

Effectiveness and Safety of Double-Blind, Placebo-Controlled, Low-Dose Oral Immunotherapy with Low Allergen Egg-Containing Cookies for Severe Hen's Egg Allergy: A Single-Center Analysis

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Keywords

Oral immunotherapy · Egg allergy · Low allergen cookies

Abstract

Introduction: The usefulness of low-dose oral immunotherapy (OIT) for the treatment of egg allergy has been unclear. **Objective:** To evaluate the efficacy and safety of OIT with low allergen cookies (LACs) containing a low dose of hen's egg. **Method:** Thirty-three patients with severe hen's egg allergy were randomly administered either OIT with LACs ($n = 21$) or placebo ($n = 12$). Two patients in the LACs group withdrew before completing OIT. The primary endpoint was the number of good responders (G-R), patients with negative results in the oral food challenge (OFC) with a final dose of 2 g hard-boiled egg whites after 4 months of OIT, in each group. Total OFC Aichi score for anaphylaxis/cumulative protein dose (TS/Pro) as the marker of severity of food allergy was also

compared. Adverse events during OIT were evaluated using patients' diaries. **Results:** The proportion of G-R in the LACs group was higher than in the placebo group (7/19 [37%] vs. 1/12 [8%], χ^2 test; $p = 0.077$). The TS/Pro after OIT in the LACs group was lower than in the placebo group (median score, 44.2 vs. 104.1, $p = 0.059$; Mann-Whitney U test). The threshold and TS/Pro before and after OIT significantly improved in the LACs group ($p = 0.015$, $p = 0.027$, respectively; Wilcoxon signed-rank test). There were 99 recorded incidences of symptoms of 1,938 intake events in the LACs group during OIT. Of these, 90 were mild; no severe symptoms occurred. **Conclusions:** OIT with LACs potentially increases the OFC threshold and decreases allergy severity and is a relatively safe treatment modality.

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Introduction

Allergen-specific immunotherapy (AIT) for immunoglobulin E (IgE)-mediated food allergies is a new therapeutic strategy for the management of allergic disorders [1–3]. AIT is classified into oral immunotherapy (OIT), sublingual immunotherapy, and percutaneous immunotherapy [2]. AIT is effective in increasing the threshold of reactivity for a range of foods in children with IgE-mediated food allergy [2]. However, a major limitation of AIT is the high frequency of adverse events [1, 2]. Yanagida et al. [3] reported on the effectiveness of low-dose OIT as a safer treatment modality for allergies to hen's egg. In our previous study, low-dose OIT using low allergen cookies (LACs) was administered to patients with severe egg allergies, and we found that their threshold level sequentially increased [4]. However, the study was a single-arm design, and the sample size was small [4]. In this study, low-dose OIT was administered using LACs to evaluate the efficacy and safety of OIT.

Materials and Methods

We conducted a double-blind, placebo-controlled study of children who had tested positive for the hard-boiled egg white (EW) oral food challenge (OFC) at Osaka Habikino Medical Center between July 2014 and July 2015.

Preparation of LACs

LACs were prepared using methods developed by Maeta et al. [4]. One LAC contained 7.9–11 mg of EW protein. The non-egg cookies were prepared using pumpkin powder instead of eggs.

Sample Size Estimation

Based on the previous study, to estimate sample size we assumed the proportions of patients with no adverse reactions to the challenge with 2 g of hard-boiled EW after OIT were 0.1 in the placebo group and 0.6 in the LACs group [4]. For the comparison of two independent binomial proportions using Pearson's χ^2 statistics, a χ^2 approximation and a two-sided significance level of 0.05 were utilized. The approximate power was 0.804, with group sample sizes of 10 in the placebo and 20 in the LACs.

Study Protocol

Patients with severe egg allergy who had a positive result in the OFC with either 0.2, 0.5, 1.0, or 2.0 g of hard-boiled EW and had provided written informed consent from their guardians were enrolled in this study (online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000502956). Symptom scores of allergic reactions were assessed after each dose by the doctor according to the modified Sampson's anaphylaxis grades in the Japanese Pediatric Guideline for Food Allergy (JPGFA) 2012 [1]. Blood samples were also taken.

Participants were randomly divided into the placebo and LACs groups at a ratio of 1:2 (online suppl. Fig S1). The allocation was made by a third person at Mukogawa Women's University, who was not related to anyone involved in this study, using the software developed by Ito. The LACs and placebo groups were provided LACs and non-egg cookies, respectively. During the OFC, the participants had to take 1, 2, 4, and 10 cookies every 20 min before OIT. Patients with negative OFC results started consuming 10 cookies daily, and those with positive OFC results started consuming fewer cookies, based on the final OFC dose and the anaphylaxis grade. Non-egg cookies or LACs were consumed for at least 4 months. Patients were instructed to ingest the provided cookies for >6 days a week. If patients were started on <10 cookies, we gradually increased the dose to reach 10 cookies per day at a rate of approximately 20% increase per week. Ten pieces of LACs contained 79–110 mg of EW protein. The guardians were asked to record the intake frequency in the patients' diaries, which was evaluated at monthly outpatient visits. Symptoms at home were confirmed through inquiries based on intake diaries from parents.

After 4 months, OFCs were performed, and blood samples were retaken. The first OFC was performed according to the described method. Patients who could ingest a final dose of 2 g of hard-boiled EW without exhibiting any allergic symptoms underwent a second OFC, where they consumed 5, 10, and 20 g of hard-boiled EW every 20 min.

Study Outcome Measures

The good responders (G-Rs) were defined as patients with no adverse reactions during the challenge with 2 g of hard-boiled EW after OIT. The proportion of G-Rs was evaluated in the two groups. A secondary measure for food allergy severity between the two groups was calculated by dividing the total maximum Aichi score for anaphylaxis (TS) by the cumulative protein dose of OFC after OIT (Pro) to generate TS/Pro [5]. The Anaphylaxis Scoring Aichi method is a scoring system to determine the severity of food allergies in OFC. Subjective and objective symptoms observed in each of the 5 systems, namely skin, respiratory, gastrointestinal, circulatory, nervous system, during the OFC test are scored according to the intensity of the symptoms. The sum of all scores is calculated to determine the total score (TS), which is indicative of the strength of the induced symptoms. TS/Pro value is the TS divided by the cumulative protein dose (Pro), which reportedly represents the severity of OFC. TS/Pro values over 31 in boiled EW challenge test are believed to be indicative of severe disease [5].

The strength of induced symptoms at home was evaluated based on classification of severities according to clinical symptoms [6].

We measured EW- and ovomucoid (OM)-specific IgE titers (Thermo Fisher Scientific-Phadia, Uppsala, Sweden) prior to treatment and 4 months after treatment. A prick test was conducted using a bifurcated needle® (Tokyo M I Commerce, Tokyo, Japan) of the EW prick solution and controls® (Torii Pharmaceutical Co., Tokyo, Japan) prior to and 4 months after treatment. A positive skin prick test was defined as a wheal which was >3 mm longer in diameter than that observed in negative controls. The diameter of the flare area was not considered when reading the outcome of the skin prick test. All methods conformed to the JP-GFA 2012 guidelines [1].

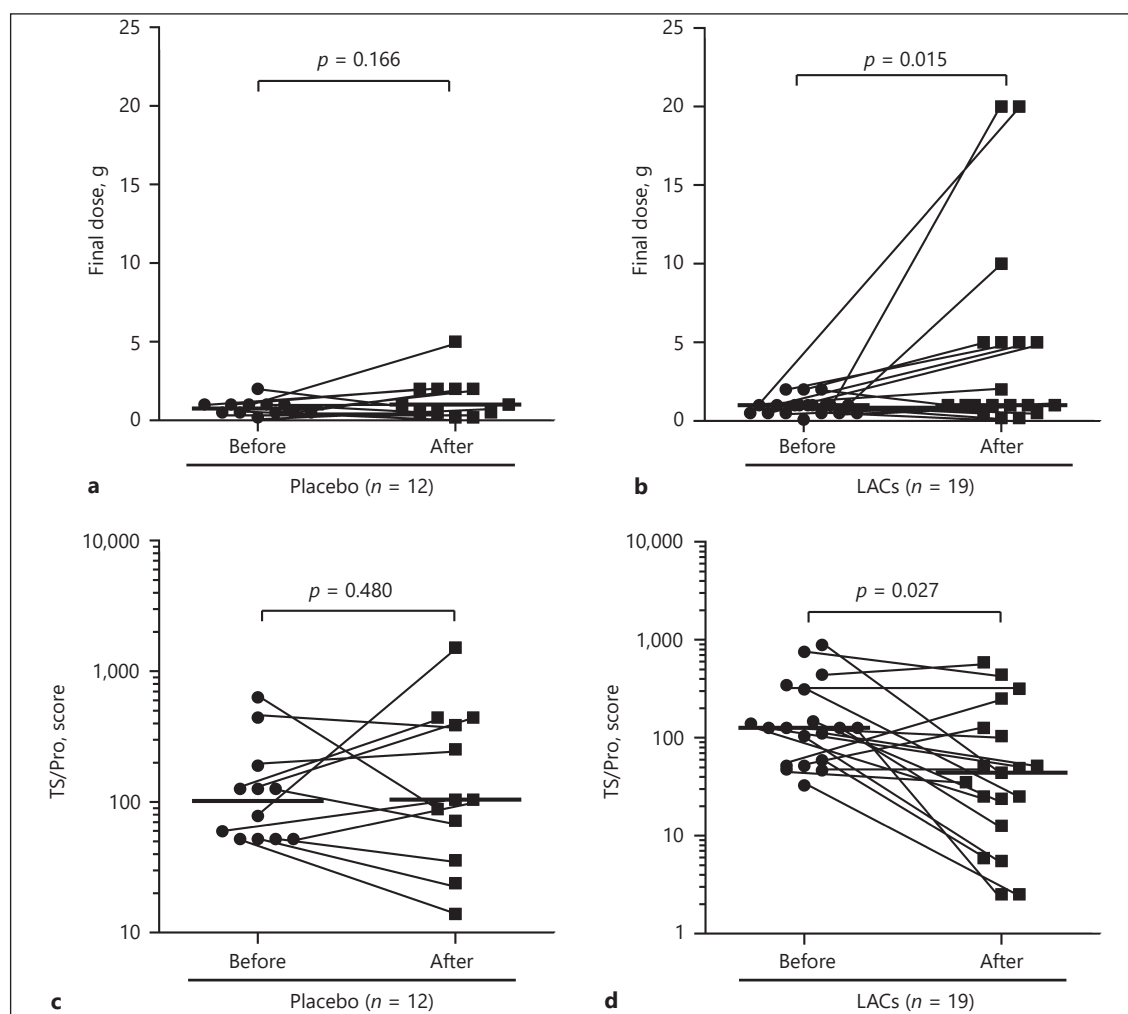


Fig. 1. The final dose of hard-boiled egg white (**a** placebo group, **b** low allergen cookies [LACs] group) and the TS/Pro values (**c** placebo group, **d** LACs group) in the oral food challenge before and after oral immunotherapy. Values indicate the raw data points and median. Raw data of final dose and TS/Pro values in the placebo and LACs groups are described in online supplementary Tables S2 and S3. TS/Pro values were calculated using Sugiura's method [5]. Statistically significant differences were assessed using Wilcoxon signed-rank test. $p < 0.05$ was considered statistically significant.

Statistical Analysis

Intent-to-treat analysis was performed. Continuous variables such as age, final dose, and TS/Pro in the oral challenge test, number of eliminated foods, antigen-specific IgE antibody values, and EW prick test values were compared between the two groups using Mann-Whitney U test. These values were also compared before and after OIT within each group using the Wilcoxon signed-rank test. Meanwhile, χ^2 tests were used to compare the number of negative results of OFC after OIT. Fisher's exact test and χ^2 tests were used to compare demographic characteristics, such as gender, induction symptoms, and number of patients with other allergic diseases, between the two groups. All statistical analyses were performed using IBM SPSS Statistics ver. 22 (New York, USA), and p values < 0.05 were considered statistically significant.

Results

Study Participants

Of the 84 severe egg allergy patients examined, 33 provided written informed consent and were enrolled in the study. Subjects were randomly assigned to the placebo ($n = 12$) and LACs groups ($n = 21$) at a ratio of 1:2 (online suppl. Fig. S1). One patient in the LACs group withdrew consent before commencing OIT, and another patient in the LACs group discontinued OIT because of rejection of LACs. OIT was completed for 12 and 19 patients in the placebo and LACs groups, respectively. The first OFC was

Table 1. Adverse events at home during oral immunotherapy

	Placebo (<i>n</i> = 12)	LACs (<i>n</i> = 19)	<i>p</i> value [†]
Number of children who developed adverse event	3/12 (25%)	5/19 (26%)	0.203
Total number of intakes	1,300	1,938	
Total number of allergic symptoms [‡]	5/1,300 (0.4%)	99/1,938 (5.1%)	0.000
Mild	5/1,300 (0.4%)	90/1,938 (4.6%)	0.000
Moderate	0 /1,300 (0%)	9 /1,938 (0.6%)	0.014
Severe	0	0	–
Symptom details (including overlapping)			
Skin	1/1,300 (0.7%)	70/1,938 (3.6%)	0.000
Gastrointestinal tract	4/1,300 (0.4%)	20/1,938 (1.0%)	0.02
Respiratory tract	0	37/1,938 (1.9%)	0.000
Cardiovascular	0	0	–
Neurological	0	0	–
Total number of medications			
Oral antihistamine	4/1,300 (0.4%)	5/1,938 (0.4%)	1.000
Oral bronchodilator	0/1,300 (0%)	5/1,938 (0.4%)	0.087
Adrenalin	0	0	–
Total number of emergency visits	0	0	–

[†] Statistically significant differences were assessed using Fisher's exact test. *p* < 0.05 was considered statistically significant. [‡] The strength of induced symptoms at home was evaluated based on classification of severities according to clinical symptoms [6]. LACs, low allergen cookies.

performed in all subjects who had undergone OIT for 4 months. The second OFC was performed for the G-Rs in the placebo (*n* = 1) and LACs groups (*n* = 7).

Patient Characteristics

The median age in the patients was 6 years, and the median final dose for OFCs with EW was 1 g. There were no significant differences in clinical and demographic characteristics and the threshold values of the OFC or the TS/Pro before OIT between the LACs and placebo groups (online suppl. Table S1).

Results of OFC after 4 Months

The challenge test results before and after OIT in both groups are shown in online supplementary Tables S2 and S3. The median starting number of cookies in both LACs and placebo groups was 10 cookies according to the results of OFC of assigned cookies (online suppl. Tables S2 and S3). If any patient did not receive 10 cookies per day, the number of cookies would gradually increase over the 4 months to reach the defined number of cookies consumed each day. The proportion of G-R was higher in the LACs group than that in the placebo group (7/19 [37%] vs. 1/12 [8%], χ^2 test; *p* = 0.077). The TS/Pro after OIT was lower in the LACs group than that in the placebo group

(median score, 44.2 vs. 104.1, *p* = 0.059, Mann-Whitney U test). In the placebo group, the final dose that evoked symptoms and the TS/Pro score in the OFC were not different before and after OIT (Fig. 1a, c). However, these indexes significantly improved after OIT in the LACs group (Fig. 1b, d).

EW- and OM-Specific IgE Antibody Titer and EW Prick Test after 4 Months

There was no significant difference in EW- and OM-specific IgE titers and wheal diameter from the EW prick tests after 4 months between the two groups (*p* = 0.205, 0.141, and 0.646, respectively; online suppl. Table S4).

Adverse Events at Home

There was no significant difference in the number of patients who experienced adverse events between the two groups (Table 1). In the LACs group, 99 (5.1%) allergic reactions occurred from a total of 1,938 intakes (Table 1); of these, 90 (91%) were mild. Antihistamine or bronchodilators were used five times during OIT. There were no adverse reactions that required epinephrine use (Table 1). In the placebo group, mild symptoms developed on five occasions from a total of 1,300 intake events; antihistamines were used five times.

Discussion/Conclusion

In the current study, we evaluated the efficacy and safety of OIT with LACs containing a low dose and low allergenicity of hen's eggs. We found that LACs may lead to a rise in threshold level and may subsequently reduce allergy severity compared with placebo.

Another report has also described low-dose OIT as being effective [3]. Yanagida et al. [3] reported that 71% of patients (15/21) exhibited sustained unresponsiveness to 1/32 of a whole egg. In our previous report, 5 of 11 patients were G-R after 4 months of OIT with LACs [4]. Thus, the findings of this study further support that low-dose OIT using LACs has a therapeutic effect against severe egg allergy.

Yanagida et al. [3] reported no severe symptoms in their cohort, and the percentage of mild to moderate adverse events were 59% (57/97) in hospital and 6% (332/5,131) at home. The number of symptoms in the current study was lower than those in Yanagida's report. In our study, there were no systemic symptoms; the number of reports of moderate and mild symptoms was 9 and 90, respectively, from a total of 1,938 total intake events. Nurmatov et al. [2], in a meta-analysis of food immunotherapy, reported that the risk of systemic symptoms increased slightly, with local symptoms also increasing, as a result of performing OIT. This led to low-dose OIT with LACs being determined as a safe treatment methodology.

In this study, patients with severe egg allergy were enrolled. The index of severe allergy is reported as TS/Pro values exceeding 31 points during OFC [5]. The calculated value for all enrolled patients prior to OIT was over 31 points, confirming that all experienced severe egg allergy. We did not define TS/Pro of G-R in this research. A negative result after ingesting 2 g of hard-boiled EW following OIT was defined as G-R, with the value of TS/Pro during OFC under 31. Based on this criterion, all patients achieved G-R except for one patient in whom the calculated value was 35.4 and slightly exceeded 31 (online suppl. Tables S3 and S4). Thus, we believe that the definition of G-R used in this study is generally appropriate.

The ratio of G-R in the LAC group, though higher than in the placebo group, did not reach statistical significance. A mouse model experiment showed that the therapeutic effect of OIT depends on the amount of allergen intake [7]. The smaller the maintenance dose, the higher the safety, but there is a possibility that the effect will decrease with low-dose OIT. Therefore, further studies should determine the optimal dose for OIT that is compatible with safety and efficacy.

There was no difference in the prick test and antigen-specific IgE before and after OIT in the LAC group. We believe these results might be due to the short duration of the OIT period in this study. Caminiti et al. [8] reported that there was no significant change in the EW-specific IgE group after 4 months of OIT with hen's egg, but the changes became significant after 10 months of immunotherapy. Yanagida et al. [3] also reported EW- and OM-specific IgE levels were significantly lower after 6 months and 12 months of OIT, respectively. Further observation in this area is needed.

The limitations of this study include the small sample size and the use of an open challenge test without eliminating the removal period to confirm the desensitized amount. Future studies should be designed for long-term observation and the use of OFC with elimination periods.

In conclusion, 4 months of OIT with LACs may potentially increase the OFC threshold and decrease severity. OIT using low-dose and low-allergenicity eggs is a relatively safe treatment modality.

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Statement of Ethics

This study was approved by the Ethics Committees of Osaka Habikino Medical Center (approval number 683, UMIN000014931) and Mukogawa Women's University (approval number 14-09, 16-77). Written informed consent was obtained from all the guardians of the patients. Informed assent was obtained from patients aged older than 6 years.

Disclosure Statement

The authors have no conflict of interest to declare.

Funding Sources

There were no funding sources.

Author Contributions

Yuri Takaoka, Shinichi Takahashi, Takahiro Muroya, Amane Shigekawa, Yuki Tsurinaga, Norihito Iba, Yukinori Yoshida, Makoto Kameda, and Satoru Doi diagnosed and treated the children with allergies and recruited the patients. Akihiro Maeta and Kyoko

Takahashi distributed the cookies. Yoichi M. Ito provided allocation software and calculated sample size. Yuri Takaoka, Akihiro Maeta, Kyoko Takahashi, and Makoto Kameda designed the research study and drafted the manuscript. All authors have read and approved the final manuscript.

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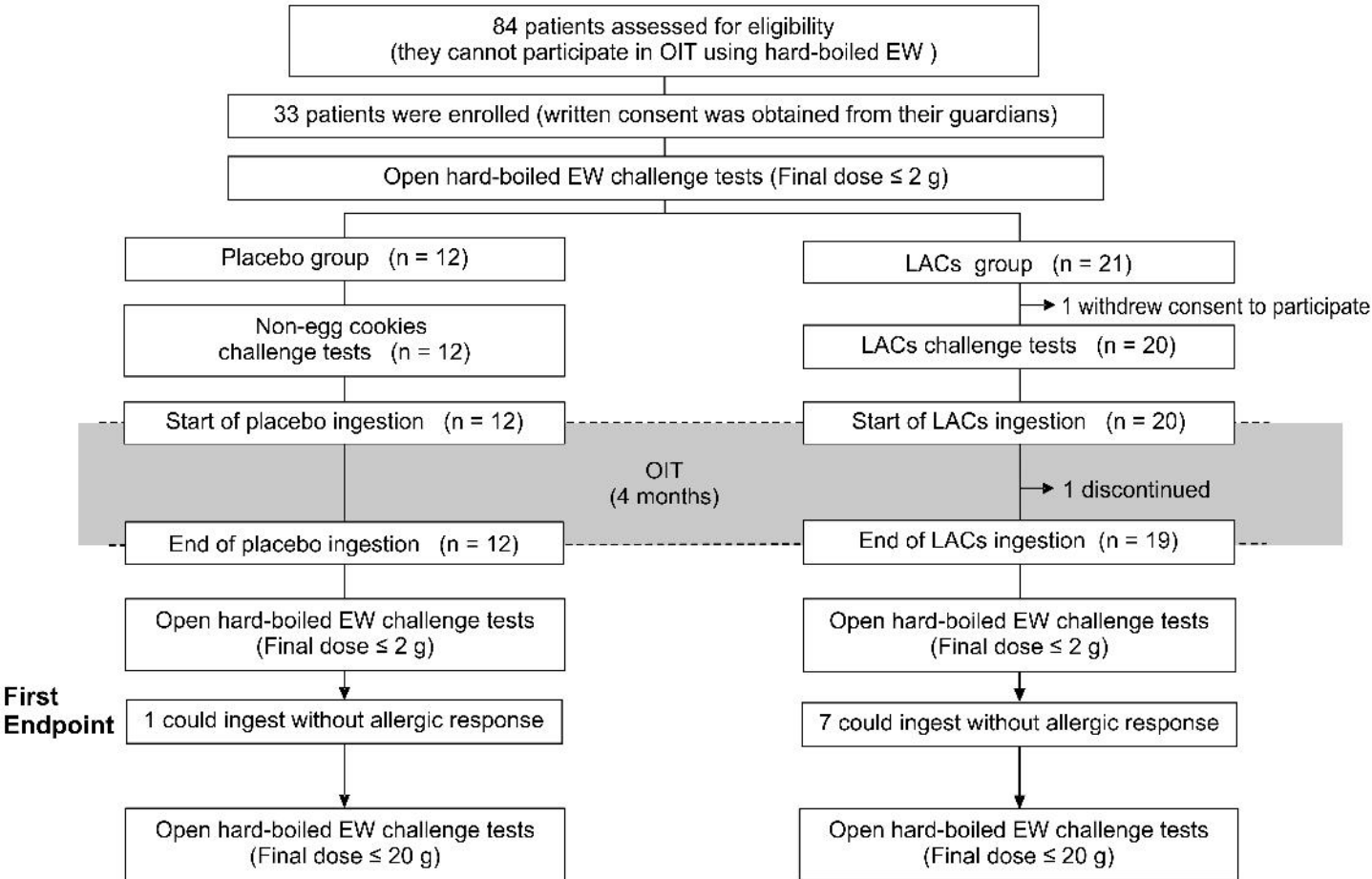


Table S1. Patient profile [†]

	Placebo (n=12)	LACs (n=19)	<i>p</i> value [‡]
Age (years)	6 (1–9)	6 (2–9)	0.3595
Sex (M:F)	10:2	15:4	1.000
Eliminated food items except for egg			
Milk (%)	5 (42%)	11 (52%)	0.473
Wheat (%)	4 (33%)	1 (5%)	0.060
Peanuts (%)	2 (17%)	2 (10%)	0.630
Other (%)	3 (25%)	3 (14%)	0.653
Number of food allergies			
1 (only egg)	4	7	0.1564
2	3	11	
≥3	5	3	
Other allergic disorders			
Atopic dermatitis	11 (92%)	15 (71%)	0.624
Allergic rhinitis	1 (8%)	1 (5%)	1.000
Allergic conjunctivitis	1 (8%)	0 (0%)	0.387
Bronchial asthma	3 (25%)	8 (38%)	0.452
Hard-boiled egg white OFC before OIT			
Final dose (g)	0.8 (0.2–2)	1.0 (0.5–2)	0.4446
TS/pro value [§]	102.3 (52.1–632.1)	126.4 (32.8–885.0)	0.8570
Laboratory data before OIT			
EW prick test (mm)	14.3 (8.0–19.5)	13.0 (9.0–20.0)	0.3006
EW-specific IgE levels (U _A /mL)	57.8 (3.1–100)	22.4 (4.2–100)	0.2289
Ovomucoid-specific IgE levels (U _A /mL)	38.3 (3.7–100)	17.0 (1.3–96.5)	0.1618

[†] Age, final dose of EW, TS/pro value, and laboratory data are presented as medians and range.

[‡] Statistically significant differences were assessed using Mann-Whitney U tests, Fisher's exact test, or Chi squared (χ^2) test. *p* <0.05 was considered statistically significant.

[§] TS/Pro values were calculated using Sugiura's method [5].

Abbreviations: OFC: oral food challenge test, OIT: oral immunotherapy, EW: egg white, LACs: low allergen cookies

Table S2. Age; sex; and the final dose, symptom scores, and TS/Pro values in the open hard-boiled egg white challenge tests before and after treatment in the low allergen cookies (LACs) group

Patient No	Age (years)	Sex	Before			OIT	After		
			Final dose (g)	Symptom scores [†] (according to Ref 1)	TS/Pro [‡] (Value, Ref 5)	Initial number of cookies (N)	Final dose (g)	Symptom scores [†] (according to Ref 1)	TS/Pro [‡] (Value, Ref 5)
O-1	7	M	0.5	G (2)	126.4	10	1.0	G (2)	49.2
O-2	6	F	1.0	R (2)	59.0	10	10.0	G (2)	5.9
O-3	5	F	0.5	G (2)	126.4	10	1.0	G (3), N (2)	104.1
O-4	4	M	2.0	G (2), R (2)	46.6	2	1.0	G (2)	52.1
O-5	3	F	0.5	G (2)	110.6	10	1.0	G (2)	52.1
O-7	1	M	1.0	G (2)	32.8	10	20.0	G (2), R (1)	2.5
O-8	4	M	2.0	R (3)	47.8	10	5.0	G (2), R (2)	35.4
O-10	6	M	0.5	G (3), C (3)	758.5	2	0.2	G (2)	442.5
O-11	8	M	1.0	G (2)	52.1	2	0.5	G (2)	126.4
O-12	2	M	1.0	S (2), R (4), C (3)	442.5	10	0.2	R (3)	590.0
O-13	6	M	2.0	S (2), C (3)	139.7	10	5.0	S (2), R (2)	25.3
O-14	6	M	1.0	G (2)	52.1	4	1.0	G (2), R (2)	252.8
O-15	3	M	1.0	S (2), C (3)	312.3	5	0.5	S (1), G (1)	25.3
O-16	9	M	1.0	S (2), R (2)	344.1	10	1.0	S (1), G (2), R (2), C (3)	317.5
O-17	6	M	0.5	G (1)	126.4	2	5.0	S (1), G (2)	12.6
O-18	4	M	1.0	G (2)	104.1	10	5.0	G (1)	5.5
O-19	6	F	0.1	G (2)	885.0	5	1.0	G (2)	44.2
O-20	5	M	1.0	S (2), R (2)	147.5	10	2.0	S (2)	23.9
O-21	6	M	0.5	G (3)	126.4	10	20.0	R (2)	2.5

OIT: oral immunotherapy

[†] Symptom scores indicated by regions of reaction (S: skin symptoms; G: gastrointestinal tract symptoms; R: respiratory tract symptoms; C: cardiovascular symptoms; N: neurological symptoms) and severity grades (1–5) according to the modified Sampson's anaphylaxis grading system in the Japanese Pediatric Guideline for Food Allergy 2012 [1]. The results of the challenge were determined by a doctor.

[‡] The TS/Pro values were calculated using Sugiura's method [5].

Table S3. Age; sex; and the final dose, symptom scores, and TS/Pro values in the open hard-boiled egg white challenge tests before and after treatment among participants in the placebo group

Patient No	Age (years)	Sex	Before			OIT	After		
			Final dose (g)	Symptom scores [†] (according to Ref 1)	TS/Pro [‡] (Value, Ref 5)	Initial number of cookies (N)	Final dose (g)	Symptom scores [†] (according to Ref 1)	TS/Pro [‡] (Value, Ref 5)
P-1	6	M	0.5	G (2)	189.6	10	0.5	G (2)	252.8
P-2	9	M	2.0	S (1), G (3),	59.8	5	1.0	C (2)	104.1
P-3	2	M	1.0	R (3)	52.1	10	1.0	R (2)	104.1
P-4	3	M	1.0	R (2)	52.1	10	5.0	S (1), G (2)	13.9
P-5	4	M	1.0	G (2), R (2)	78.1	10	0.5	R (4), C (3)	1517.1
P-6	6	F	0.2	G (2)	442.5	10	2.0	S (2), R (3)	387.2
P-7	8	M	0.5	S (2), G (3), R (2)	632.1	10	2.0	S (2), G (2), R (2)	88.5
P-8	6	M	0.5	S (2)	126.4	10	2.0	S (2), G (2), R (2)	71.8
P-9	6	M	1.0	G (2)	52.1	10	2.0	S (1), G (2),	35.9
P-10	8	M	0.5	S (2)	126.4	10	0.2	G (2)	442.5
P-11	8	M	0.5	G (2)	126.4	10	0.2	G (2)	442.5
P-12	4	F	1.0	G (2)	52.1	10	2.0	G (2), N (2)	23.9

OIT: oral immunotherapy

[†] Symptom scores indicated by regions of reaction (S: skin symptoms; gastrointestinal tract symptoms; R: respiratory tract symptoms; C: cardiovascular symptoms; N: neurological symptoms) and severity grades (1–5) according to the modified Sampson's anaphylaxis grading system in the Japanese Pediatric Guideline for Food Allergy 2012 [1]. The results of the challenge were determined by a doctor.

[‡] The TS/Pro values were calculated using Sugiura's method [5].

Table S4. Laboratory data before and after oral immunotherapy[†]

	Placebo (n=12)			LACs (n=19)		
	Before	After	<i>p</i> value [‡]	Before	After	<i>p</i> value [‡]
EW prick test (mm, Min–Max)	14.3 (8.0–19.5)	13.3 (9.0-18.0)	0.722	13.0 (9.5–14.0)	13.5 (5.5-24.0)	0.962
EW-specific IgE (U_A/mL, Min–Max)	57.8 (3.1–100)	45.6 (1.9-100)	0.038	22.4 (9.7–56.5)	23.8 (1.9-100)	0.084
Ovomucoid-specific IgE (U_A/mL, Min–Max)	38.3 (3.7–100)	36.2 (5.8-100)	0.695	17.0 (6.2–41.8)	15.2 (5.0-87.3)	0.227

[†] Values are displayed as median and range.

[‡] Statistically significant differences were assessed using Wilcoxon signed-rank tests in each group. *p* <0.05 was considered statistically significant.

Abbreviations: EW: egg white, LACs: low allergen cookies