after test/placebo food ingestion. Safety evaluation items comprised adverse events and adverse reactions, which were evaluated based on the physical test, blood test, and urinalysis.

Primary Endpoints

Primary Endpoints were evaluated using the JRQLQ[12-15].

Secondary Endpoints

Secondary endpoints were evaluated using the severity grading of allergic rhinitis symptoms, nasal discharge test (nasal discharge eosinophil count), blood test (unspecific IgE, specific IgE [*Dermatophagoides pteronyssinus*, Japanese cedar, Hinoki cypress, house dust]), and a homemade questionnaire.

Statistical Analysis

For comparison between the placebo group and the test food group, the difference in the score of each test parameter between the score at the baseline and the score at 2 weeks after the start of test/placebo food ingestion and upon completion of test/placebo food ingestion was subjected to repeated measures ANOVA. The baseline served as the covariable and the time point, group, and time-group interactions served as factors. An adjustment was not made for multiple tests involving multiple items and multiple time points. Mann-Whitney U-test was employed for inter-group comparison of responses to questions or the classified values. For inter-group comparison at the baseline, the chi-square test (on gender and age) and unpaired *t*-test (on cognitive function test) were employed. Statistical analysis was performed using the software SPSS Statistics, ver.23.0 (IBM Corp., Armonk, NY, USA), and the significance level was set at $p<0.05\%$ (two-tailed) in each test.

In the safety analysis, an inter-group comparison was conducted on the data from physical measurement (excluding height), physical examination, and peripheral blood test at 2 and 4 weeks after starting test/placebo food ingestion using ANCOVA in which the baseline served as the covariable, and the group served as a factor.

Results

Subjects Analyzed

A flow diagram of subjects is given in Figure 1. The study was carried out as scheduled, without any protocol change. Of the 90 individuals having consented to participate in the study, 66 satisfying all the inclusion criteria and falling under none of the exclusion criteria were enrolled in the study. Afterward, three subjects were found to fall under any of the exclusion criteria, and another three quit the study after the start of the intervention. Thus, 60 subjects were eventually included in the per-protocol set and the analysis. The safety analysis set consisted of 64 subjects, and the per-protocol set consisted of 60 subjects. In terms of sex, the test food group consisted of 18 women and 12 men, and the placebo group consisted of 15 women and 15 men. In terms of age, the test food group consisted of three subjects at age 20-29 years, three at age 30-39 years, 12 at 40-49 years, six at 50 years 59 years, five at 60-69 years and one at 70-79 years. The placebo group consisted of three subjects at age 20-29 years, five at 30-39 years, 10 at 40-49 years, seven at 50-59 years, two at 60-69 years, and two at 70-79 years. Background variables and blood test results of subjects are given in Table I. There was no significant difference between the two groups.

Efficacy Evaluation

Primary endpoints

Table II shows the absolute value of each JRQLQ score in both groups. Table II gives the

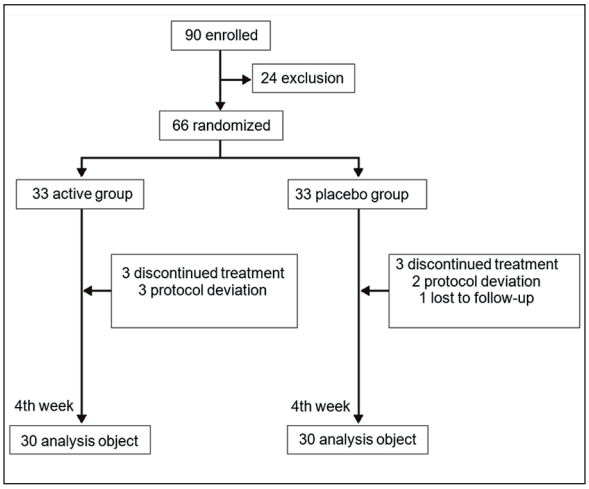

Figure 1. Flow diagram of the enrolled subjects.

Table I. Background variables and blood test results of subjects.

Variable	Active group (n = 32)	Placebo group (n = 32)	*p*-value
Sex (male/female)	13/19	17/15	0.45
Age (years)	46.8 (13.0)	46.9 (13.0)	0.92
Height (cm)	165.4 (8.0)	165.2 (8.6)	0.92
Weight (kg)	61.3 (13.0)	65.0 (13.9)	0.27
BMI (kg/m^2)	22.3 (3.5)	23.7 (3.7)	0.13
Body-fat percentage (%)	23.4 (6.4)	24.7 (6.0)	0.41
Blood pressure (mmHg):			
• Systolic	118.5 (14.3)	115.0 (12.4)	0.30
• Diastolic	75.7 (10.6)	74.7 (10.8)	0.71
Pulse74.0 (9.0)	74.7 (8.4)	0.75	
Blood biochemistry:			
• AST (GOT) (U/L)	21.3 (9.2)	22.3 (5.8)	0.60
• ALT (GPT) (U/L)	19.2 (13.0)	20.4 (10.8)	0.69
• γ-GTP (IU/L)	25.6 (16.9)	26.6 (21.2)	0.84
• Total cholesterol (mg/dL)	208.8 (27.3)	211.8 (35.7)	0.71
• Triglyceride (mg/dL)	78.8 (35.8)	95.4 (56.3)	0.17
• Glucose (mg/dL)	86.6 (9.5)	83.8 (7.8)	0.21
• HbA1c (NGSP) (%)	5.3 (0.2)	5.4 (0.3)	0.64
• HDL cholesterol (mg/dL)	71.9 (17.4)	62.3 (15.5)	**0.02**
• LDL cholesterol (mg/dL)	118.4 (29.7)	129.4 (31.6)	0.16

Values in bold are significant ($p<0.05$). Continuous variables are shown as mean (SD). SD, standard deviation.

magnitude of change in each score at 2 weeks (2W) and 4 weeks (4W) after starting ingestion from the pre-start baseline. The absolute values of JRQLQ total score, Quality Of Life (QOL) total score, sleep score, and physical score were significantly lower in the test food group than in the placebo group (Table II). The magnitude of change in sleep and physical score at 4W was significant between the two groups (Table II).

The decrease in the score of each item of JRQLQ indicates a tendency for improvement. The absolute value of sleep score (mean ± SD) in the test food group was 1.7 ± 1.2 before starting ingestion, 1.1 ± 0.9 at 2W, and 0.8 ± 0.8 at 4W. The corresponding value in the placebo group was 1.5 ± 1.1 before starting ingestion, 1.2 ± 0.8 at 2W, and 1.4 ± 0.9 at 4W. Thus, the magnitude of change in sleep score was significantly smaller in the test food group (4W; -1.0 ± 0.9) than in the placebo group (4W; -0.1 ± 1.0; p=0.00), as shown in Table II. The absolute value of the physical score in the test food group was 3.6 ± 2.5 before starting ingestion, 2.3 ± 1.6 at 2W, and 1.5 ± 1.7 at 4W. The corresponding value in the placebo group was 3.8 ± 2.1 before starting ingestion, 2.9 ± 1.6 at 2W, and 2.8 ± 1.6 at 4W.

Thus, as shown in Table II, the magnitude of change in physical scores was significantly smaller in the test food group (4W; -2.1 ± 1.8) than in the placebo group (4W; -1.0 ± 2.0; p=0.03).

Of the JRQLQ items showing significant inter-group differences, the following had significantly lower scores in the test food group than in the placebo group, as shown in Table III: "sneezing" (p=0.04), "decrease in thinking power" (difficulty in focusing; p=0.01), "disturbance in outdoor activities, such as sports and picnics" (p=0.01), "sleep disorder" (p=0.01), "malaise" (p=0.00), "tendency to fatigue" (p=0.02), and "face scale" (p=0.03).

Secondary endpoints

Of the items showing significant inter-group differences in severity grading of allergic rhinitis, the following had significantly lower scores in the test food group than in the placebo group, as shown in Table IV: "sneezing" (p=0.01), "rhinorrhea" (p=0.01), and "disturbance in daily living" (p=0.01). The "ocular itching sensation" score was significantly low at 1 week after the start of ingestion (p=0.02).

In the nasal discharge test, the nasal discharge eosinophil count at 2W was significantly lower in the test food group than in the placebo group. Still, there was no significant difference in this parameter between the two groups when examined at 4W (Table V). Blood test (unspecific IgE, specific IgE [*Dermatophagoides pteronyssinus*, Japanese cedar, Hinoki cypress, and house dust])

Table II. Influence on Japanese Rhino-conjunctivitis Quality of Life Questionnaire expressed for both absolute values and magnitude of change.

Variable	Absolute value			Magnitude of change		
	Active group (n = 30)	Placebo group (n = 30)	*p*-value	Active group (n = 30)	Placebo group (n = 30)	*p*-value
JRQLQ						
2W	25.8 (14.6)	29.7 (13.1)	0.23	-8.9 (15.5)	-5.0 (16.9)	0.36
Total score						
4W	19.1 (12.1)	26.3 (15.0)	**0.04**	-15.6 (17.5)	-8.4 (18.5)	0.13
Nose/eye symptoms						
2W	8.9 (4.7)	9.8 (4.7)	0.47	-1.2 (4.9)	-0.5 (4.7)	0.59
4W	7.4 (4.1)	8.7 (4.8)	0.28	-2.6 (5.1)	-1.6 (5.3)	0.44
Nose score						
2W	5.9 (3.6)	6.8 (3.6)	0.27	-1.1 (3.2)	-0.2 (3.6)	0.34
4W	4.8 (2.4)	6.0 (3.4)	0.13	-2.1 (3.4)	-1.1 (3.5)	0.27
Eye score						
2W	3.0 (1.8)	3.0 (1.7)	0.85	-0.1 (2.0)	-0.3 (1.8)	0.74
4W	2.6 (2.0)	2.8 (1.9)	0.78	-0.5 (2.3)	-0.5 (2.2)	0.95
QOL total score						
2W	16.9 (11.1)	19.9 (9.8)	0.21	-7.7 (11.6)	-4.5 (13.9)	0.34
4W	11.6 (9.1)	17.6 (11.4)	**0.02**	-12.9 (13.8)	-6.8 (14.1)	0.10
Activities of daily living score						
2W	5.5 (4.0)	5.9 (2.8)	0.61	-1.9 (4.7)	-1.5 (3.8)	0.72
4W	3.9 (3.0)	5.4 (3.3)	0.06	-3.5 (4.8)	-2.1 (4.2)	0.21
Outdoor activity score						
2W	2.0 (2.1)	2.2 (1.3)	0.73	-0.4 (1.8)	-0.3 (2.2)	0.90
4W	1.1 (1.4)	1.8 (1.3)	0.09	-1.2 (1.9)	-0.7 (2.2)	0.34
Social activity score						
2W	2.1 (2.1)	2.9 (1.8)	0.13	-0.9 (2.3)	-0.2 (2.8)	0.31
4W	1.5 (1.7)	2.1 (1.9)	0.20	-1.5 (3.0)	-1.0 (2.5)	0.46
Sleep score						
2W	1.1 (0.9)	1.2 (0.8)	0.34	-0.6 (0.9)	-0.3 (0.9)	0.24
4W	0.8 (0.8)	1.4 (0.9)	**0.00**	-1.0 (0.9)	-0.1 (1.0)	**0.00**
Physical score						
2W	2.3 (1.6)	2.9 (1.6)	0.12	-1.3 (1.9)	-0.9 (2.0)	0.43
4W	1.5 (1.7)	2.8 (1.6)	**0.00**	-2.1 (1.8)	-1.0 (2.0)	**0.03**
Mental life score						
2W	3.8 (3.4)	4.7 (3.6)	0.13	-2.6 (3.0)	-1.3 (4.0)	0.14
4W	2.8 (2.8)	4.1 (3.7)	0.08	-3.6 (3.7)	-1.9 (4.3)	0.11

revealed no significant difference in any parameter between the test food group and the placebo group.

According to the original QOL questionnaire results using the visual analogue scale (VAS) method, there was no significant inter-group difference in any item, as shown in Table VI (absolute values). However, the scores of "itching sensation other than ocular/nasal itching sensation" (p=0.04) and "rash (skin below the nose, etc.)" (p=0.01) were significantly lower in the test food group than in the placebo group as a result of interactions. In addition, in terms of the magnitude of change (Table VI), no item showed a significant inter-group difference reflecting improvement in the test food group. Yet, the score of "rash, (the skin below the nose, etc.)" (p=0.02) was significantly lower in the test food group than in the placebo group as a result of interactions.

Safety Evaluation

Of the items showing significant inter-group differences in physical measurement and physical examination, the following had significantly lower scores in the test food group than in the placebo group: "systolic blood pressure" (p=0.003) and "diastolic blood pressure" (p=0.015). No item of urinalysis showed a significant difference between the test food group and the placebo group. The blood test showed only blood glucose level to be significantly lower in the test food group than in the placebo group (p=0.021). All the

Table III. Influence on Japanese Rhino-conjunctivitis Quality of Life Questionnaire items.

Variable (Question item score)		Active group (n = 30)			Placebo group (n = 30)			p-value
		Median	Q1	Q3	Median	Q1	Q3	
Runny nose	B	2.0	1.0	2.0	1.5	1.0	2.0	0.64
	2W	1.0	1.0	2.0	2.0	1.0	2.0	0.20
	4W	1.0	1.0	1.0	1.0	1.0	2.0	0.23
Sneezing	B	1.5	1.0	2.0	2.0	1.0	2.8	0.65
	2W	1.0	1.0	2.0	1.5	1.0	2.8	0.39
	4W	1.0	1.0	2.0	1.5	1.0	2.0	**0.04**
Nasal congestion	B	2.0	1.0	2.0	2.0	1.0	3.0	0.95
	2W	1.0	1.0	2.0	1.0	1.0	2.8	0.38
	4W	1.0	1.0	1.0	1.0	1.0	2.0	0.51
Nasal itching sensation	B	1.0	1.0	2.0	2.0	1.0	2.8	0.46
	2W	1.0	1.0	2.0	1.5	1.0	2.0	0.37
	4W	1.0	1.0	2.0	1.0	1.0	2.0	0.87
Ocular itching sensation	B	2.0	1.0	2.0	2.0	1.0	3.0	0.52
	2W	2.0	1.0	3.0	2.0	1.0	3.0	0.84
	4W	1.5	1.0	2.0	2.0	1.0	2.0	0.55
Teary eyes	B	1.0	1.0	2.0	1.0	1.0	2.0	0.79
	2W	1.0	0.3	1.8	1.0	0.3	1.0	0.80
	4W	1.0	0.0	1.8	1.0	0.0	1.0	0.98
Disturbed learning/job/housework	B	1.0	1.0	2.0	1.0	1.0	2.0	0.84
	2W	1.0	0.0	2.0	1.0	1.0	1.8	0.63
	4W	1.0	0.0	1.0	1.0	1.0	1.8	0.21
Difficulty in mental focusing	B	2.0	1.0	2.0	2.0	1.0	2.0	0.83
	2W	1.0	1.0	2.0	1.0	1.0	2.0	0.36
	4W	1.0	0.0	1.0	1.0	1.0	2.0	0.12
Decrease in thinking power (difficulty in focusing)	B	1.0	1.0	2.8	1.0	1.0	2.0	0.81
	2W	1.0	0.0	1.0	1.0	1.0	2.0	0.13
	4W	1.0	0.0	1.0	1.0	1.0	1.0	**0.01**
Inconvenience in reading newspapers/books	B	1.0	1.0	2.8	1.0	1.0	2.0	0.45
	2W	1.0	1.0	2.0	1.0	1.0	2.0	0.96
	4W	1.0	0.0	1.0	1.0	0.0	1.0	0.46
Reduced memory	B	1.0	0.3	2.0	1.0	1.0	2.0	0.88
	2W	1.0	0.0	1.0	1.0	1.0	1.0	0.82
	4W	1.0	0.0	1.0	1.0	0.3	1.0	0.27
Disturbance in outdoor activities (sports, picnics, etc.)	B	1.0	0.3	2.0	1.0	0.3	2.0	0.74
	2W	1.0	0.0	1.8	1.0	1.0	2.0	0.34
	4W	0.0	0.0	1.0	1.0	0.0	1.8	**0.01**
Disturbance in going out (tendency to avoid going out)	B	1.0	0.0	2.0	1.0	1.0	2.0	0.61
	2W	1.0	0.0	1.8	1.0	1.0	1.8	0.32
	4W	0.5	0.0	1.0	1.0	0.0	1.0	0.38
Disturbance in social activity (tendency to avoid it)	B	1.0	0.0	2.0	1.0	0.0	1.0	0.82
	2W	0.5	0.0	1.0	1.0	1.0	1.0	0.17
	4W	0.0	0.0	1.0	1.0	0.0	1.0	0.10
Disturbance in conversation/ telephone call with/to other people	B	1.0	0.0	2.0	1.0	1.0	1.8	0.63
	2W	1.0	0.0	1.0	1.0	0.3	1.0	0.28
	4W	0.0	0.0	1.0	1.0	0.0	1.0	0.53
Nervous with surrounding people	B	1.0	0.0	2.0	1.0	0.0	2.0	0.49
	2W	1.0	0.0	1.0	1.0	0.3	1.0	0.08
	4W	0.0	0.0	1.0	0.5	0.0	1.0	0.34

Continued

Table III *(Continued)*. Influence on Japanese Rhino-conjunctivitis Quality of Life Questionnaire items.

Variable (Question item score)		Active group (n = 30)			Placebo group (n = 30)			*p*-value
		Median	Q1	Q3	Median	Q1	Q3	
Sleep disorder	B	2.0	1.0	3.0	1.5	1.0	2.0	0.44
	2W	1.0	0.3	2.0	1.0	1.0	2.0	0.66
	4W	1.0	0.0	1.0	1.0	1.0	2.0	**0.01**
Malaise	B	1.5	1.0	3.0	2.0	1.0	3.0	0.54
	2W	1.0	1.0	1.0	1.0	1.0	2.0	0.08
	4W	0.0	0.0	1.0	1.0	1.0	2.0	**0.00**
Tendency to fatigue	B	2.0	1.0	3.0	2.0	1.0	3.0	0.85
	2W	1.0	1.0	2.0	2.0	1.0	2.0	0.13
	4W	1.0	0.0	1.0	1.0	1.0	2.0	**0.02**
Feeling gloomy	B	2.0	1.0	3.0	2.0	1.0	2.8	0.94
	2W	1.0	0.0	1.8	1.0	0.3	2.0	0.26
	4W	1.0	0.0	1.0	1.0	1.0	2.0	0.09
Irritation	B	1.5	1.0	2.0	1.0	1.0	2.0	0.63
	2W	1.0	0.0	1.0	1.0	0.0	2.0	0.31
	4W	1.0	0.0	1.0	1.0	0.0	1.8	0.30
Depressed mood	B	2.0	1.0	2.0	1.0	1.0	2.0	0.40
	2W	1.0	0.0	1.0	1.0	0.0	2.0	0.61
	4W	1.0	0.0	1.0	1.0	0.0	1.0	0.66
Discontent with daily life	B	1.0	1.0	2.0	1.0	1.0	2.0	0.95
	2W	1.0	0.0	1.8	1.0	1.0	2.0	0.25
	4W	1.0	0.0	1.0	1.0	0.0	1.0	0.19
Face scale	B	2.0	1.3	3.0	2.0	2.0	3.0	0.90
	2W	2.0	1.0	2.0	2.0	2.0	3.0	0.23
	4W	1.0	1.0	2.0	2.0	1.0	3.0	**0.03**

Values in bold are significant ($p<0.05$). JRQLQ, Japanese Rhino-conjunctivitis Quality of Life Questionnaire. B, before start of ingestion. 2W, 2 weeks after start of ingestion. 4W, 4 weeks after start of ingestion.

significant inter-group differences observed in parameters were variations within the criterion range of the parameters concerned. No adverse event attributable to ingestion of the test food was observed during the study period.

Discussion

Allergy-like symptoms of type I allergy are caused by excessive mucus secretion and mast cell degranulation due to excessive IgE formation upon allergen invasion into the living body[16].

Type 1 allergy is involved in allergic rhinitis, which presents with nasal symptoms (sneezing, nasal discharge, nasal congestion, etc.) and other symptoms, such as headache and difficulty concentrating, resulting in reduced QOL[13]. In the eyes, conjunctivitis can develop, resulting in congestion, ocular itching sensation, lacrimation, and so on[14,17]. Allergic rhinitis also involves increasing nasal discharge eosinophil count arising from exposure to allergens. This change is used as one of the signs of diagnosing allergic rhinitis[18]. Suppression of degranulation[16] and inhibition of the binding of histamine (released as a result of degranulation) to the receptor[19] are known as valid approaches to alleviation or suppression of these symptoms of allergic reactions.

Regarding the physiological activity of quercetin contained in the test food for this study, numerous scholars[8] using cells and animals demonstrated its effects in inhibiting mast cell activity and histamine release, suppressing inflammation induced by eosinophil increase or activation, adjusting the immune function, etc. Additionally, quercetin inhibits the formation and release of histamine and other allergic/proinflammatory substances by stabilizing the mast cell membrane[19]. It has also been reported[20] that quercetin inhibits the activation

Table IV. Influence on severity grading of allergic rhinitis.

Variable (score)		Active group (n = 30)			Placebo group (n = 30)			*p*-value
		Median	Q1	Q3	Median	Q1	Q3	
Sneezing	B	1.0	1.0	2.0	2.0	1.0	2.0	**0.04**
	1W	1.0	1.0	2.0	1.0	1.0	2.0	0.19
	2W	1.0	1.0	2.0	1.0	1.0	2.0	0.44
	3W	1.0	1.0	1.0	1.0	1.0	2.0	**0.04**
	4W	1.0	1.0	1.0	1.0	1.0	2.0	**0.01**
Rhinorrhea	B	1.0	1.0	2.0	2.0	1.0	3.0	**0.04**
	1W	1.0	1.0	2.0	2.0	1.0	3.0	**0.01**
	2W	1.0	1.0	2.0	2.0	1.0	2.0	0.05
	3W	1.0	1.0	1.0	1.5	1.0	2.0	**0.01**
	4W	1.0	1.0	1.0	1.5	1.0	2.0	**0.01**
Nasal congestion	B	1.0	0.0	1.0	1.0	0.0	2.0	0.40
	1W	1.0	0.0	1.0	1.0	0.0	2.0	0.19
	2W	1.0	0.0	1.0	1.0	0.0	1.8	0.45
	3W	0.0	0.0	1.0	1.0	0.0	1.8	0.12
	4W	0.0	0.0	1.0	1.0	0.0	1.8	0.22
Ocular itching sensation	B	1.0	1.0	2.0	2.0	1.0	2.0	0.09
	1W	1.0	1.0	2.0	2.0	1.0	2.0	**0.02**
	2W	1.0	0.3	2.0	1.0	1.0	2.0	0.20
	3W	1.0	0.3	2.0	1.5	1.0	2.0	0.08
	4W	1.0	0.0	2.0	1.0	1.0	2.0	0.22
Lacrimation	B	1.0	0.0	1.0	1.0	0.0	1.0	0.97
	1W	1.0	0.0	1.0	0.5	0.0	1.0	0.82
	2W	0.0	0.0	1.0	0.0	0.0	1.0	0.75
	3W	0.0	0.0	1.0	1.0	0.0	1.0	0.38
	4W	0.0	0.0	1.0	0.5	0.0	1.0	0.64
Disturbance in daily living	B	1.0	0.0	1.0	1.0	0.3	2.0	0.42
	1W	1.0	0.0	1.0	1.0	0.3	2.0	0.20
	2W	0.5	0.0	1.0	1.0	1.0	1.8	**0.03**
	3W	0.0	0.0	1.0	1.0	1.0	2.0	**0.00**
	4W	0.0	0.0	1.0	1.0	1.0	1.0	**0.01**

Values in bold are significant ($p<0.05$). B, before start of ingestion (1 week). 1W, 1 week after start of ingestion. 2W, 2 weeks after start of ingestion. 3W, 3 weeks after start of ingestion. 4W, 4 weeks after start of ingestion. SD, standard deviation.

of human mast cells by suppressing the inflow of calcium ions, releasing inflammatory cytokines, and activating protein kinase, which is involved in allergic reactions. In a study[21] using mast cells derived from the nasal mucosa of Japanese patients with perennial allergic rhinitis, the addition of quercetin reduced the amount of histamine release significantly. Therefore, ingestion of quercetin-containing food is expected to manifest an anti-allergic activity through suppression of mast cell activity, inhibition of histamine release, and suppression of eosinophil increase.

Allergic rhinitis can be divided into two types (seasonal and perennial). Seasonal allergic rhinitis often assumes the form of pollinosis with pollen serving as an allergy, while perennial allergic rhinitis is primarily caused by allergens, such as house dust and house mites[14]. In Tokyo, Japanese cedar (Sugi) pollen prevails in mid-February through late March, and Hinoki cypress pollen increases in late March to mid-April[22]. Therefore, we can predict that the pollen spread level gets higher in late February to early April than in other seasons, and symptoms of seasonal allergic rhinitis are more likely to be induced during this period. For this reason, the present study was designed to evaluate the influence of 4-week continuous ingestion of quercetin-containing test

Table V. Influence on nasal discharge neutrophil count and specific IgE in nasal discharge test and blood test.

Variable (score)		Active group (n = 30)			Placebo group (n = 30)			*p*-value
		Median	Q1	Q3	Median	Q1	Q3	
Nasal discharge eosinophil count	B	0.0	0.0	1.0	0.0	0.0	1.0	0.70
	2W	0.0	0.0	1.0	1.0	0.0	1.0	**0.02**
	4W	0.0	0.0	1.0	1.0	0.0	2.0	0.09
Dermatophagoides pteronyssinus	B	1.5	0.0	3.0	3.0	0.0	3.0	0.12
	2W	1.5	0.0	3.0	3.0	0.0	3.0	0.12
	4W	1.0	0.0	3.0	3.0	0.0	3.0	0.08
Japanese cedar	B	3.0	1.0	3.8	3.0	2.0	3.8	0.83
	2W	2.5	1.0	3.8	3.0	2.0	3.8	0.47
	4W	3.0	1.0	3.8	3.0	2.0	4.0	0.47
Hinoki cypress	B	1.0	0.0	2.8	1.5	0.0	2.0	0.94
	2W	1.0	0.0	2.0	2.0	0.0	2.0	0.74
	4W	1.0	0.0	2.0	2.0	0.0	2.0	0.57
House dust	B	1.5	0.0	3.0	3.0	0.0	3.0	0.18
	2W	1.5	0.0	3.0	2.5	0.0	3.0	0.19
	4W	1.5	0.0	3.0	2.5	0.0	3.0	0.21

Values in bold are significant ($p<0.05$). B, before start of ingestion. 2W, 2 weeks after start of ingestion. 4W, 4 weeks after start of ingestion.

food on subjective symptoms related to allergic reactions to allergens in Japanese adults of both sexes having eye and/or nose discomfort during the period from late February to early April (the intervention period).

In the evaluation of each score of JRQLQ as a primary effect of the test food, significant improvement was noted in QOL total score (absolute value), sleep score (absolute value and magnitude of change), and physical score (absolute value and magnitude of change) (Table II). In a post-hoc analysis, the JRQLQ total score (absolute value), QOL total score (absolute value), sleep score (absolute value and magnitude of change), and physical score (absolute value and magnitude of change) at 4 weeks after the start of ingestion were significantly lower in the test food group than in the placebo group (Table II) Since the JRQLQ total score in this study is the total of nose symptom score and QOL total score, it seems likely that the significant difference in QOL total score was reflected in the significant difference in JRQLQ total score. A lower JRQLQ score indicates better QOL; ingestion of the test food has improved the QOL[23].

The JRQLQ is designed to calculate each of the nose/eye symptom score, nose score, and eye score as the average in each field based on the 5-grade rating of six symptoms, i.e., runny nose, sneezing, nasal congestion, nasal itching sensation, ocular itching sensation, and teary eyes[23]. In addition, the 17 items related to QOL (5-grade rating) are classified by field, and the mean score of each of them is evaluated. The daily living score is calculated as the mean of the scores of "disturbance in learning/job/housework," "difficulty in mental focusing," "decrease in thinking power (difficulty in focusing)," "inconvenience in reading newspapers/books," and "decrease in memory". The outdoor activity score is calculated as the mean of the sores of "disturbance in sports, picnics, etc." and "disturbance in going out (tendency to avoid going out)." The social activity score is calculated as the mean of scores of "disturbance in social activity (tendency to avoid it)," "disturbance in conversation/telephone call with/to other people," and "nervous with surrounding people." The sleep score is the mean of the score of "sleep disorder." The mental quality of life score is the mean of scores of "feeling gloomy," "irritation," "depressed mood," and "discontent with daily living"[23]. The scores in these six fields are added to yield a total QOL score. The face scale, which indicates the comprehensive status

Table VI. Influence on original QOL questionnaire with VAS expressed for both absolute values and magnitude of change.

Variable (score)		Absolute value			Magnitude of change		
		Active group (n = 30	Placebo group (n = 30)	*p*-value	Active group (n = 30	Placebo group (n = 30)	*p*-value
Itching sensation other than ocular/nasal itching sensation	2W	42.2 (21.3)	35.9 (23.7)	0.06	-3.5 (25.1)	-18.0 (24.0)	**0.03**
	4W	35.6 (26.4)	41.6 (27.8)	0.56	-10.1 (28.5)	-12.3 (36.9)	0.80
Blurred vision	2W	47.7 (28.1)	39.8 (25.1)	0.32	-12.3 (27.0)	-16.9 (28.3)	0.52
	4W	38.7 (25.8)	38.6 (22.8)	0.84	-21.3 (25.6)	-18.1 (27.9)	0.65
Rash (skin below the nose, etc.)	2W	28.8 (27.0)	27.2 (25.2)	0.34	-10.1 (26.2)	-19.8 (25.2)	0.15
	4W	19.6 (22.6)	29.8 (24.5)	0.22	-19.2 (25.5)	-17.2 (27.0)	0.77
Difficulty in breathing	2W	32.9 (24.2)	34.6 (20.7)	0.96	-14.5 (28.5)	-16.7 (23.4)	0.74
	4W	26.8 (22.1)	35.6 (24.8)	0.20	-20.6 (24.0)	-15.8 (29.3)	0.49
Nasal bleeding	2W	9.3 (19.5)	12.8 (22.1)	1.00	-13.2 (21.3)	-17.0 (22.1)	0.49
	4W	10.3 (19.0)	10.1 (16.2)	0.62	-12.2 (24.0)	-19.7 (26.2)	0.25
Coarse voice	2W	17.7 (22.5)	25.1 (23.2)	0.22	-25.7 (23.2)	-20.7 (26.0)	0.43
	4W	19.6 (21.1)	27.2 (23.4)	0.20	-23.8 (21.7)	-18.6 (31.2)	0.45
Ocular discharge	2W	32.3 (29.7)	34.8 (26.5)	0.73	-19.1 (26.5)	-17.2 (29.6)	0.79
	4W	26.5 (26.4)	30.2 (23.9)	0.56	-24.9 (22.7)	-21.8 (28.9)	0.65
Cough	2W	22.3 (25.2)	27.0 (23.6)	0.44	-26.0 (28.1)	-21.9 (30.0)	0.58
	4W	22.0 (23.7)	24.4 (23.7)	0.69	-26.3 (24.5)	-24.4 (31.5)	0.80
Daytime drowsiness	2W	34.4 (26.5)	40.9 (22.6)	0.33	-21.8 (22.3)	-16.8 (30.2)	0.47
	4W	31.4 (24.8)	40.4 (21.0)	0.15	-24.8 (22.2)	-17.4 (31.4)	0.30
Ocular congestion	2W	38.8 (32.4)	38.3 (25.1)	0.95	-13.3 (27.1)	-12.1 (28.5)	0.87
	4W	30.3 (31.4)	32.8 (21.5)	0.59	-21.7 (26.9)	-17.5 (27.6)	0.55
Throat discomfort	2W	32.6 (25.6)	37.2 (27.4)	0.24	-26.9 (25.6)	-16.4 (28.8)	0.14
	4W	28.0 (26.7)	37.0 (25.0)	0.09	-31.5 (28.9)	-16.5 (30.6)	0.06
Malaise	2W	34.4 (27.0)	37.2 (23.4)	0.74	-21.5 (23.7)	-20.6 (29.8)	0.89
	4W	25.8 (23.8)	34.6 (24.2)	0.17	-30.1 (19.8)	-23.2 (32.0)	0.32
Depth of sleep	2W	38.7 (27.1)	45.5 (29.2)	0.16	-22.0 (18.9)	-13.2 (26.6)	0.14
	4W	34.5 (26.1)	41.9 (24.1)	0.15	-26.2 (23.9)	-16.7 (26.7)	0.15
Ease in falling asleep	2W	36.7 (25.7)	41.7 (27.2)	0.26	-18.6 (20.3)	-12.1 (24.5)	0.27
	4W	30.5 (24.5)	41.6 (25.9)	0.05	-24.8 (26.6)	-12.2 (28.7)	0.08

Continuous variables are shown as mean (SD). Values in bold are significant ($p<0.05$). 2W, 2 weeks after start of ingestion. 4W, 4 weeks after start of ingestion. SD, standard deviation.

of QOL, is used in combination with the score of each field related to nose/eye symptoms and QOL for evaluation of QOL related to allergic rhinitis. In the present study, the scores of "sneezing," "decrease in thinking power (difficulty in focusing)," "disturbance in outdoor activities such as sports and picnics," "sleep disorder," "malaise," "tendency to fatigue," and "face scale" at 4W were significantly lower in the test food group than in the placebo group (Table III). These items are used to calculate the nose/eye symptom score, daily living score, outdoor activity score, sleep score, physical score, and JRQLQ total score and were approximately consistent with the items showing significant differences when fields analyzed the scores. The JRQLQ has also been used to evaluate the efficacy of the drugs for treating of allergic rhinitis, thereby making an "effective" judgment if the evaluation of scores for fields and items shows a significant reduction[13,23]. In an analysis of individual responses of subjects to the questionnaire, all items whose score was significantly lower in the test food group than in the placebo group had a larger number of subjects

answering 0, meaning "absent (no)", or 1, meaning "mild" in the test food group, indicating that these changes were clinically significant. These results of JRQLQ suggest that ingestion of the test food reduced the adverse influence of allergic rhinitis on QOL, primarily the impact on sleep and the body.

In severity grading of allergic rhinitis evaluated as a secondary outcome, the scores of "sneezing" (3W and 4W), "rhinorrhea" (1W, 3W, and 4W), "ocular itching sensation" (1 week after starting ingestion), and "disturbance in daily living" (2-4W) were significantly lower in the test food group than in the placebo group (Table IV). Severity grading of allergic rhinitis was conducted by asking each subject to select a concrete number from a given range reflecting the daily frequency of sneezing and nose-blowing, the intensity of nasal congestion, and the percentage of oral breathing[15]. This is a more subjective evaluation method than JRQLQ. According to the criteria for evaluation of the efficacy of treatment given in the Guidelines for Nasal Allergy Management, one-level reduction in the severity grade is deemed as "improved," and two levels or more reduction as "markedly improved" or "symptom disappeared"[14]. In the present study, the mean scores of "rhinorrheas" (1W) and "ocular itching sensation" (1W) were 1.0 (meaning "mild") in the test food group and 2.0 (meaning "moderate") in the placebo group. This difference by one level of severity grade between the two groups can be interpreted as clinically significant. In the nasal discharge test, the nasal discharge eosinophil count at 2W was significantly lower in the test food group than in the placebo group (Table V). Regarding the subjective symptoms related to sleep, the post-hoc analysis of the responses to the original questionnaire revealed a significantly lower score of "ease in falling asleep" (absolute value) at 4W in the test food group than in the placebo group (p=0.049) (Table VI). Thus, in the evaluation of secondary outcomes, alleviation of allergy-like symptoms was demonstrated in both subjective evaluation (severity of allergic rhinitis and nasal discharge neutrophil count) and subjective evaluation (primarily disturbances in sleep and daily living), approximately consistent with the results from JRQLQ (primary outcomes).

In addition to alleviation of eye/nose symptoms mentioned above, the original questionnaire revealed significant inter-group differences in the scores of "itching sensation other than ocular/nasal itching sensation" (absolute value) and "rash (skin below the nose, etc.)" (absolute value and magnitude of change) (Table VI). In the analysis of changes over time of the scores of "itching sensation other than ocular/nasal itching sensation" and "rash (skin below the nose, etc.)" which showed significant inter-group differences, the scores tended to decrease constantly in the test food group. In contrast, the placebo group showed elevation of the scores at 4W after decreasing at 2W. Under the original questionnaire employed in this study, lower scores indicate better subjective ratings. Thus, the test food appears to have alleviated subjective symptoms throughout the intervention period.

Quercetin affects the recruitment of immunocompetent cells to the skin and prevents secondary infection outbreaks following the destruction of the skin barrier[4,8]. In previous studies[8,24,25] in which healthy adults of both sexes ingested the same test food as that used in the present study, containing quercetin in the same quantity or smaller quantity (100 mg/day), wheal, redness, skin thickness, and microcirculation after histamine injection was reduced significantly, suggesting that quercetin also has an immunomodulating activity. Therefore, it seems likely that in the present study, subjective symptoms, such as itching sensation and rash, were alleviated by the effect of quercetin (contained in the test food) in suppressing the immune reactions in the skin.

Symptoms of allergic rhinitis, both perennial and seasonal, have been reported to aggravate QOL, such as sleep, anxiety, and a depressed mood[26]. Regarding perennial allergic rhinitis, a study[27] designed to evaluate the influence on the score of SF-36 (a questionnaire for evaluation of health-related QOL) in Japanese patients with perennial allergic rhinitis demonstrated that among sneezing, nasal discharge, and nasal congestion, QOL was most affected by nasal congestion. Moreover, the influence of the severity of this symptom is reportedly greater than that of any other symptom[27]. Regarding seasonal allergic rhinitis, a study[28] in Japanese patients with pollinosis demonstrated that improved sleep quality had a significant positive correlation with nasal discharge, sneezing, nasal congestion, and nasal/ocular itching sensation. Also, alleviation in difficulty falling asleep had a significant positive correlation with ocular itching sensation[28]. The present study suggests that if patients with perennial or seasonal allergies orally ingest the quercetin-containing test food during the pollen-spreading season, it will help reduce ocular/nasal symp-

toms and preserve their QOL. The alleviation of allergy-like symptoms following ingestion of the test food probably improved the sleep score (an indicator of sleep disorder), accompanied by improvement in QOL, i.e., primarily alleviation of subjective symptoms, such as decreased thinking power, disturbance in outdoor activities, and malaise. Quercetin combined with the new delivery system used in the present study has been reported[10] to have recorded a plasma level after an oral dose in humans 20-times higher than that of ordinary quercetin, thanks to the high solubility and trans-oral absorption. Therefore, an elevation in the bioavailability of quercetin seems to have contributed to the alleviation of allergic symptoms in the present study. Ingestion of this form of quercetin was previously shown to alleviate asthmatic patients' symptoms and suppress the inflammation following subcutaneous injection of histamine[24,25]. The results from the present study are also consistent with previous findings[8].

In the present study, the intervention was conducted when the pollen-spreading level was probably higher than usual, and the symptoms of seasonable allergic rhinitis were more likely to be induced. Even during such a period, ingestion of the test food resulted in significant improvement in both objective evaluation (severity of allergic rhinitis and nasal discharge eosinophil count) and subjective evaluation (primarily influence on sleep and body). This allowed us to confirm that the influence of allergic rhinitis on QOL can be significantly alleviated. However, it was not possible in the present study to check significant changes in unspecific IgE and specific IgE levels in the blood following test food ingestion. In a previous study, the nasal discharge IgE level correlated more closely with the nasal eosinophil count than with serum IgE level in patients with Japanese cedar pollinosis[29]. Serum IgE levels can elevate in cases complicated by other illnesses, such as asthma, atopic dermatitis, and parasitosis. However, the level of nasal discharge IgE (formed in the nasal mucosa) is considered to be less affected by other illnesses[29]. Furthermore, lacrimal fluid IgE measurement has been used as a new diagnosis method for allergic conjunctivitis induced by type I allergy, similarly to allergic rhinitis[29,30]. Therefore, if IgE contained in nasal discharge, lacrimal fluid, and blood is measured, we may expect that the test food's influence on antibody formation may be verified.

For safety evaluation, the physical measurement, physical examination, urinalysis, and peripheral blood test were conducted as the screening and pre-ingestion test and at 2 and 4 weeks after starting ingestion. Significant inter-group differences were sporadically noted in the physical measurement parameters, physical examination, and peripheral blood test. Yet, all these changes were within the criterion or optimum range, posing no medical problem[31-34]. In ingestion, part of the subjects showed values outside the criterion range. Still, the overall evaluation of such data and the other data allowed a judgment that there was no medical problem. In the peripheral blood test, the mean of each parameter was within the criterion range. The subjects showing some parameters outside the criterion range were judged to have no medical problem based on overall evaluation and the data on the other parameters[34-36]. Adverse events developed in some of the subjects, but all events subsided following oral medication or application of an ophthalmic solution. They were confirmed to have no causal relationship to the test food. Thus, the test food was shown to involve no safety problem.

Conclusions

The present study evaluated the influence of 4-week continuous ingestion of quercetin-containing test food on subjective symptoms related to allergic reactions to allergens in Japanese adults of both sexes having eye and/or nose discomfort. Ingestion of the test food resulted in significant improvement in subjective evaluation (primarily the influence on sleep and the body) and objective evaluation (severity of allergic rhinitis and nasal discharge eosinophil count), thus significantly alleviating the effect of allergic rhinitis on QOL.

Conflict of Interest

There is no conflict of interest which can affect the results of this study or interpretation of the results among all individuals involved in this study, their family members, all contractors, Indena Japan Co., Ltd. and Indena S.p.A.

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Availability of Data and Material
Data will be available upon reasonable request.

Authors' Contribution
All authors contributed equally and gave their approval to submit.

Ethics Approval
The study protocol was approved on 19 November 2019 by the Medical Corporation Seishinkai, Takara Clinic (Approval No. 1911-1911-IJ01-01-TC) and registered with UMIC-CTR (UMIN000038765) on 3 December 2019. It was carried out under sufficient consideration of human rights, safety and well-being of individual participants in accordance with the Declaration of Helsinki, the Clinical Study Act, and the Clinical Study Act Enforcement Regulations. Written informed consent was obtained from all subjects before enrollment in the study.

Trial Registered Number
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