

Early sustained unresponsiveness after short-course egg oral immunotherapy: a randomized controlled study in egg-allergic children

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Summary

Background No studies have evaluated the potential of egg oral immunotherapy (egg-OIT) to induce sustained unresponsiveness after discontinuing therapy following short-term treatments.

Objective We assessed the efficacy of short-course egg-OIT to induce sustained unresponsiveness.

Methods Sixty-one egg-allergic children, 5 to 17 years old, with positive double-blind placebo-controlled food challenge (DBPCFC) to dehydrated egg white (EW) were randomized to receive egg-OIT (OITG) for 3 months (maintenance dose one undercooked egg every 48 hours) or to continue egg avoidance diet (control group, CG) for 4 months. Children who completed egg-OIT avoided egg for 1 month. At 4 months, both groups underwent a DBPCFC. OITG participants who passed this challenge were instructed to add egg to their diet *ad libitum*. Immune markers were studied at different time points.

Results Ninety-three percent (28/30) of OITG children were desensitized in a median of 32.5 days (IQR, 14 days). At 4 months, 1/31 (3%) in CG passed DBPCFC and 11/30 (37%) of OITG (95% CI, 14 to 51%; $P = 0.003$), all of them were consuming egg at 36 months. A decrease in EW, OVA and OVM skin test results and OVA-specific IgE (sIgE) levels was observed on OITG at 4 months ($P = 0.001$). EW-, OVA- and OVM-sIgE levels prior to the start of egg avoidance diet were lower in OITG children who passed DBPCFC at 4 months than in those who did not pass it. EW- and OVM-sIgE showed the best diagnostic performance in predicting DBPCFC result at 4 months. Levels above optimal EW-sIgE cut-off of 7.1 kU/L indicated 90% probability of failing DBPCFC.

Conclusion This is the first demonstration of sustained unresponsiveness with a three-month egg-OIT protocol. Almost all treated subjects were desensitized and 37% achieved sustained unresponsiveness. EW-sIgE levels at the end of treatment predicted sustained unresponsiveness. This protocol shows a new approach to OIT for egg-allergic children.

Keywords clinical tolerance, desensitization, egg allergy, food allergy, oral immunotherapy, sustained unresponsiveness

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Introduction

Food allergy is an important health concern that has become increasingly prevalent in the past 10 years [1]. Up to 10% of one-year-old children are food allergic [2]. Egg and milk are the most common causes of food allergy in childhood, affecting 1.6% to 8.9% of children between 1 and 3 years of age [2–4]. In addition, egg allergy persists in 49–88% of children at 6 years of age [5, 6] and in up to 32% at 16 years of age [5]. Food

allergy impairs the quality of life of allergic children [7], impacts on the daily activities of children and their families [8] and is a life-threatening condition [9] where severe allergic reactions can occur [10].

To provide a better treatment rather than the limited current standard of care based on food avoidance, new therapeutic strategies, such as oral immunotherapy (OIT), are being researched. Food immunotherapy represents a potential etiological treatment and one step further in the treatment of food allergy.

Egg-OIT is a great developing treatment in the last 6 years [11–20]. To date, most studies have focused on desensitization [11–20], where the food tolerance is maintained only due to its uninterrupted regular intake. Only scattered studies have analysed sustained unresponsiveness (SU) [11, 14, 16, 18], in which tolerance is maintained after a withdrawal of the intake for an extended period of time. Unlike other OIT protocols to date [11, 14, 16, 18], this study evaluates the SU after a short-course OIT with a target maintenance dose of one raw egg white.

We present a randomized controlled study of the safety and efficacy of an egg-OIT protocol to induce SU in a short period of time in children over 5 years old with egg allergy.

Methods

Trial design

Controlled randomized trial investigating the safety and efficacy of egg-OIT treatment for 3 months to obtain SU compared with egg avoidance diet, in children with IgE-mediated egg allergy.

Participants

Patients aged between 5 and 17 of both genders, egg-allergic children who followed egg avoidance diet including baked and extensively heated egg, were invited to participate in the study and were consecutively recruited at the Department of Allergy, Hospital Infantil Universitario Niño Jesús in Madrid, Spain. Patients were recruited between January and October 2009. Full follow-up was completed in February 2013.

Inclusion criteria were as follows: history of symptoms of allergic reaction immediately or within two hours following egg ingestion, positive skin prick test (SPT) (≥ 3 mm) and specific IgE (sIgE) levels ≥ 0.7 kU/L for egg white (EW), ovalbumin (OVA) and/or ovomucoid (OVM), and a positive double-blind placebo-controlled food challenge (DBPCFC) to dehydrated EW at enrolment. Participants were excluded if they had a history of extremely severe anaphylaxis after egg consumption (e.g. hypoxia, hypotension, loss of consciousness), egg non-IgE-mediated adverse reactions, eosinophilic esophagitis, malignant diseases, autoimmune diseases, severe immune deficiencies, any baseline disease contraindicating the use of epinephrine and/or allergy to any component of the placebo-controlled challenge.

Baseline parameters were recorded as individual potential predictors to developing SU (variables considered: baseline age, gender, history of asthma, history of previous anaphylaxis to egg ingestion).

Randomization and generation of study groups

The allocation of patients to the intervention and control groups was performed after their acceptance to participate in the study and provided they met all the selection criteria and none of the exclusion criteria. Participants were assigned to groups in a 1 : 1 ratio using a computer-generated randomization table (Fig. 1).

Intervention group (OITG): Children received egg-OIT for 3 months followed by egg avoidance diet for 1 month. Control group (CG): Children continued egg avoidance diet during 4 months.

At baseline, immune markers were analysed in OITG and CG. Three months after starting egg-OIT, prior to the start of egg avoidance diet for 1 month, immune markers were analysed in OITG. Four months after the study had begun, children in OITG and CG underwent a DBPCFC and immune markers were determined.

Study supervision

The study protocol and consent forms were approved by the Institution's Independent Ethics Committee. Written informed consent was signed by the parents or guardians according to the ethical guidelines of the institution for research in children, and written informed assent was obtained from children over 11 years.

The authors assure the accuracy of data and analysis, as well as compliance with the established protocol.

Double-blind placebo-controlled food challenge

The DBPCFC active and placebo challenges were performed on different days [21]. The allergen source used for active challenge was dehydrated EW (OVOSEC SA, Valladolid, Spain), also called dried EW or EW powder [11, 15, 16, 18]; the product retains allergenicity of raw EW proteins as we confirmed in a previous study [22]. According to the manufacturer, 3600 mg of product is equivalent to one medium size EW and its protein content is at least 78% (2808 mg).

In each of the 2 days of the DBPCFC, nine doses were administered. The dosage of verum oral challenge consisted of the administration of 7, 13, 32, 65, 129, 259, 388, 583 and 1332 mg of EW protein (cumulative dose 2808 mg) at 20-min intervals. Passing meant subjects successfully consumed the maximum dose of dehydrated EW. The test was considered positive if objective symptoms developed (i.e. hives, sneezes, runny nose, cough, and respiratory distress), if subjective but mild–moderate symptoms (oral pruritus, abdominal pain) persisted over 40 min or if subjective but severe symptoms (severe abdominal pain) appeared regardless of its

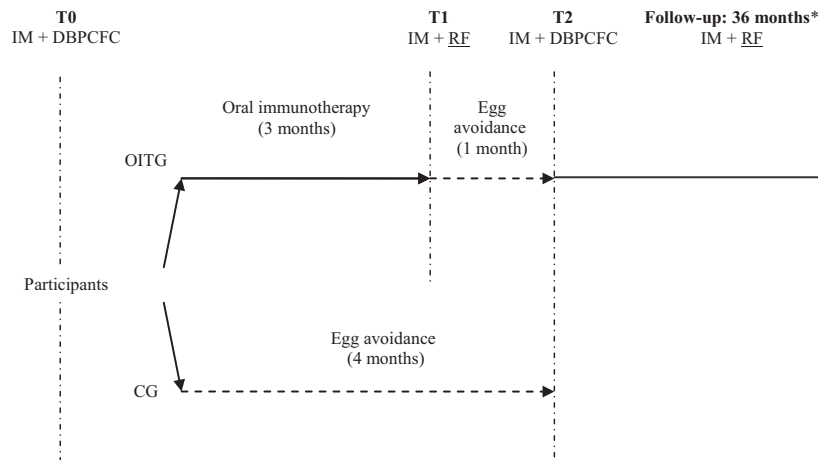


Fig. 1. Study outline. T0, Baseline, T1, 3 months after starting egg-OIT; T2, 4 months after the study had begun; IM, immune markers (skin tests, sIgE and sIgG₄); DBPCFC, double-blind placebo-controlled food challenge; OITG, intervention group; CG, control group; RF, registry forms (egg-OIT compliance and record of adverse reactions). *OITG children who passed DBPCFC at T2. Adverse reactions related to egg ingestion, the frequency, presentation and amount of egg intake were recorded and immune markers were analysed every 6 months.

duration. Patients remained under observation in the hospital at least two hours after the last dose administration or two hours after symptoms had totally subsided in children who failed.

Egg-OIT protocol

The allergen source used in the initial-day dose escalation and build-up (BP) phases of the egg-OIT was dehydrated EW. The egg-OIT protocol followed the procedure described by Staden et al. [14] with some modifications and consisted of three phases: i. initial-day dose escalation phase, which consisted in the administration in 1 day of 0.08, 0.2, 0.3, 0.5, 1, 2, 5, 9, 17, 35, 70 and 140 mg of EW protein (cumulative dose 280 mg) at intervals of 20 minutes, ii. BP, which consisted of increases in the dose of 0.02, 0.3, 3, 14, 68, 188, 352, 1404 mg and 2808 mg of EW protein on a weekly basis, and iii. maintenance phase (MP), which consisted of eating at least one undercooked egg (fried egg, scrambled or undercooked omelette) compulsory every 48 hours. Moreover, during this phase, the subject could freely take any other foodstuffs containing raw, cooked or heated egg (i.e. candies, sauces and ice cream).

Elapsed time between baseline DBPCFC and egg-OIT initial-day dose escalation phase was less than 2 weeks. The initial-day dose escalation was carried out in the hospital. The end of this phase was determined by the onset of an allergic reaction to any of the 12 doses or after administration of the last dose (140 mg). Every patient was kept under surveillance for two hours after the last dose was administered. Achieving a minimum amount of EW without symptoms in the initial-day

dose escalation phase was not required to qualify for BP.

The BP started with the highest egg dose tolerated in the initial-day dose escalation phase. Dose increases were performed once a week in the hospital. If the dose was tolerated, the patient continued with the home daily intake of the same amount for the following week. In case of an adverse reaction during BP at increasing dosage, the previously tolerated dose was prescribed daily for the following week and a new attempt of increasing the dose was performed on the next week.

The MP was started only if the child tolerated 2808 mg of EW protein at BP. In case of an adverse event during MP, the subject was evaluated the next day at the unit, and personalized strategies were undertaken according to the severity of the event.

Follow-up

Egg-OIT compliance and recording of adverse reactions were collected by parents/guardians in registry forms during egg-OIT.

Adverse events were classified according to severity as mild (oropharyngeal symptoms [OFS], skin, digestive and/or rhinitis manifestations), moderate (mild adverse events plus mild respiratory distress) and severe (severe respiratory distress and/or symptoms of hypotension) [23]. Parents were trained for prompt recognition and treatment of adverse reactions.

Participants, who had received egg-OIT and passed DBPCFC at 4 months, were instructed to add egg to their diet ad libitum. The children could freely take undercooked egg and any foodstuffs containing raw, cooked

or heated egg. Egg consumption and recording of adverse events that could occur during the 36 months following the DBPCFC at 4 months were verified by clinical visits every 6 months. During the visits, adverse reactions related to egg ingestion, the frequency, presentation and amount of egg intake were recorded. Participants who failed DBPCFC at 4 months were re-desensitized following a modified egg-OIT protocol in which the BP was started 1 day after the DBPCFC with the last tolerated dose in the active challenge.

Participants assigned to CG were invited to follow egg-OIT if failed DBPCFC at 4 months.

Immune markers

Skin prick test and determinations of serum sIgE for EW, OVM (Gal d 1) and OVA (Gal d 2), and determinations of serum-specific IgG₄ (sIgG₄) for EW were performed at baseline and after 3 and 4 months. SPT was performed according to the standard procedure for prick [24] with EW (1 mg/mL), OVA (1 mg/mL) and OVM (1 mg/mL) (Leti, Madrid, Spain). A wheal mean diameter of 3 mm or more compared to the saline control was defined as a positive reaction. Determinations of serum EW-sIgE, OVM-sIgE and OVA-sIgE and determinations of serum EW-sIgG₄ were performed (ImmunoCAP, Thermo Fisher Scientific, Waltham, MA, USA).

Outcomes

The primary study outcome was to assess the induction of SU after 3 months of OIT with egg. We define SU as the ability to consume 2808 mg of EW protein without symptoms in a DBPCFC at 4 months, after OIT for 3 months and egg avoidance diet throughout the entire fourth month. Secondary objectives were to assess: (i) the induction of desensitization, defined by the patient's ability to eat at least one undercooked egg (fried egg, scrambled or undercooked omelette) compulsory every 48 h during MP without adverse reactions; (ii) safety (number and severity of adverse reactions); (iii) individual potential predictors to develop SU (variables considered: baseline age, gender, history of asthma, history of previous anaphylaxis to egg ingestion, frequency and severity of adverse events during OIT, dose triggering symptoms in DBPCFC at baseline and EW-, OVA- and OVM-sIgE at 3 months); (iv) changes in the DBPCFC threshold; and (v) immunological changes throughout the trial.

Sample size

The necessary sample size was predetermined to estimate the proportions of patients with SU after egg-OIT and without it; an α of 95% and a statistical power (1- β) of 90%; an expected proportion of 36% in OITG,

based on the study of Staden *et al.* [14] and 2% in the CG. Assuming a rate of 15% of subjects lost to follow-up in each group, the minimum number of subjects in each group required to perform these estimates was 30.

Statistical analysis

To assess the differences between OITG and CG at baseline and 4 months, intragroup differences in OITG and CG between at baseline and 4 months, with respect to variables related to the selection for the study and immune markers, the Student *t*-test was used for quantitative variables and the chi-square test was applied for qualitative variables.

Fisher's exact test was performed to determine whether the proportions of patients achieving the SU were different between OITG and CG.

All clinical outcomes were assessed by intention-to-treat analysis.

To evaluate the variation of the threshold between baseline and 4 months of OITG patients who did not pass DBPCFC at 4 months, the Wilcoxon signed-rank test was performed. The Mann-Whitney *U*-test was performed to assess differences between the threshold of OITG and CG children who did not pass DBPCFC at 4 months.

Receiver operating characteristic (ROC) curve analysis studied the diagnostic performance of egg-sIgE at 3 months to predict the DBPCFC result at 4 months. Specificity, Youden index (YI) and likelihood ratio (LR) were used to determine the most appropriate cut-off point. After identifying the cut-off, positive and negative predictive values were calculated to determine the likelihood of passing or failing DBPCFC at 4 months, above or below, respectively, for said cut-off.

Results

Study participants

Sixty-one patients of 91 (67%) who were invited to participate were randomized (Fig. 2). Thirty patients were randomized to OITG and 31 to CG. Sixty-three percent of children were male (73% in OITG and 52% in CG). The age of patients ranged from 5 to 17 years (median, 8 years; IQR, 6 years). Eighty-seven percent of children had a history of atopic dermatitis, 59% of rhinitis, 41% asthma and 63.9% other food allergy. Twenty-three percent (7/30) of the patients in OITG had a history of anaphylaxis due to egg consumption. Seventeen percent (5/30) of OITG and 10% (3/31) of CG had EW-sIgE levels higher than 50 kU/L (ranges: 53.7–245 and 50.5–73 kU/L, respectively). There were no significant differences between OITG and CG in baseline clinical characteristics, except for EW-sIgE levels that were significantly higher in OITG (Table 1).

Outcomes

Clinical outcomes. Ninety-three percent (28/30) of OITG patients were desensitized in a median of 32.5 days (IQR, 14 days). The median duration of the BP in children who needed less than 3 months to be desensitized (83%, 25/30) was 30 days (IQR, 10 days). Ten percent (3/30) of OITG children needed more than 3 months to be desensitized (mean 121 days, SD 16.8 days) due to adverse reactions during the immunotherapy. These patients did not perform an elimination diet or DBPCFC at 4 months and were included in the group of children who did not pass the DBPCFC at 4 months for the statistical analysis. Seven percent of OITG children (2/30) were withdrawn from the study for non-severe (mainly abdominal pain and vomiting) repeated allergic reactions.

At 4 months, 37% (11/30) of OITG children passed the DBPCFC, compared with 3% (1/31) of CG patients who passed it (95% CI for the difference in the response rate, 14 to 51%; $P = 0.003$). All OITG children who

passed the DBPCFC at 4 months regularly consumed foodstuffs containing raw, cooked or heated egg and at least 2 undercooked eggs per week during the 36-month follow-up. Baseline age, gender, history of asthma, history of previous anaphylaxis to egg ingestion, frequency and severity of adverse events during egg-OIT, and the dose that triggered symptoms in DBPCFC at baseline, were not potential predictors of DBPCFC results at 4 months.

The OITG patients ($n = 14$) who did not pass DBPCFC at 4 months increased their threshold mean dose from 100.8 mg EW protein (SD, 96.3 mg) at baseline to 481.3 mg (SD, 417.5 mg) at 4 months ($P = 0.002$). In addition, this dose was significantly higher than the threshold dose in CG patients at 4 months (mean 256.2 mg, SD 425.3 mg) ($P = 0.02$). CG patients showed a non-significant increase in their threshold from baseline (mean 218.3 mg, SD 405.5 mg) to 4 months (mean 256.2 mg, SD 425.3 mg) ($P = 0.41$). There were no differences in threshold at baseline

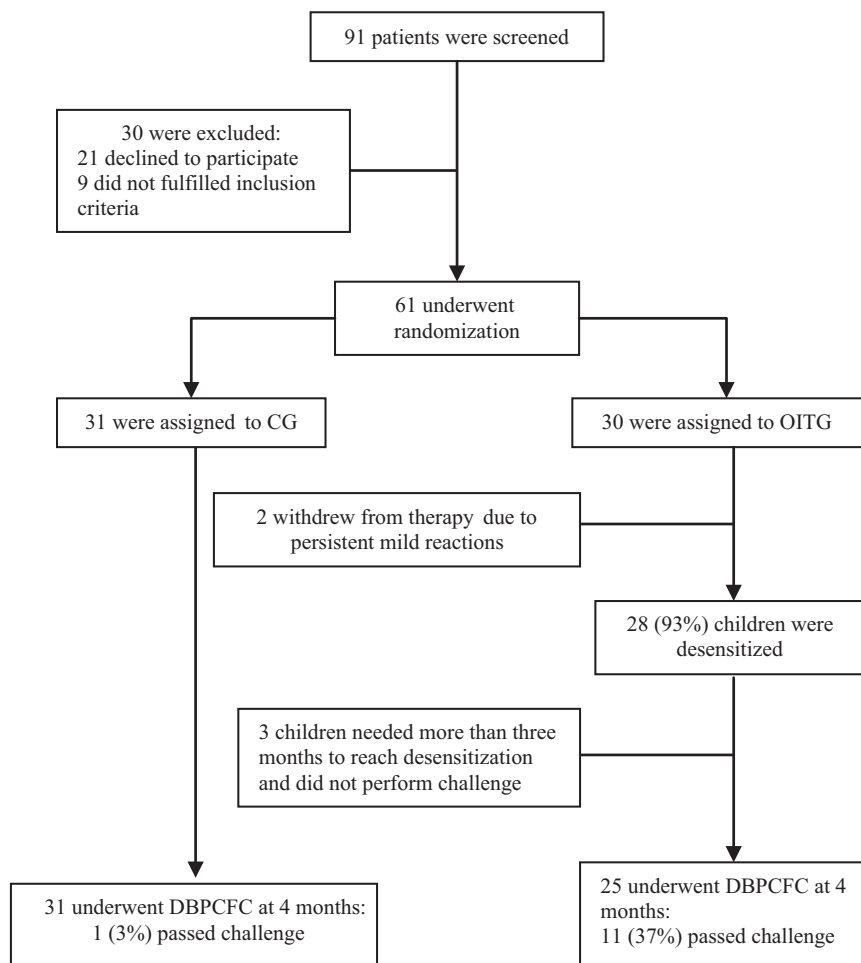


Fig. 2. Study enrolment, randomization, and outcomes. OITG, Intervention group; CG, Control group; DBPCFC, Double-blind placebo-control food challenge.

Table 1. Baseline clinical characteristics, baseline double-blind placebo-controlled food challenge, according to study group. Student's *t*-test and chi-square test

Characteristics	CG (N = 31)	OITG (N = 30)	P
Age – years mean (SD)	8.71 (2.6)	7.80 (2.9)	0.2
Atopic dermatitis – percent	83.8	90	0.7
Asthma – percent	41.9	40	0.9
Allergy to other foods – percent	67.7	60	0.6
Total IgE antibody – kU/L	370.5	603	0.2
median (range)	(33.2–3582)	(143–5000)	
EW-sIgE – kU/L	2.2 (0.7–73)	6.4 (1–245)	0.02
median (range)			
OVA-sIgE – kU/L	2.2 (0.7–43.5)	6.3 (0.7–185)	0.1
median (range)			
OVM-sIgE – kU/L	2.3 (0.7–89.1)	3.1 (0.7–162)	0.4
median (range)			
EW-sIgG ₄ – mg/L	0.2 (0.1–3.6)	0.45 (0.1–6.2)	0.2
median (range)			
Wheal diameter on EW skin prick test – mm	6 (3–12)	6 (3–11)	0.2
median (range)			
Age at first allergic reaction to egg – months mean (SD)	15.7 (13.8)	18.1 (14.8)	0.5
Cumulative dose that elicited symptoms at DBPCFC at T0 – mg of EW protein	162 (9–3600)	72 (9–1708)	0.3
median (range)			

CG, control group; OITG, intervention group; SD, standard deviation; EW, egg white; sIgE, specific IgE; OVA, ovalbumin; OVM, ovomucoid; DBPCFC, double-blind placebo-controlled food challenge.

There were no significant differences between OITG and CG in baseline clinical characteristics, except for EW-sIgE levels that were significantly higher in OITG.

between OIT patients who did not pass DBPCFC at 4 months (mean 100.8 mg, SD 96.3 mg) and CG patients (mean 218.3 mg, SD 405.5 mg) ($P = 0.29$). There were no statistical differences in threshold at baseline between OIT patients who did not pass DBPCFC at 4 months (median 135 mg, IQR 171 mg) and OIT patients who passed the challenge (median 45 mg, IQR 135 mg) ($P = 0.98$).

Adverse events. There were 145 allergic reactions during egg-OIT, 21 (14.5%) in the initial-day dose escalation phase, 79 (54.5%) in BP and 45 (31%) in MP in 70% (21/30) of patients (Table 2).

Initial-day dose escalation phase. Doses triggering reactions on this day were as follows: 140 mg (24%), 70 mg (24%), 35 mg (19%), 17 mg (9%), 9 mg (9%), 5 mg (5%), 2 mg (5%) and 1 mg (5%). No participants reacted with doses of 0.08, 0.2, 0.3 and 0.5 mg.

Build-up phase. There were gastrointestinal symptoms in 82% (65/79) of the reactions, OFS in 21.5% (17/79), rhinitis in 11.4% (9/79), respiratory distress in 6.3% (5/79) and generalized urticaria in 3.8% (3/79). One patient required epinephrine in this phase due to rhinitis, urticaria and mild respiratory distress after a 2404 mg dose. In this case, due to the intensity of the reaction, the protocol was modified and the egg dose was reduced by 50%. He tolerated the new dose every day during 1 week. Further increases were performed following the protocol without reactions. Two children (7%) were withdrawn from the study for non-severe repeated allergic reactions (abdominal pain and vomiting) during BP. These two patients had EW-sIgE levels of 5.6 and 245 kU/L, respectively.

Maintenance phase. All reactions were mild; there were gastrointestinal symptoms in 44% (20/45) of the reactions, OFS in 53% (24/45), rhinitis in 24% (11/45) and generalized urticaria in 9% (4/45). Fifty-seven percent (16/28) of children who completed treatment declared/admitted that they disliked the taste and texture of undercooked egg. However, they continued taking egg according to the protocol.

Follow-up. During the 36-month follow-up of OITG children who passed the DBPCFC at 4 months, one participant referred one single episode of unilateral eye redness and itching after eating fried egg and two other participants reported occasional OFS after eating omelette or fried egg, only in the first 6 months after the DBPCFC at 4 months. In all cases, the symptoms resolved without treatment. The three of them continued taking egg normally until the end of the follow-up period.

The baseline EW-, OVA- and OVM-sIgE levels (kU/L) were higher in patients who suffered any adverse event [median and range: 11.9 (1–245), 8.2 (0.7–185) and 8.6 (0.7–162), respectively] than in those who had not [median and range: 4.0 (1–11.5), 4.9 (0.7–15.5) and 4.7 (0.7–15.5), respectively] ($P < 0.05$). The median EW-, OVA- and OVM-sIgE levels of the three children who needed more than 3 months to be desensitized was 85.3 (range 28.3–164), 69.9 (range 8.4–116) and 45 kU/L (range 15.7–76.5), respectively. Finally, the dose that elicited symptoms at baseline for these participants was 9 mg in two patients and 45 mg in the other one.

Immunological changes. A significant decrease at 4 months compared to baseline was observed in the size of EW, OVA and OVM SPT in OITG ($P = 0.001$) (Table 3). The size of SPT at 4 months was smaller in OITG than in CG, although statistical differences were observed only for OVA and OVM.

There was also a decrease in EW-, OVA- and OVM-sIgE levels in OITG at 4 months compared to baseline,

Table 2. Oral doses associated with symptoms during egg-OIT

Variable		Initial-day Dose Escalation Phase	Build-up Phase	Maintenance Phase	All
Patients with symptoms – <i>n</i> (%)		21 (70%)	16 (53%)	10 (33%)	21 (70%)
Total doses – <i>n</i>		358	1300	792	2450
Any symptom – <i>n</i> (% of doses per phase)		21 (5.9%)	79 (6.1%)	45 (5.7%)	145 (5.9%)
Symptom type – <i>n</i>	OFS	12 (3.4%)	17 (1.3%)	24 (3%)	53 (2.2%)
(% of doses per phase)	Generalized	0	3 (0.2%)	4 (0.5%)	7 (0.3%)
	Urticaria				
	Rhinitis	12 (3.4%)	9 (0.7%)	11 (1.4%)	32 (1.3%)
	Respiratory	0	5 (0.4%)	0	5 (0.2%)
	Distress				
	Gastrointestinal	12 (3.4%)	65 (5%)	20 (2.5%)	97 (4%)
Severity – <i>n</i> (% of doses with any symptom per phase)	Mild	21 (100%)	76 (96%)	45 (100%)	142 (98%)
	Moderate	0	3 (4%)	0	3 (2%)
	Severe	0	0	0	0
Epinephrine treatment – <i>n</i> (% of doses per phase)		0	1 (0.08%)	0	1 (0.04%)

OFS, oropharyngeal symptoms.

which was only significant for OVA-sIgE ($P < 0.001$). However, no differences were observed in sIgE between OITG and CG at 4 months. An increase in EW-sIgG₄ levels occurred in OITG comparing baseline vs. 4 months ($P < 0.001$) and also comparing OITG vs. CG at 4 months ($P = 0.001$).

Egg white-, OVA- and OVM-sIgE levels in OITG at 3 months were lower among patients who passed DBPCFC at 4 months than those with a positive challenge ($P < 0.02$) (Table 4). There were no differences in either SPT or EW-sIgG₄ levels in 3 months between patients who failed or passed the DBPCFC at 4 months.

Identification of a sIgE cut-off to predict sustained unresponsiveness. Egg white- and OVM-sIgE at 3 months showed the best diagnostic performance. The area

under the curve (AUC) for EW- and OVM-sIgE was 0.78 ($P = 0.02$) and 0.75 ($P = 0.03$), respectively (Fig. 3). The AUC for OVA-sIgE was not statistically significant. The cut-off that showed the best values was 7.1 kU/L for EW-sIgE and 1.7 kU/L for OVM-sIgE. The probability of failing the DBPCFC with EW- or OVM-sIgE levels above the cut-off was 90% and 73%, respectively. The probability of passing the DBPCFC if EW- or OVM-sIgE levels were below the cut-off point was 67% and 70%, respectively.

Discussion

The present randomized controlled trial provides important evidence of early development of sustained unresponsiveness to egg in a significant percentage of

Table 3. Skin prick test median wheal diameter, sIgE and sIgG₄

	OITG			CG			OITG vs. CG 4 months <i>P</i>
	Baseline	4 months	<i>P</i>	Baseline	4 months	<i>P</i>	
Median SPT – mm (range)							
EW	6 (3–11)	5 (3–8)	0.001	6 (3–12)	5.5 (0–13)	0.45	0.16
OVA	5 (0–9)	3 (0–8)	0.001	5 (0–13)	4 (0–11)	0.02	0.03
OVM	5.5 (0–11)	3.5 (0–9)	0.001	6 (0–11)	5.5 (0–14)	0.14	0.02
Median sIgE – kU/L (range)							
EW	6.4 (1–245)	3.1 (0.5–432)	0.15	2.2 (0.7–73)	2.2 (0.4–60.2)	0.47	0.31
OVA	6.3 (0.7–185)	2.2 (0.3–192)	< 0.001	2.2 (0.7–43.5)	1.3 (0.3–32.3)	0.01	0.28
OVM	3.1 (0.7–162)	2.1 (0.3–163)	0.11	2.3 (0.7–89.1)	1.7 (0.3–76.7)	0.17	0.31
Median sIgG ₄ – mg/L (range)							
EW	0.4 (0.1–6.2)	4.4 (0.1–30)	0.0001	0.2 (0.1–3.6)	0.4 (0.1–7.5)	0.02	0.001

SPT, skin prick test; OITG, Intervention group; CG, Control group; EW, egg white; OVA, ovalbumin; OVM, ovomucoid.

persistent egg-allergic patients after a short-course egg-OIT. Thirty-seven percent ($n = 11$) of patients achieved this state after 3 months of treatment compared to only 3% ($n = 1$) of controls who continued an avoidance diet during the same period. Children who developed sustained unresponsiveness continued taking egg ad libitum during 36-month follow-up. The results showed that egg-allergic patients could achieve a state of sustained unresponsiveness after a short period of treatment.

So far, trials of egg-OIT have mainly analysed the efficacy to desensitize to the food [11–20]. Only few studies analysed sustained unresponsiveness after egg-OIT after prolonged periods of treatment [11, 14, 16, 18], all of them with protocols longer than ours. These studies describe sustained unresponsiveness in 28–100% of the population after 21–33 months of egg-OIT. However, if we only consider randomized controlled studies, this percentage ranges between 28% [18] and 36% [14]. These differences might be related to differences in baseline egg-sIgE levels, egg-OIT doses and the treatment duration before sustained unresponsiveness. In our active group, the median baseline EW-sIgE was lower (6.4 kU/L) than reported in the patients treated by Buchanan [11] (8.2 kU/L), Burks [18] (10.3 kU/L) and Vickery [16] (12.5 kU/L). Regardless of the lower sIgE, the higher dose during maintenance (2808 mg of protein) in our study compared to others (300 mg [11] and 1600 mg [18]) might have positively influenced the high ratio of sustained unresponsiveness despite of our shorter protocol.

Table 4. Immune markers of OITG at 3 months according to DBPCFC responses at 4 months

OITG			
Immune markers at 3 months	DBPCFC at 4 months		
	Negative ($N = 11$)	Positive ($N = 17$)*	<i>P</i>
Median SPT – mm (range)			
EW	4.5 (3–8)	4.5 (3–8)	0.76
OVA	3 (0–4)	3 (3–7.5)	0.13
OVM	3 (0–9)	3 (0–6.5)	0.67
Median sIgE – kU/L (range)			
EW	1.8 (0.7–9.3)	9.1 (1–58.8)	0.001
OVA	0.8 (0–6.7)	4.2 (0.3–29.8)	0.02
OVM	0.6 (0–6.7)	6.4 (0–49.7)	0.004
Median sIgG ₄ – mg/L (range)			
EW	3.2 (0.4–25.7)	6.9 (0.7–30)	0.53

SPT, skin prick test; EW, egg white; OVA, ovalbumin; OVM, ovomucoid.

*Data including the three OITG group children who failed achieving maintenance phase in 3 months time. These patients did not undergo the elimination diet or the DBPCFC at 4 months. Data were included in the group of children with positive DBPCFC.

In this study, giving higher doses of egg than other protocols sought two therapeutic targets: i. eliminate the risk of allergic reactions due to accidental food ingestion, and ii. normalize the diet, that is, the patient could freely take egg-containing food in addition to the doses established as egg-OIT maintenance. However, a drawback of the treatment is that 57% of the participants receiving egg-OIT referred difficulty in taking food during the maintenance phase because they disliked either the appearance, the texture and/or the taste of the egg. This has also been observed in other studies [25] and might increase the risk of loss of adherence in longer protocols. The state of sustained unresponsiveness enables patients to take food in any form, any amount and whenever they want, without the fear of an allergic reaction. In this state, the necessity to take the food with strict periodicity disappears, unlike what happens with the state of desensitization. Nevertheless, a total avoidance of the food is not recommendable as it has been proven with a peanut-OIT model [26] that sustained unresponsiveness is transient in most of the cases. In the present study, only three patients who passed DBPCFC at 4 months, had occasional mild reactions that resolved without treatment in the first 6-month follow-up, safely consuming egg throughout the rest of the follow-up period.

During maintenance, egg was given as undercooked egg to preserve its allergenicity. This point is particularly important because the administration of extensively heated egg does not imply tolerance of raw egg during OIT. Itoh et al. [15] stated that 50% of patients who received OIT with 60 g of heated egg twice a week for more than 9 months did not tolerate 1 g of raw powdered egg after treatment. Therefore, performing both dose increase and maintenance phases with raw and/or nearly raw egg facilitates the tolerance to the most allergenic form of egg, which is raw, and provides protection in a larger extent.

Our treatment proved to be highly effective in desensitizing patients (93% success) compared with other studies (48–92% success) [11–14, 16–20]. Moreover, in patients who were desensitized but did not reach sustained unresponsiveness, significant increases in the threshold of egg in the DBPCFC were observed after egg-OIT. The data are consistent with Skripak et al. [27] who demonstrated that milk OIT is effective in increasing the threshold for reactions to milk [27].

Regarding the safety of the procedure, over a third of patients had no adverse events at all throughout the treatment. Despite the relative high rate of adverse events with this protocol (5.9% of the administered doses), we found it to be lower than reported by other studies in which reactions occurred in between 7.6% [28] and 25% [18] of the administered doses. Elevated egg-sIgE levels were associated with a higher frequency of adverse events during egg-OIT, as described Vaz-

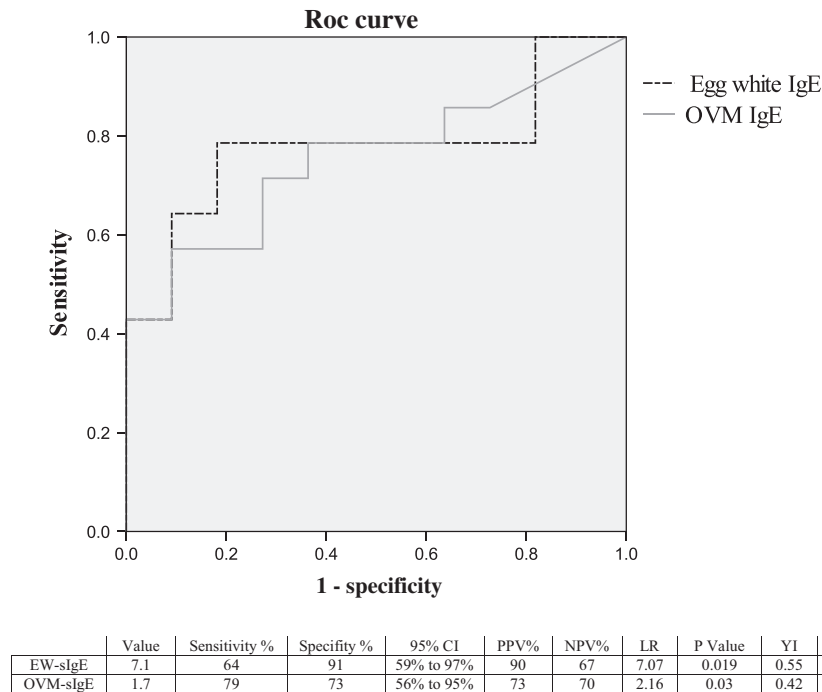


Fig. 3. The discriminative potential of sIgE at 3 months after starting egg-OIT on DBPCFC results after egg-OIT at 4 months. Receiver operating characteristic curves are shown in the figure. Optimal cut points for EW-IgE and OVM-IgE are shown in tabular form below. EW, Egg white; OVM, Ovomucoid; PPV, Positive predictive value; NPV, Negative predictive value; LR, Likelihood ratio; YI, Youden index; AUC, Area under the curve.

quez-Ortiz et al. [28]. In this same sense, and in concordance with the observations of this study, the three children who needed more than 3 months to become desensitized had higher egg-sIgE and a lower threshold at baseline DBPCFC than the patients who completed the build-up phase in less than 3 months.

Although more than 95% of the adverse events observed were mild, 7% (2/30) of patients had to discontinue egg-OIT due to mild but persistent allergic reactions, mainly abdominal pain and vomiting. Accounting with our own previous experience of eosinophilic esophagitis due to milk OIT [29], it was proposed to perform oesophageal endoscopy, but the parents refused it. However, such symptoms subsided without treatment immediately after the OIT was withdrawn. No correlation between withdrawals and specific IgE levels to egg allergens was observed. The withdrawal rate due to adverse reactions was lower than the observed in other studies (12–36%) [14, 18]. These differences in the rate of adverse events and withdrawals due to adverse reactions might be related to the lower egg-sIgE levels in our population compared to these studies. On the other hand, there may be other factors such as asthma, which can influence the frequency of adverse events during egg-OIT. In our population, 41% of the participants had asthma, vs. 64% of the subjects in the study published by Vazquez-Ortiz et al. [28]. Unfortunately, the work of Burks et al. does not offer this information, and we are unable to determine whether the high frequency of

adverse events described in their study was due to an increased frequency of asthma among the treated patients, or to other not covered reasons.

Moreover, although the cumulative dose of egg white protein administered during challenges (2808 mg) was lower than the recommended dose of food protein proposed by PRACTALL consensus [30] for DBPCFC (4443 mg of food protein), no significant adverse events were reported after the completion of 36-month follow-up. Only three patients had very mild and transient symptoms during the first 6 months, but that did not prevent them continue taking egg during the follow-up.

In our study, the changes in egg-specific immune response, as evidenced by decreased wheal size on SPT to egg allergens, were only observed in children receiving egg-OIT and not in the control group. However, there were no differences in SPT in 3 months between patients who failed or passed the DBPCFC at 4 months, in contrast to other OIT studies in which wheal size on SPT was inversely related to the probability of sustained unresponsiveness [18, 31]. On the other hand, sIgE levels for EW, OVA and OVM were lower in children who achieved sustained unresponsiveness than in those who did not pass DBPCFC at 4 months. This variable did not correlate with sustained unresponsiveness in the study of Burks et al., [18] but our results in the allergen-specific IgE levels are consistent with those of Vickery et al. [31] where patients who successfully passed a challenge 1 month after stopping peanut-OIT

had lower peanut sIgE levels at baseline and at the time of challenge compared with the subjects who failed [31]. The study concluded that smaller skin test results and lower peanut sIgE levels were predictive of a successful outcome. In the present study, EW-sIgE levels after the 3 months of egg-OIT showed the best capacity to predict the outcome of the DBPCFC 1 month after stopping treatment. Levels above the EW-sIgE cut-off of 7.1 kU/L indicated 90% probability to fail the DBPCFC. This cut-off for EW-sIgE is similar to the diagnostic decision point proposed by Sampson [32] who demonstrated that egg-specific IgE levels above 7 kU/L identify patients with > 95% probability of reacting to egg [32]. However, these results need to be interpreted with caution, as Sampson referred to diagnostic procedure in regular patients, whilst our cut-off regards to OIT-treated patients evaluating sustained unresponsiveness after an elimination diet preceded by an egg-OIT. Therefore, levels of egg-sIgE throughout egg-OIT could be used to establish the most appropriate time to stop the treatment and confirm sustained unresponsiveness after an avoidance diet period. Specific IgE levels were used previously to draft the build-up phase dosage by other authors [16], and according to our data, it could be used also to tailor the duration of maintenance phase and choose the best moment to assess sustained unresponsiveness in each individual. Immune markers were analysed in children who developed sustained unresponsiveness every 6 months during the 36-month follow-up. SPT and sIgE to egg allergens progressively decreased along this period (data not shown).

An increase in egg white-specific IgG₄ levels occurred in egg-OIT patients comparing baseline with 4 months and also comparing egg-OIT with control patients at 4 months. In other egg-OIT protocols [18], patients who achieved sustained unresponsiveness had higher specific IgG₄ levels than patients who did not reach this state. However, in our study, we failed to detect such differences. We consider it could be due to our shorter protocol. As sIgG₄ changes start to become significant after 3 or 4 months [33], it is more likely that it was too early to detect such changes rather than these changes did not happen, although there is evidence of a lack of significant increase in this biomarker in other OIT trials [26].

One of the weaknesses of the study is the absence of a placebo group; however, the use of DBPCFC before enrolment and after OIT, and the randomization process, minimized this weakness. Moreover, the lower level of sIgE to egg proteins compared to other studies could have lead to a greater success ratio. Therefore, the robust evidence provided in this study enables its

extrapolation to patients with a similar profile, but should be cautiously applied in the case of patients with higher sIgE levels. Another weakness of this study could be the low sample size to assess predictors for sustained unresponsiveness, but considering the differences between groups, it seems to be enough to ascertain those differences.

In conclusion, this study shows how 37% of patients with persistent egg allergy developed sustained unresponsiveness following oral immunotherapy during 3 months with higher doses of raw egg than other protocols. Also, this strategy was safe and effective in 93% of children to induce desensitization. The protocol also allowed patients to take any other foodstuffs containing raw, cooked or heated egg during the state of desensitization. Levels of egg-sIgE throughout egg-OIT could be used as potential predictors of sustained unresponsiveness. No correlation between other variables and development of sustained unresponsiveness was observed.

Future needs arise from this study such as the identification of other immune markers to predict the likelihood of sustained unresponsiveness, the reduction in the frequency of adverse events and the customization of the treatment duration.

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Contributors

CE and MDI conceived, designed and directed the study. NP and CG contributed to the statistical design and data analysis of the study. CE, PRR, SSG, IPR and MDI carried out the clinical procedures. All authors contributed to and reviewed the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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