

Low-Dose Oral Immunotherapy Using Low-Egg-Allergen Cookies for Severe Egg-Allergic Children Reduces Allergy Severity and Affects Allergen-Specific Antibodies in Serum

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Keywords

Oral administration · Low-allergen cookies · Egg allergy · Allergen-specific immunotherapy

Abstract

Background: We evaluated the safety and efficacy of low-egg-allergen cookies (LAC) as low-dose oral immunotherapy (OIT) in children with severe egg allergy. We also examined the relationship between mild desensitization by low-dose OIT and serum biomarkers of allergy. **Methods:** We enrolled 13 children with egg allergy who could not receive OIT with hard-boiled egg white (EW). For 11 participants, OIT was carried out using LAC for 3–4 months. Open food challenges with hard-boiled EW and blood samplings were performed before and after OIT. Participants were divided into 2 groups: high effect (H-E) and no/low effect (N/L-E). Serum levels of total IgE and egg yolk-, EW-, and ovomucoid (OM)-specific IgE, ovalbumin (OVA)- and OM-specific IgG₄, IgA₁, and IgA₂, and the percentage of CD 203c⁺ were measured. **Results:** Allergic severity was reduced in 7 patients, who were assigned to the H-E group. Moreover, no study participants were taken off the intake of LAC during OIT. In

the H-E group, OVA-specific IgA₂ levels after OIT were significantly higher than before OIT. The ratios of OM-specific IgG₄/OM-specific IgE and OM-specific IgA₂/OM-specific IgE in the H-E group after OIT were significantly higher than before OIT. **Conclusion:** Our findings suggest that low-dose OIT using LAC is an effective and safe treatment for patients with severe egg allergy.

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Introduction

Egg allergy is the most common food hypersensitivity in Japan [1]. Oral immunotherapy (OIT) has been studied as a new treatment, and uses small amounts of food allergen to desensitize the patient [1]. However, it is not often recommended because it carries a risk of exacerbating allergic symptoms [1]. It is necessary to develop a safe OIT method for such cases.

When applied to patients with egg allergy, egg OIT generally uses egg white (EW) powder [2–4]. However,

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Table 1. The age and sex of all subjects, and final dose and symptom scores in the open challenge using hard-boiled EW before and after treatment

Patient No.	Age ^a	Sex	Before treatment		After treatment		Group ^c
			final dose that evoked symptoms	symptom score ^b according to [10]	final dose that evoked symptoms	symptom score ^b according to [10]	
1	5, 7	M	1.0 g	GS (2)	2.0 g	no symptoms	H-E
2	5, 11	M	1.0 g	GS (2)	2.0 g	GS (2)	H-E
3	6, 0	M	0.2 g	GS (2)	2.0 g	no symptoms	H-E
4	5, 10	M	0.2 g	RS (2)	2.0 g	no symptoms	H-E
5	3, 10	F	0.5 g	GS (3)	2.0 g	no symptoms	H-E
6	4, 8	F	1.0 g	GS (2), NS (2)	2.0 g	no symptoms	H-E
7	6, 11	M	1.0 g	GS (2)	2.0 g	GS (2)	H-E
8	8, 7	M	1.0 g	SS (1), RS (2), GS (2)	1.0 g	RS (2), GS (2)	N/L-E
9	4, 1	M	0.1 g	GS (2)	1.0 g	RS (4), GS (2), CS (3)	N/L-E
10	8, 6	M	2.0 g	SS (2), GS (3)	2.0 g	RS (3), GS (2), SS (2)	N/L-E
11	5, 11	F	1.0 g	SS (2)	1.0 g	RS (2), GS (2), SS (1)	N/L-E
12	6, 0	M	0.5 g	GS (2)		no treatment	
13	4, 8	F	0.1 g	GS (2)		no treatment	

Grades of severity (1–5) are according to the modified Sampson anaphylaxis grades in the Japanese Pediatric Guideline for Food Allergy [10]. The challenge results were determined by a doctor. M, male; F, female; GS, gastrointestinal tract symptoms; SS, skin symptoms; RS, respiratory tract symptoms; CS, cardiovascular symptoms; NS, neurological symptoms.

^a In years, months. ^b Indicates the area where a reaction occurred. ^c The high effect (H-E) group could ingest 2 g of hard-boiled EW despite allergic reactions (no symptoms) or proceed to the final increased hard-boiled EW dose without the allergic symptoms worsening before OIT; the no/low effect (N/L-E) group included the other subjects.

patients with severe egg allergy cannot safely eat even 0.5 g of hard-boiled EW. Thus, egg OIT carries a large risk for such patients. In recent egg OIT reports, cooked foods such as baked egg muffins have been used to diminish allergen activity [5]. However, no studies have focused specifically on patients with severe egg allergy, for whom safe OIT methods have not yet been developed. Takahashi [6] reported on heated meringue prepared in a domestic pressure cooker and developed a cookie recipe using this meringue [6]. Following up on this work, we used these cookies for OIT prior to beginning the use of hard-boiled EW.

Recently, low-dose OIT was evaluated [7–9]. However, overall evidence on low-dose OIT is sparse. Therefore, we evaluated whether low-dose OIT using low-egg-allergen cookies (LAC) for patients with severe egg allergy was safe and effective. We also examined the relationship between mild desensitization by low-dose OIT and serum biomarkers of allergy.

Materials and Methods

Study Participants

Participants included children with egg allergy, who, by their treating doctor's recommendation, could not receive OIT using hard-boiled EW (online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000485891). Participants were recruited at the Osaka Habikino Medical Center. A detailed clinical history of all participants (age, sex, family and personal history of allergies, symptoms) was obtained from the clinical records.

Preparation of LAC

Ingredients for LAC were a hen's egg, water, sugar, potato starch, and baking powder, all of which can be purchased from a general supermarket. The heated meringues were prepared by Takahashi's method [6]. Ingredients were mixed using a ratio of 1.0 g heated meringue, 0.2 g egg yolk, 1.64 g sugar, 29 mg baking powder, 0.3 mL soft water, and 4.0 g potato starch. The mixture, which then weighed 0.5–0.7 g, was rolled, and then baked at 160°C for 45 min in an oven (GMO-5910: Osaka Gas Co., Ltd, Osaka, Japan). After baking, the cookies were kept in the oven for 15 min, and then left at room temperature for about 10 min. All LAC were made in our lab, and 1 LAC contained approximately 7.9–11 mg of EW protein.

Table 2. The final dose of hard-boiled EW in the open food challenge and levels of allergic biomarkers in the serum in the N/L-E and H-E groups

	N/L-E group (n = 4)		H-E group (n = 7)		p value		
	before	after	before	after	group	time	G × T
Final dose that evoked symptoms	1.0 (0.3–1.8) ^b	1.0 (1.0–1.8) ^{a, b}	1.0 (0.2–1.0) ^b	2.0 (2.0–2.0) ^a	0.288	0.001	0.012
Total IgE, IU/mL	705 (401–1150)	1,067 (605–2081)	846 (254–1609)	655 (368–1814)	0.967	0.082	0.661
EY-IgE, U _A /mL	13.3 (2.1–79.5)	16.4 (2.3–81.8)	10.2 (8.6–65.0)	10.0 (8.5–40.8)	0.885	0.648	0.232
EW-IgE, U _A /mL	47.0 (15.7–91.4)	64.2 (14.9–96.9)	43.6 (26.9–100)	30.5 (19.3–100)	0.933	0.675	0.055
OVA-IgG ₄ , µgA/mL	0.225 (0.160–1.92)	0.345 (0.180–2.23)	1.31 (0.21–1.57)	2.02 (1.25–2.68)	0.417	0.384	0.414
OVA-IgA ₁ , ref. %	22.3 (8.3–51.0)	22.7 (6.7–83.1)	15.1 (5.2–59.1)	24.1 (17.0–129)	0.484	0.368	0.705
OVA-IgA ₂ , ref. %	19.5 (6.0–31.1)	24.0 (9.3–38.1)	12.8 (7.8–22.8) ^A	36.2 (26.4–66.8) ^B	0.427	0.087	0.155
OM-IgE, U _A /mL	39.1 (14.0–69.7)	47.3 (12.5–69.1)	18.9 (12.1–87.1)	14.7 (10.8–46.8)	0.709	0.520	0.268
OM-IgG ₄ , µgA/mL	0.265 (0.138–2.32)	0.300 (0.128–3.04)	1.04 (0.18–1.78)	1.52 (1.02–2.72)	0.439	0.414	0.455
OM-IgA ₁ , ref. %	55.9 (7.4–134)	62.2 (10.6–293)	59.1 (23.0–315)	107 (36–344)	0.412	0.921	0.620
OM-IgA ₂ , ref. %	14.4 (4.8–27.2)	17.9 (7.0–48.3)	13.5 (9.5–86.5)	33.0 (27.5–64.5)	0.417	0.386	0.470
CD203c ⁺ , %	67.9 (62.1–75.0)	33.6 (5.3–67.8)	34.9 (5.31–45.0)	28.0 (6.57–62.4)	0.119	0.246	0.159

Values are displayed as the median and 25th–75th percentile. Statistically significant differences were assessed using a repeated two-way ANOVA followed by the Bonferroni multiple-comparisons test. When two-way ANOVA indicated the presence of a group-time interaction, one-way ANOVA was conducted followed by the Tukey multiple-comparisons test across all columns. When the interaction was significant, different superscript letters indicate significant differences ($p < 0.05$). When the interaction was not significant, different superscript letters (^{a, b} [before vs. after in N/L-E group] and ^{A, B} [before vs. after in H-E group]) indicate significant differences ($p < 0.05$). G, group; T, time; EY, egg yolk; EW, egg white; OVA, ovalbumin; OM, ovomucoid; ref., reference.

Open Food Challenge and OIT Schedule

The detailed study design is illustrated in online supplementary Figure S1. Prior to conducting OIT, participants were screened with open food challenge with hard-boiled EW and blood samples were taken. In the challenge tests, participants were administered 0.1, 0.2, 0.5, 1.0, and 2.0 g of hard-boiled EW at 20-min intervals, and the results were determined by the doctor. Symptom scores of allergic reaction were assessed according to the modified Sampson's anaphylaxis grades in the Japanese Pediatric Guideline for Food Allergy [10]. The initial numbers of LAC were determined by the open food challenge with LAC. Participants were administered 1, 2, 4, and 10 LAC at 20-min intervals, and the results were determined by the doctor (online suppl. Table S1). The low-dose OIT was performed for 4 months (online suppl. Fig. S1 and Table S2). The maximum number of LAC consumed daily was 10 (each containing 79–110 mg of EW protein). After OIT, the open food challenge with hard-boiled EW was carried out and blood samples were retaken. Participants were then divided into a high-effect (H-E) group and a no/low-effect (N/L-E) group (Table 1). Those in the H-E group had either no allergic reaction to ingesting 2 g of hard-boiled EW or could proceed to the final increased dose of hard-boiled EW (compared to before the intervention), with no worsening of allergic symptoms. The other participants were assigned to the N/L-E group.

Measurement of Allergic Biomarkers in Serum

Levels of serum total IgE and egg yolk-, EW-, and ovomucoid (OM)-specific IgE were measured using the ImmunoCAP 100 instrument (Thermo-Fisher Scientific, MA, USA). The percent-

age of CD203c⁺ basophils in whole blood was measured using an allergenicity kit (Beckman Coulter, Inc., CA, USA). These biomarkers were analyzed at the Osaka Habikino Medical Center. Moreover, serum ovalbumin (OVA)- and OM-specific, IgG₄, IgA₁, and IgA₂ were measured by indirect enzyme-linked immunosorbent assay (ELISA) at the Mukogawa Women's University. Coating antigens (500 µg/mL) were used: OVA (OVA grade VI, Sigma-Aldrich Co. LLC, MO, USA) and OM, which was purified from an egg white by the method of ethanol precipitation [6]. The following secondary antibodies were used: biotin-conjugated mouse monoclonal anti-human IgG₄ (Sigma-Aldrich Co., LLC, MO, USA) and horseradish peroxidase-conjugated mouse monoclonal B3506B4 anti-human IgA₁, mouse monoclonal A9604D2 anti-human IgA₂, and streptavidin (all from Abcam, Cambridge, UK). We used human pool serum as a reference standard (OVA-specific IgG₄, 15.3 µgA/mL; OM-specific IgG₄, 15.6 µgA/mL). The levels of OVA- and OM-specific IgA₁ and IgA₂ in serum were calculated against the reference human pool serum as 100%.

Statistical Analysis

Statistically significant differences were assessed using a repeated two-way analysis of variance (ANOVA) followed by the Bonferroni multiple-comparisons test. When two-way ANOVA indicated the presence of a group-time interaction, one-way ANOVA was conducted followed by the Tukey multiple-comparisons test. $p < 0.05$ was considered statistically significant. All analyses were conducted using GraphPad Prism v5.0 (GraphPad Software, San Diego, CA, USA).

Results

Reduction in Allergic Severity for Low-Dose OIT Using LAC

In the center, 45 out of 116 patients could participate in the OIT using hard-boiled EW; 13 of these 45, assessed for eligibility, were enrolled in the study. Two patients were excluded because they showed an allergic reaction after eating 1–2 LAC (online suppl. Table S1). After the OIT, 5 participants were able to eat 2 g of hard-boiled EW without an allergic reaction (no symptoms) and 2 could proceed to the final increased dose of hard-boiled EW without a worsening of symptoms (Table 1). These 7 participants in the H-E group improved to a level at which they could start OIT using hard-boiled EW. Four participants did not show any improvement in response to OIT (Table 1). In addition, none of the study participants had to stop their intake of LAC during OIT (online suppl. Table S2).

Influence of the Allergic Biomarkers for Low-Dose OIT Using LAC

The serum levels of allergic biomarkers before OIT were not significantly different in the N/L-E and the H-E groups (Table 2). The EW- and OM-specific IgE levels in the H-E group did not differ before and after OIT. In addition, the OVA- and OM-specific IgG₄ concentrations in the H-E group were not significantly different before and after OIT. However, the ratio of OM-specific IgG₄/OM-specific IgE was significantly higher in the H-E group than in the N/L-E group after OIT (the ratio in the H-E group did not differ before and after OIT) (Fig. 1d). Serum levels of OVA-specific IgA₂ were significantly higher after OIT compared to before OIT. Moreover, the ratio of OM-specific IgA₂/OM-specific IgE in the H-E group was significantly higher after OIT compared to before OIT (Fig. 1f). The median increase in the ratio of OVA-specific IgA₂/EW-specific IgE in the H-E and N/L-E groups was 4.1-fold and 1.7-fold, respectively (Fig. 1c).

Discussion

Low-dose OIT has been presented in several recent reports [7–9]. Although egg OIT generally uses EW powder [2–4], Yanagida et al. [8] reported that low-dose (i.e., 1/100–1/32 of a whole egg [62–192 mg of egg protein] in scrambled form once a day) OIT induced sustained unresponsiveness to 1/32 and 1/2 of a whole egg

in scrambled form, with no severe symptoms. Vickery et al. [11] reported that sustained unresponsiveness was achieved at high rates with OIT, both low-dose (300 mg) and high-dose (3,000 mg) peanut flour once a day, in young peanut-allergic children. However, in an allergic mouse model, the intake of a small amount of allergen did not induce oral desensitization [12].

In this study, 7 out of 11 participants improved to a level at which they could begin OIT using hard-boiled EW. They could safely eat 0.5 g of hard-boiled EW after the OIT without an allergic reaction (no symptoms). Therefore, it is suggested that low-dose OIT can reduce the risk of allergic symptoms by accidental ingestion of food including egg, and improve the quality of life of allergic children and their parents. No patients in our study needed to stop their intake of LAC and no adverse reactions during OIT were reported (data not shown). Our results thus confirm that low-dose OIT using LAC is an effective and safe treatment against severe egg allergy.

Several authors have reported on serum biomarkers to predict clinical outcomes [13, 14]. Allergen-specific IgG₄ is the most frequently used serum biomarker in studies of OIT because it could inhibit allergen IgE binding to B cells [15], and increased it when oral desensitization was induced [2–8, 14]. Wright et al. [14] reported that the ratio of serum OM-specific IgG₄/OM-specific IgE was significantly higher in the group that achieved sustained unresponsiveness whereas this was not the case for OVA. Our results are consistent with theirs. IgA is well known for its role in mucosal immunity and is particularly important for antigen elimination in the gastrointestinal tract [16]. Kulis et al. [17] reported that the concentration of peanut-specific IgA measured in saliva or serum was found to be higher in patients who achieved desensitization after 12 months of peanut sublingual immunotherapy. Moreover, serum EW-specific IgA₂ has been associated with the development of tolerance in egg allergy [18]. Pilette et al. [19] reported that the IgA response to immunotherapy is selective for IgA₂, is correlated with increased local TGF- β expression, and induces monocyte IL-10 expression. In a mouse model of allergy, IgE-mediated systemic anaphylaxis and mast cell degranulation induced by antigen ingestion were suppressed by antigen-specific IgA in serum, but not by IgA in the gut lumen [20]. In our study, the level of OVA-specific IgA₂ and the ratios of OVA-specific IgA₂/EW-specific IgE and OM-specific IgA₂/OM-specific IgE were higher in the H-E group after OIT than before OIT. However, Vazquez-Ortiz et al. [21] reported that serum

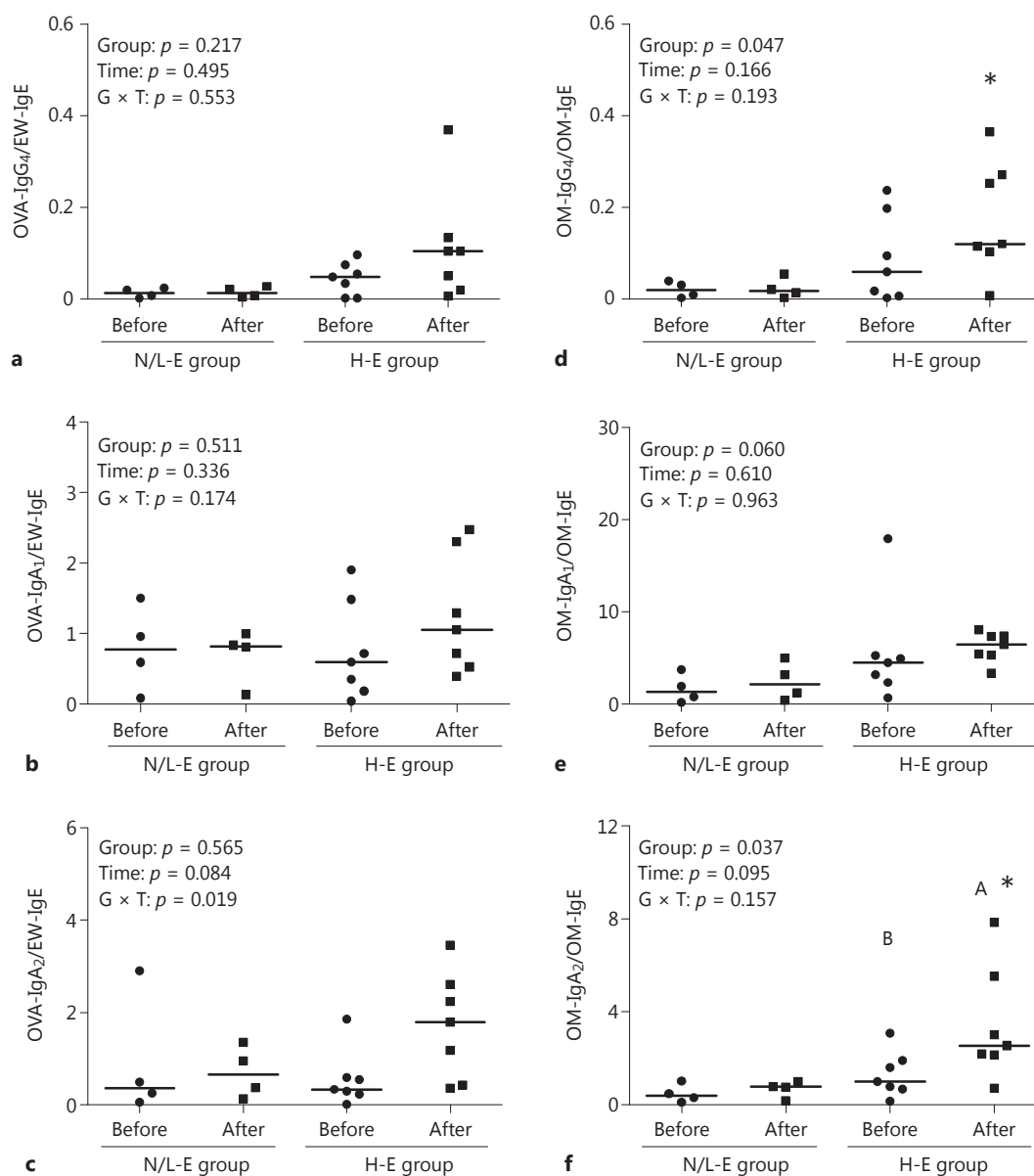


Fig. 1. Ratios of OVA-specific IgG₄ (a), IgA₁ (b), and IgA₂ (c) to EW-specific IgE, and OM-specific IgG₄ (d), IgA₁ (e), and IgA₂ (f) to OM-specific IgE in serum before and after OIT. Values indicate the raw data points and medians. Statistically significant differences were assessed using a repeated two-way ANOVA followed

by the Bonferroni multiple-comparisons test. “A” and “B” indicate significant differences ($p < 0.05$). When the interaction was not significant, the asterisks (N/L-E group vs. H-E group at one time point) in the H-E group indicate significant differences ($p < 0.05$). G, group; T, time.

allergen-specific IgA was not associated with a natural or induced tolerance to egg in children with egg allergy. Our results indicate the relationship between mild desensitization by low-dose OIT and the production of allergen-specific IgG₄ and IgA₂ in the serum.

There were several limitations to this study. The most important limitation is the absence of a placebo group, so we could not distinguish between the effect of OIT and natural tolerance causing the improvement in the egg-allergic patients. However, it is difficult to explain the improvement as being only due to natural tolerance for 3–4 months. The number of patients in the H-E and N/L-E groups was not comparable, and the total number of subjects was small. Moreover, the concentrations of IgA₁ and IgA₂ did not display a percentage against the control pool serum of egg-allergic patients.

Our findings suggest that low-dose OIT using low-allergen food is an effective and safe treatment, and an adequate prior step to OIT using hard-boiled EW for patients with a severe egg allergy. Moreover, it was suggested that a small amount of antigen administration influences the immunologic function. In the future, we recommend that a randomized, double-blind, placebo-controlled trial of LAC be carried out in order to eliminate the effect of natural tolerance.

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Statement of Ethics

The study protocol was approved by the Ethics Committees of the Mukogawa Women’s University (Permit No.: 11–52) and Osaka Habikino Medical Center (Permit No.: 577). Written informed consent was obtained from the parents of all the study participants.

Disclosure Statement

The authors have no conflict of interest to declare.

Author Contributions

A.M., M.A., Y.T., and K.T. designed the research. Y.T. and M.K. diagnosed the children with allergy and recruited the study participants. N.M. and M.A. made the LAC. A.M. and M.M. analyzed the serum samples at the Mukogawa Women’s University. A.M., Y.T., M.K., and K.T. drafted the manuscript. All authors read and approved the final manuscript.

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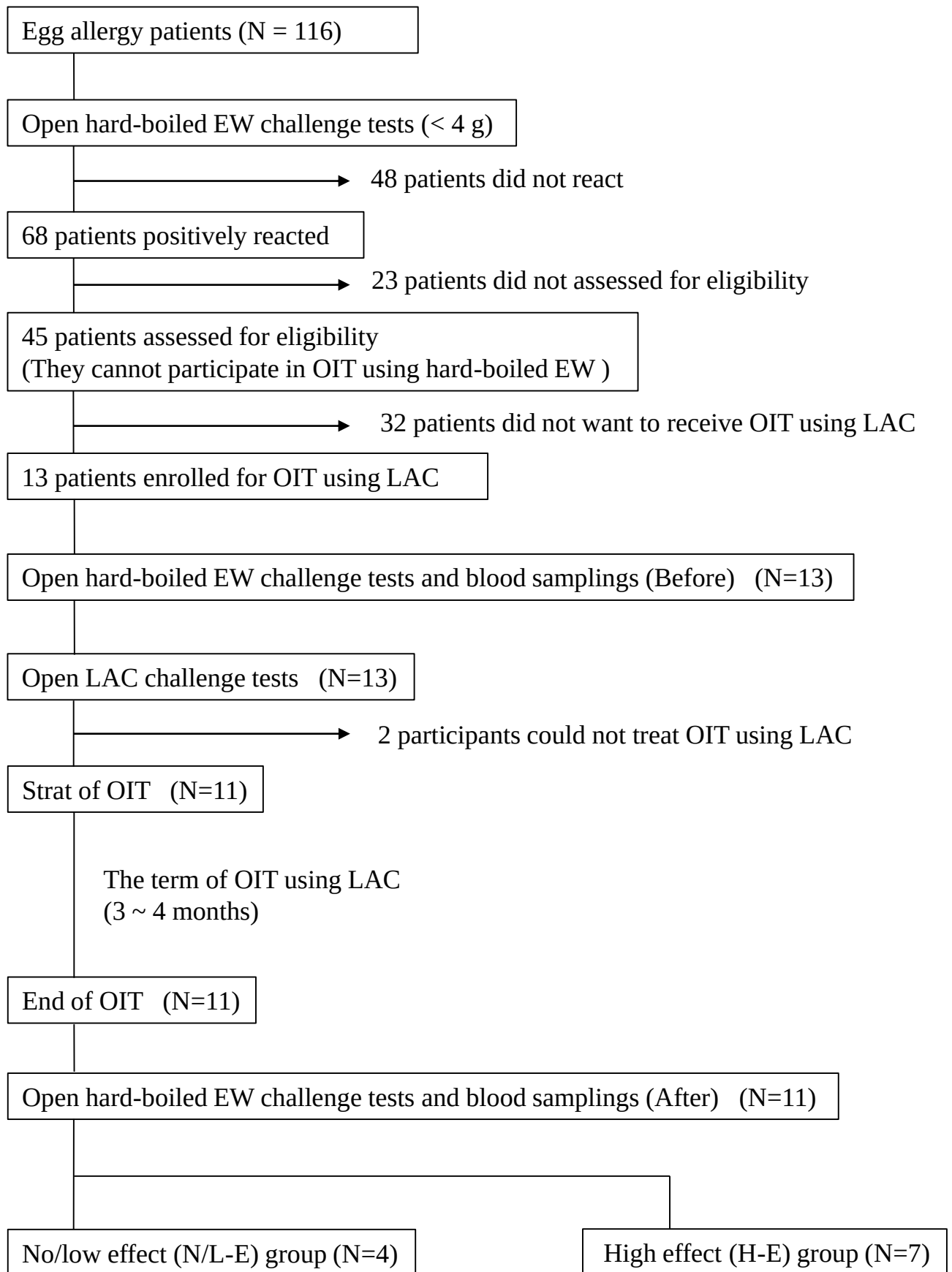


Fig. S1. Study design. Participants could not receive oral immunotherapy (OIT) with hard-boiled EW. The low-dose OIT with low-egg allergen cookies (LAC) was performed for 3–4 months. After the second open hard-boiled EW challenge, participants were divided high effect (H-E) and no/low effect (N/L-E) groups. Participants in the H-E group were those who could ingest 2 g of hard-boiled egg white (EW) despite allergic reactions and for whom the final hard-boiled EW dose could be increased without allergic symptoms worsening before OIT. The other participants were assigned to the N/L-E group.

Table S1. The final dose and symptom scores in open low-egg allergen cookies (LAC) challenge tests before treatment, and the initial numbers of LAC once a day

Patient No	Before treatment		The initial numbers of LAC once a day
	Final dose of LAC that evoked symptoms	Symptom scores [†] (according Ref 10)	
1	10	NS (2)	2
2	10	No symptoms	10
3	10	GS (3)	1
4	5	RS (2)	1
5	10	RS (2)	2
6	10	No symptoms	10
7	10	No symptoms	10
8	10	No symptoms	10
9	5	GS (2)	1
10	5	RS (2)	1
11	10	SS (1) RS (3) GS (2)	1
12	1	SS (1) GS (2)	No treatment
13	2	GS (2)	No treatment

[†] Symptom scores indicated reacted region (GS; Gastrointestinal tract Symptoms SS; Skin Symptoms, RS; Respiratory tract Symptoms, CS; Cardiovascular Symptoms, NS; Neurological Symptoms) and severity grades (1–5) according to the modified Sampson’s anaphylaxis grades in the Japanese Pediatric Guideline for Food Allergy [10] and these challenge results were determined by a doctor.

Table S2. The intake amount of low-egg allergen cookies (LAC) during oral immunotherapy (OIT)

Patient	Amount / week for OIT																Total
No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1	14	14	21	21	28	28	35	42	49	56	63	70	70	70			581
2	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70		1050
3	7	7	14	14	21	21	28	28	35	42	49	56	63	70	70		525
4	7	7	14	14	21	21	28	28	35	42	49	56	63	70	70		525
5	14	14	21	21	28	28	35	42	49	56	63	70	70	70			581
6	70	70	70	70	70	70	70	70	70	70	70	70	70	70			980
7	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70		1050
8	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	1120
9	7	7	14	14	21	21	28	28	35	42	49	56	63	70			455
10	7	7	14	14	21	21	28	28	35	42	49	56	63	70			455
11	7	7	14	14	21	21	28	28	35	42	49	56	63	70	70		525