Received: 10 December 2017 | Revised: 7 October 2018 | Accepted: 9 October 2018

DOI: 10.1111/pai.13002

ORIGINAL ARTICLE

Food Allergy & Anaphylaxis

WILEY

Egg OIT in clinical practice (SEICAP II): Maintenance patterns and desensitization state after normalizing the diet

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Edited by: Philippe Eigenmann

Abstract

Background: It is unknown which are the most suitable maintenance pattern and egg consumption to maintain the desensitization state after ending the oral immunotherapy (OIT). This multicenter, randomized, controlled trial compared two OIT maintenance patterns with pasteurized egg white (PEW), evaluating the egg consumption effect on the desensitization state after ending the OIT.

Methods: One hundred and one children with confirmed egg allergy were randomized: 25 to an egg-free diet (CG) and 76 to an OIT year with PEW and two maintenance patterns, 38 patients to daily 3.3 g proteins (AG) and 38 to every two days (BG). PEW challenge (DBPCFC), adverse reactions, and immune markers were assessed at baseline, at the end of the OIT, and at 6 and 12 months later on ad libitum egg consumption (T0, T12, T18, and T24). A questionnaire evaluated the egg consumption at T18.

Results: At T12, 64 of 76 (84.21%) OIT patients had reached total desensitization (32 AG and 32 BG) vs 4 of 25 (16.00%) CG who passed the PEW DBPCFC. Thirty (93.75%) AG vs 25 (78.12%) BG patients completed an OIT year. At T18, 27 of 29 (93.1%) AG vs 20 of 24 (83.3%) BG passed the PEW DBPCFC, 96% consuming at least two egg servings/week. At T24, 97.43% OIT patients passed the challenge. Most patients had adverse reactions, more frequent in the BG patients; frequency and severity of reactions decreased through the study. PEW skin prick test wheal and sIgE antibody serum levels similarly decreased in AG or BG, but AG patients had greater increase in PEW sIgG4 ($P < 0.05$).

Conclusions: Daily OIT maintenance achieves better adherence, effectiveness, and safety. Two egg servings/week ensure maintained desensitization after the end of an OIT year.

KEYWORDS

desensitization state, effects of the egg consumption after ending the OIT, egg allergy, egg consumption after OIT and desensitization state, egg OIT, evolution of adverse reactions through OIT and 12 months after ending OIT, OIT maintenance patterns

1 | INTRODUCTION

Most young children with egg allergy reach natural tolerance by the age of 5; however, this tendency decreases until adolescence.[1-3] Oral immunotherapy (OIT) aims to normalize the diet of the patients with food allergy in a safe and effective way. This treatment induces a state of desensitization that provides specific protection from allergic reactions by increasing the threshold of the allergic response. However, some studies have shown that 30%-75% of patients lose the achieved state of desensitization after a period of food avoidance.[4-6]

Variability in the studies (eg, the age of the patients, baseline confirmation of egg allergy, egg material used, target dose, maintenance interval, and length of OIT), along with the possibility of loss of desensitization state after stopping OIT, makes it difficult to compare and choose the best protocol for implementation in clinical practice.[6-16]

The Spanish Pediatric Society Clinical Immunology, Allergy, and Asthma (SEICAP) conducted a multicenter, randomized, controlled study of OIT in children with proven persistent egg allergy to determine the best OIT strategy, the most effective and safe protocol to reach total desensitization and to maintain this stage once the diet has been normalized.

The study was divided into two parts: The first part, SEICAP I, assessed the effectiveness and safety of OIT to induce desensitization to 3.3 g protein of pasteurized egg white (PEW) vs an egg-free diet for one year to reach natural tolerance; the main objectives of this second part (SEICAP II) were to assess and compare the effect of two maintenance patterns and the ad libitum egg consumption on the desensitization state, 6 and 12 months after ending the OIT.

2 | METHODS

2.1 | Study design objectives, participant selection, and randomization

The primary aims of this study were to: (a) compare adherence, safety, and effectiveness of a daily (A) vs an every 2 days (B) OIT maintenance pattern; and (b) assess the effect of the maintenance pattern and the egg consumption to maintain the desensitization state (30 mL or 3.3 g PEW protein) 6 and 12 months after finishing one year of OIT.

Secondary objectives were to study the clinical and immunologic markers (tolerance to cooked egg, threshold PEW dose, egg white skin prick test wheal, and egg sIgE and sIgG4 antibody serum levels) at the beginning of the OIT associated with the persistence of the desensitization state.

Adherence is described as compliance with the maintenance pattern assigned to the patient in the randomization. The effectiveness of OIT was assessed according to the rate of patients reaching and maintaining total desensitization. Total desensitization or maintenance of the state of desensitization was defined as the ability to pass a DBPCFC with 3.3 g PEW proteins (equivalent to a medium-sized egg). Safety was evaluated according to the rate of total dose adverse reactions (DARs) and their grades, which were assessed by the study coordinator according to Sampson's grading.[17] Egg consumption in the last week (number of whole eggs or egg servings) was evaluated by a questionnaire at T18.

2.1.1 | Sample size, patient selection, and randomization

We calculated (see Statistical analysis) that a sample of 101 patients randomized, 76 to one year of OIT (38 assigned to daily and 38 to every two days maintenance) and 25 control group (CG) to an egg-free diet for one year would be enough to assess the objectives of the study.

Patients with a diagnosis of egg allergy were recruited from the allergy units of the Spanish children's hospitals of the public healthcare system; their parents were informed and invited to participate in this study.

Inclusion criteria were as follows: (a) Children aged 6-9 years with a previous diagnosis of egg allergy and at least one allergic reaction to egg over the last year, having at the time of inclusion; (b) signed informed consent to participate in the study; (c) positive skin prick test (SPT) to an egg white (EW) solution 10 mg/mL, mean diameter wheal >3 mm; (d) sIgE serum levels above 0.35 kU/L to EW, ovalbumin (OVA), or ovomucoid (OVM); and (e) egg allergy confirmed by a pasteurized egg white double-blind, placebo-controlled food challenge (PEW DBPCFC).

Exclusion criteria were as follows: (a) severe or uncontrolled asthma[18]; (b) severe atopic dermatitis according to the objective severity scoring of atopic dermatitis index[19]; (c) esophagitis symptoms; (d) autoimmune, cardiovascular, or neuropsychiatric diseases; (e) beta-blocker treatment; (f) food OIT during the last year; and (g) immunotherapy with airborne allergens in the start-up phase.

2.2 | Study protocol

The study protocol and consent forms were approved by the institutional review board of the Spanish public healthcare system (EC3250) of La Paz University Hospital (Madrid) and then by the rest of the participating hospitals. Written informed consent was obtained from the parents or guardians, with assent from children older than 7 years (Table 1).

The study was carried out in five stages over 24 months, from T0 or inclusion and randomization time to T6, T12, T18, and T24 (6, 12, 18, and 24 months after inclusion, respectively) with the corresponding weekly visits on induction period and the follow-up visits in the five stages. Visits included review by the researches of the patients' symptom diaries, clinical history, physical examination, spirometry, immune markers, and egg challenge to confirm the allergy or desensitization state.

A total of 101 patients meeting all the inclusion and none of the exclusion criteria were included and randomized by means

TABLE 1 Study protocol: 101 patients who met all inclusion and none exclusion criteria were included in the study and randomized to egg OIT (AG and BG) or control (CG) groups. At T0, AG and BG patients started induction period of OIT with PEW until reaching target dose (3.3 g protein) and then completing a year of OIT with this daily dose (AG) or every two days (BG). Control patients (CG) completed a year on an egg-free diet. CG patients with persistent egg allergy at T12 could start OIT at this time (OIT CG patients), which ended when patients reached target dose. All patients started ad libitum egg consumption when they reached the target dose. Clinical and immunologic markers and egg challenge were assessed/performed throughout the study in five stages: at inclusion and at 6, 12, 18, and 24 months later (T0, T6, T12, T18, and T24)

	SEICAP I		SEICAP II		
	T0 ◄——► T12				T24
Patients´ inclusion and randomization	Induction / Maintenance A►……OIT…… ◄…….daily……... ◄…… *ad libitum* egg consumption…… ◄ B►……OIT…… ◄……...every two days……. ◄…… *ad libitum* egg consumption …….... ◄ C◄……egg-free diet……...► OIT CG.. Induction….. *ad libitum* egg consumption…….◄				
	T0	**T6**	**T12**	**T18**	**T24**
Clinical history	AG, BG, CG	AG, BG	AG, BG, CG	AG, BG, CG	AG, BG, CG
Spirometry	AG, BG, CG	AG, BG	AG, BG, CG	AG, BG, CG	AG, BG, CG
10 mg/ml EW skin prick test	AG, BG, CG	AG, BG	AG, BG, CG	AG, BG, CG	AG, BG, CG
Total and specific (EW, OVA, OVM) sIgE serum antibody levels	AG, BG, CG	AG, BG	AG, BG, CG	AG, BG, CG	AG, BG, CG
EW sIgG4 serum antibody levels	AG, BG, CG	----------	AG, BG, CG	----------	----------
Boiled egg and PEW DBPCFC **PEW DBPCFC**	AG, BG, CG ----------	---------- ----------	CG AG, BG	---------- AG, BG, OIT CG	---------- AG, BG, OITCG
Open raw egg challenge	When OIT patients reached total desensitization and when patients passed PEW DBPCFC at T12				
Egg consumption questionnaire	----------	----------	----------	AG, BG, OIT CG	----------

DBPCFC: double-blind, placebo-controlled food challenge; EW: egg white; OVA: ovalbumin; OVM: ovomucoid; PEW: pasteurized egg white; SEICAP I: multicenter, randomized, controlled study to assess induction of desensitization; SEICAP II: multicenter, randomized, controlled study to assess OIT maintenance, and observational study about egg consumption and desensitization state after the end of OIT. [Colour table can be viewed at wileyonlinelibrary.com]

of a centralized computer algorithm at T0: 76 to one year of OIT, 38 were assigned to daily maintenance (AG) and 38 to every two days (BG), and 25 control group (CG) to an egg-free diet for one year (Supporting Information Table S1 in the repository). At T12, a DBPCFC with 3.3 g PEW protein was performed to all CG and OIT patients who had reached total desensitization (Table 1). CG patients with confirmed egg allergy at T12 could, if they required it, start OIT.

2.2.1 | Immunologic markers

Skin prick tests (SPTs) were performed with EW extract (10 mg/mL), saline, and histamine solutions as negative and positive controls (Diater Laboratories SA, Leganes, Madrid, Spain). The wheal size was calculated using the average of the largest and the perpendicular midpoint diameter and then subtracting the size of the saline wheal. Total IgE, (EW, OVA, OVM) sIgE, and EW sIgG4 antibody serum levels were measured with the use of ImmunoCAP 100 (Thermo Fisher Scientific, Madrid, Spain).

2.2.2 | Oral food challenge

Egg allergy and the desensitization state were confirmed by an egg DBPCFC blinded with potato, carrot, and olive oil mashed together. Challenges were performed in a hospital setting and supervised by a physician. At T0, all patients performed DBPCFC with one boiled EW at 100°C for 10 min, starting with a dose of 2.5 g and at 30-minute intervals 5, 10 and 25 g (0.183, 0.366, 0.733 and 1.833 g protein) up to an accumulated dose of 45 g (3.30 g protein). If the patient passed this challenge, on the following day, a second PEW DBPCFC was performed, starting with 1 mL and at 30-minute intervals 2, 4, 8, and 15 mL (0.11, 0.22, 0.44, 0.88, and 1.65 g protein, respectively), up to an accumulated dose of 30 mL (3.3 g protein), which is equivalent to one medium-sized EW. After two hours, the patient was discharged, if allergic symptoms did not developed. The challenge was stopped if the patient developed urticaria/edema, severe abdominal pain, vomiting, rhinitis, bronchospasm, or hypotension; symptoms were treated, and the patient was discharged 6 hours after controlling the reaction.

An open raw egg challenge at breakfast time with a milk shake (cow's milk or soy milk or oat milk in case of cow's milk or soy allergy) was performed to all OIT patients 24 hours after reaching total desensitization and at T12 to all patients who passed the PEW DBPCFC.

2.2.3 | Oral immunotherapy protocol

PEW (Guillen, Valencia, Spain), whose allergenicity has been proven equivalent to raw EW [20], was the material used for the OIT (Supporting Information Table S2 in the repository), which was performed in three phases. (a) The initial dose escalation phase performed in the hospital, starting with 1 mL of a 1/1000 water solution of PEW; if the patient did not develop allergic symptoms, a double dose was administered every 30 minutes until reaching undiluted PEW; if the patient did not develop DARs, they were discharged 2 hours later and the desensitization protocol was continued the next day. (b) A build-up phase (PI or PII pattern), based on 30% weekly increments in the hospital over the last tolerated dose; moreover, PI patients were given at home daily increments of 5%. Total desensitization was considered to have occurred when 30 mL (3.3 g protein) of PEW was reached without any reaction. The next day, an open controlled food challenge was carried out in the hospital to confirm total desensitization. (c) Then, the maintenance phase was initiated; patients assigned to AG ingested the target dose (3.3 g protein PEW) daily at home and those patients assigned to BG the same dose every two days, up to complete one year of OIT. Control patients with confirmed persistent egg allergy at T12 who started OIT, finished it when they reached the target dose. All OIT patients (AG, BG, and CG) started ad libitum egg consumption when reaching 3.3 g PEW proteins.

Treatment for asthma control was continued during the study, and no other medications were administered.

If dose adverse reactions (DARs) occurred, the protocol was readapted (Supporting Information Table S3). During the induction phase, the patients and their families were instructed to avoid potential risk factors for DARs: The use of nonsteroidal anti-inflammatory drugs other than paracetamol was not permitted, and if necessary, the patient was evaluated by the researcher; intense exercise was not permitted between 1 hour before and 4 hours after taking the OIT; patients were observed for at least 4 hours after receiving each dose, and no other egg consumption than OIT was permitted before reaching total desensitization.

Parents were trained in the recognition and treatment of reactions according to European Academy of Allergy and Clinical Immunology Anaphylaxis Guidelines.[21] Epinephrine auto-injectors and instructions on their use were provided to the parents. Parents were asked to complete daily home diaries, including administered dose, symptoms, and treatment required to control them. These diaries were reviewed during the visits by the investigators, who graded the reactions according to the Sampson grading.[17] DARs were analyzed in five periods: every 3 months until T6 and then every 6 months until T24 (T0-T3, T3-T6, T6-T12, T12-T18, and T18-T24).

2.3 | Statistical analysis

A two-group continuity-corrected chi-squared test calculated that a sample of 101 participants (76 receiving oral immunotherapy, 38 A maintenance and 38 B maintenance, and 25 assigned to an egg-free diet for one year) would provide 80% power, at one-sided alpha level of 0.05, to detect a significant between-group difference in the rate of sustained unresponsiveness, assuming an estimated success rate of 20% in the control group and an estimated 50% rate in the oral immunotherapy group. Clinical outcome was assessed by per-protocol analysis. The chi-squared test was used to compare patients reaching total desensitization in the OIT group or achieving natural tolerance in the CG over 1 year. The Mann–Whitney test was used to test for differences between the OIT and CG and the PI and PII groups. The Wilcoxon rank-sum test was used to evaluate between-group differences in the SPT (wheal size) and in immunoglobulin levels. Evolution of the immunologic markers through the study was analyzed by a mixed-model regression analysis to control the effect of repeated measurements, factoring for group (OIT, CG) and measurement period (T0, T1, T12, T18, and T24). We studied the main effect and interaction between factors (a significant interaction indicated that the profiles of the groups had different shapes). Firstly, we studied the main effects of each factor, that is, the behavior of the average values obtained with each group or the average value for marker at each time point. "Post hoc" comparisons were performed using the Bonferroni method. All analyses were performed with the use of SAS software 9.3 (SAS Institute, Cary, NC, USA).

3 | RESULTS

Nine allergy units of the Spanish public healthcare system participated in the study enrolling 101 children with egg allergy proven by DBPCFC and with median age 6 years and 9 months (mean 80.02 ± 12.87 months; Figure 1). They were randomized at inclusion: 76 to OIT (38 AG, 38 BG) and 25 (CG) to an egg-free diet over one year. Patients in CG resulted younger than those in AG, BG, or AG + BG ($P < 0.05$); nevertheless, the remaining clinical and immunologic characteristics were similar (Supporting Information Table S4 in the repository).

3.1 | Clinical response

3.1.1 | T0-T12

At T12, 18 of 25 (72.00%) or 18 of 22 (81.81%) of the CG patients that carried out the PEW DBPCFC failed it; 16 of them also failed the boiled egg challenge and 4 of 22 (18.8%) control patients passed the boiled egg and PEW DBPCFC vs 64 of 76

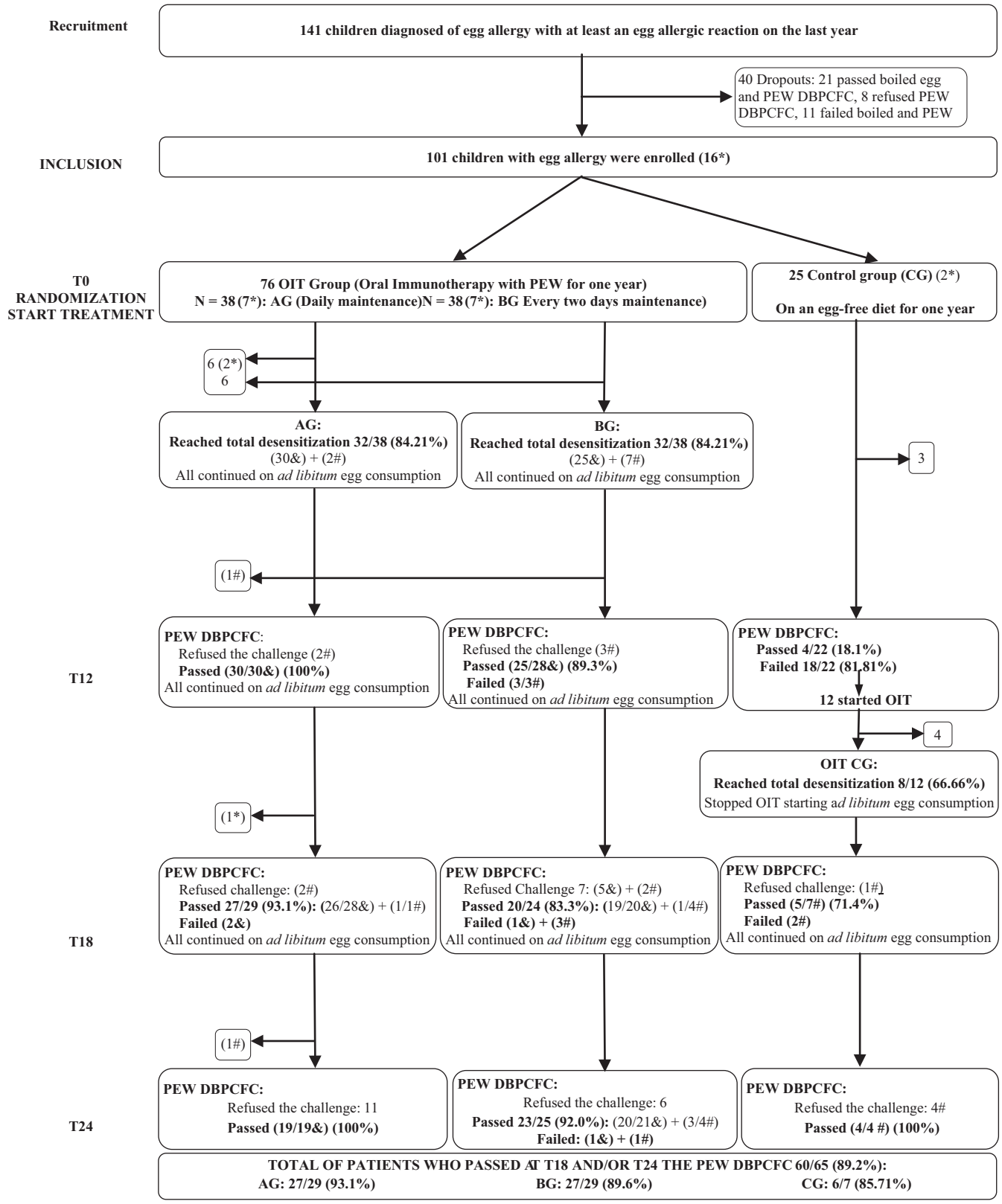

FIGURE 1 Flow diagram. Evolution of patients through the study. *: patients who passed boiled egg DBPCFC at inclusion or the start of the OIT; DBPCFC: double-blind, placebo-controlled food challenge; OIT: oral immunotherapy; PEW: pasteurized egg white; PEW OIT: oral immunotherapy with pasteurized egg white (target dose 3.3 g PEW proteins); Failed PEW DBPCFC: immediate allergic reaction after ingesting ≤3.3 g PEW proteins; Pass PEW DBPCFC: patients who did not develop allergic symptoms into 2 hours after ingesting target dose; &: patients reaching target dose and completing a year of OIT; #: patients reaching target dose but not completing one year of OIT

(84.2%) OIT patients (32 AG and 32 BG) who had reached target dose or total desensitization ($P = 0.000$). Then, all of these patients passed the open raw egg challenge and started ad libitum egg consumption continuing with this maintenance dose up to complete one year of OIT. Twelve of the egg-allergic CG patients at this point of time requested and started PEW OIT; eight of them (66.66%) reached total desensitization, passed the open raw challenge, finished OIT, and started ad libitum egg consumption.

Finally, 72 of 88 (81.81%) patients who started OIT had reached total desensitization, mean induction period 121.12 ± 91.43, and median 98.00 (7-329) days. Only 55 of them completed one year of OIT, 30 of 32 (96.87%) AG vs 25 of 32 (78.12%) BG, with a similar maintenance period (252.18 ± 61.96, median 273.50 (94-330) AG vs 244.75 ± 65.68, median 256.50 (1-329) BG ($P > 0.05$)). All patients who completed 1 year of OIT passed at T12 the PEW DBPCFC and the open raw egg challenge. Nine patients discontinued OIT maintenance: one patient from BG who suffered during the grade 4 DAR phase and dropped out and eight patients (2 from AG and 6 from BG) who continued in the study consuming egg ad libitum. Five of these patients (2 from AG and 3 from BG) refused the challenge, and three from BG failed it; all of them had discontinued the OIT maintenance at least 6 months before the challenge.

3.1.2 | T18

At T18, 70 of 72 (95.83%) children who had achieved total desensitization continued on ad libitum egg consumption (31 from AG, 31 from BG, and 8 from CG). One patient from AG, who did not completed one year of OIT, refused the PEW DBPCFC and dropped out at this time. Most patients, 52/60 (86.6%), that performed the PEW DBPCFC at T18, passed it: 45/48 (93.75%) passed the PEW challenge six months after ending one year of OIT, 26/28 (92.85%) AG and 19/20 (95.00%) BG, vs 7/12 (58.33%) who had not completed one year of OIT ($P = 0.006$; Figure 1).

The analysis of the egg consumption questionnaire on the last week showed that most patients who passed the PEW DBPCFC at this time (96.07%) liked eggs, it suited them better and they had consumed more egg servings (at least 2) than those who failed the challenge (50.05%) ($P < 0.05$). Nevertheless, those patients who did not complete 1 year of OIT and passed the challenge at T18 had consumed at least three egg servings on the last week and those who had not completed the year and failed the DBPCFC PEW had eaten two or less (Table 2 and Figure 2).

Patients who passed the PEW DBPCFC at T18 had, at T0, greater threshold dose-response that those who failed it ($P = 0.035$; Table 3). All who failed the PEW DBPCFC at T18 had increased their threshold dose-response ($P = 0.000$; Table 4, Figure 3). Nine patients refused the PEW DBPCFC at this time; eight of them reached T24 on ad libitum egg consumption.

3.1.3 | T24

Sixty-nine of 72 (95.83%) children, who reached total desensitization, continued at T24 on ad libitum egg consumption. At this time, 46 of 48 (95.83%) patients passed the PEW DBPCFC: 39 of 40 (97.5%) twelve months after completing one year of OIT (19/19 AG vs 20/21 BG) vs 7 of 8 (87.5%) who did not complete it. Two patients in BG did not pass the challenge: one of them had not completed one year of OIT and he also failed the egg challenge at T18; and another had completed 1 year of OIT and passed the egg challenge at T18, he recogniced that since then, he had only eaten foods with baked eggs; both of these patients responded at T24 with a greater dose of PEW than at T0. Twenty-one patients refused the PEW DBPCFC at T24; 13 of them had passed the challenge at T18, 4 did not pass it,

TABLE 2 Egg consumption questionnaire at T18: patients passing vs failing the PEW DBPCFC at that time (Fisher's exact and Mann-Whitney tests). PEW DBPCFC Refused 12

	PEW DBPCFC Refused N = 12		
T18 Egg consumption questionnaire	**Passed N = 52**	**Failed N = 8**	***P* value**
Does the patient like eggs?	40 (78.4%)	1 (12.5%)	0.005
Does the patient feel good when he eats egg?	47 (90.4%)	1 (12.5%)	0.002
Does the patient eat soft-boiled egg?	10 (19.6%)	1 (12.5%)	0.420
Does the patient eat omelette?	49 (96.1%)	3 (30.0%)	0.001
Does the patient eat boiled egg?	40 (78.4%)	1 (12.5%)	0.005
How many egg servings has the patient eaten during the last week?			
½	0 (0%)	1 (10%)	<0.001
1	2 (3,9%)	4 (40%)	
2	9 (17.6%)	2 (20%)	
3	37 (72.5%)	1 (30%)	
4	3 (5.8%)	0	

and 4 also had rejected it at that time. Table 4 shows characteristics and evolution of patients who did not completed one year of OIT and those who did not pass PEW DBPCFC at T18 or T24.

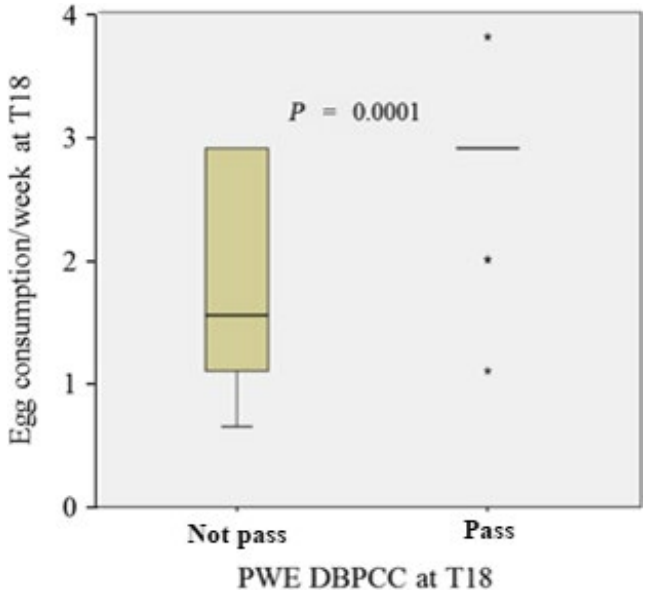

FIGURE 2 PWE DBPCFC at T18 and egg consumption on last week (Mann-Whitney U test). PEW DBPCFC: pasteurized egg white double-blind, placebo-controlled food challenge [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Immune markers

At T0, immune markers were similar in the AG, BG, and CG patients (Supporting Information Table S4 in the repository). Nevertheless, the patients who passed the boiled egg DBPCFC at T0 had higher EW sIgG4 but lower OVM sIgE antibody serum levels ($P < 0.05$) than those patients who failed it (Supporting Information Figure S1 in the repository).

OIT patients who passed the PEW challenge at T18 had at T0 higher threshold dose-response than those that failed it ($P = 0.035$), but immunologic markers did not show differences (Table 3). Patients who passed PEW DBPCFC at T18 and T24 showed a similar and significant decrease in the EW SPT wheal and (EW, OVA, and OVM) sIgE antibody serum levels from T0 to T24 ($P = 0.000$). EW sIgG4 antibody serum levels increased in AG and BG OIT patients from T0 to T12 ($P < 0.001$), but patients on daily maintenance (AG) had a greater increase from T6 to T12 than those with every two days maintenance or BG ($P = 0.036$); CG patients on an egg-free diet did not show changes from T0 to T12 (Figures 3 and 4 and Supporting Information Table S5 in the repository).

3.3 | Safety

From T0 to T12, 66 of 76 (86.84%) OIT patients (AG or BG) developed DARs vs 8/22 (27%) of the CG patients over an egg-free

TABLE 3 Threshold responding dose and immunologic markers at T0 in OIT patients passing vs failing the PEW DBPCFC at T18 (Mann-Whitney test)

		N	Mean ± SD	Median (Min-Max)	*P* value
T0 Threshold responding dose (EW g protein)					
T18 PEW DBPCFC	Passed	52	0.636 ± 0.671	0.440 (0.022-2.50)	0.035
	Failed	8	0.215 ± 0.143	0.205 (0.080-.550)	
T0 10 mg/mL EW SPT wheal					
T18 PEW DBPCFC	Passed	52	7.894 ± 2.428	8.250 (4.0-13.15)	0.061
	Failed	8	9.350 ± 2.186	9.750 (6.0-12.0)	
T0 total IgE KU/L					
T18 PEW DBPCFC	Passed	52	833.71 ± 1612.5	391.00 (10-10801)	0.510
	Failed	8	647.89 ± 500.28	429.00 (186-1507)	
T0 EW sIgE KU/L					
T18 PEW DBPCFC	Passed	52	46.479 ± 280.0	3.980 (0.02-2045.0)	0.148
	Failed	8	13.16 ± 15.62	6.63 (1.9-52.6)	
T0 OVA sIgE KU/L					
T18 PEW DBPCFC	Passed	52	33.00 ± 206.95	2.16 (0.02-1496.0)	0.075
	Failed	8	7.52 ± 7.55	4.02 (0.6-22.9)	
T0 OVM sIgE KU/L					
T18 PEW DBPCFC	Passed	52	8.60 ± 16.81	1.77 (0.03-89.0)	0.100
	Failed	8	13.864 ± 17.11	6.73 (0.3-54.1)	
T0 EW sIgG4 mg/L					
T18 PEW DBPCFC	Passed	47	0.251 ± 5.445	0.510 (0.04-.310)	0.774
	Failed	8	0.832 ± 0.888	0.390 (0.07-2.19)	

EW: egg white; OVA: ovalbumin; OVM: ovomucoid; PEW DBPCFC: pasteurized egg white double-blind, placebo-controlled food challenge; SPT wheal: skin prick test wheal (D[mm] + d[mm])/2.

TABLE 4 Evolution of patients who reached total desensitization and did not complete 1 year of OIT. Threshold responding dose (g PEW protein), induction and OIT periods (weeks), and egg consumption. Patients are named by their number of inclusion and the group to which they were randomized

	8C	17C	19C	20C	28B	33B	36B	38C	41B	43B	57A	60C	61C	67A	70B	73C	85B	94A	95B
T0 Threshold dose PEW g	0.66	0.08	0.08	0.22	2.5	0.27	1.1	0.02	0.11	0.11	0.27	0.25	1.1	0.55	0.16	0.66	0.55	0.16	0.22
Induction period (wk)	24	25	10	11	17	15	5	10	21	24	12	20	7	2	16	17	12	17	9
OIT period (wk)	24	25	10	11	18	23	17	10	27	52	52	20	7	27	26	17	25	52	52
T18 Egg consumption	3	3	1	3	3	1	3	3	2	2	1/2	2	3	3	3	3	1	2	1
T18 PEW DBPCFC	Pass	Ref	0.55	Pass	Pass	1.65	Ref	Pass	0.33	1.65	0.44	0.88	Pass	Pass	Ref	Pass	2.4	0.88	Pass
T24 PEW DBPCFC	Ref	Ref	Pass	Pass	Pass	Ref	Pass	Pass	0.33	Pass	Ref	Ref	Pass	Ref	Ref	Ref	Pass	Ref	1.1*

Egg consumption: egg consumption the last week before T18; PEW DBPCFC: pasteurized egg white double-blind, placebo-controlled food challenge; Ref: refused; T0: inclusion, randomization and starting in patients randomized to OIT; T18: 18 months after inclusion; Threshold dose: threshold responding dose (g PEW proteins); *: This patient had only eaten foods with baked egg since T18.

diet, who developed reactions related to inadvertent egg ingestion ($P < 0.001$). DARs decreased in number and severity throughout the OIT and throughout the study ($P < 0.05$; Figure 5, Table 5).

Most OIT patients (90.78%) developed DARs during the build-up phase; 420 of 8448 (4.9%) doses caused immediate allergic reactions, mean 5.3 ± 7.9, median 3.0 (0-56): 74.53% were grade 1 or 2; 21.90% were grade 3; and 3.57% were grade 4 reactions, which occurred in 7 patients. During the maintenance phase, 54 patients (26 AG vs 28 BG) reported 87 reactions related to dose [0.76 ± 1.85 (0-7) AG vs 2.1 ± 3.49 (0-7) BG ($P < 0.05$)]. Seventy-two (82.76%) were grade 1-2 and 15 (17.24%) were grade 3 reactions. One patient developed a grade 4 reaction after 2 months on B maintenance; she had stopped the OIT for 4 days because of gastroenteritis, and ten minutes after taking the restart dose, 15 mL PEW, she developed intense generalized pruritus with erythema, urticaria, abdominal pain, and vomiting requiring a dose of adrenaline to control the symptoms. One patient developed symptoms of esophagitis between T12 and T24, but the macroscopic and microscopic examination of his esophagus was normal.

No patient had moderate or severe adverse reactions because of egg consumption once they had finished the OIT, but at least 17 patients reported immediate oral pruritus or isolated mild abdominal pain, which spontaneously disappeared in a few minutes; these symptoms had subsided by T24.

4 | DISCUSSION

This multicenter, randomized, controlled assay examines a strategy to normalize the diet, maintaining the desensitization state after finishing OIT. We assessed the effect of one year of OIT with PEW, equivalent to one raw egg, comparing the effectiveness and safety of two different OIT maintenance patterns: daily or every two days

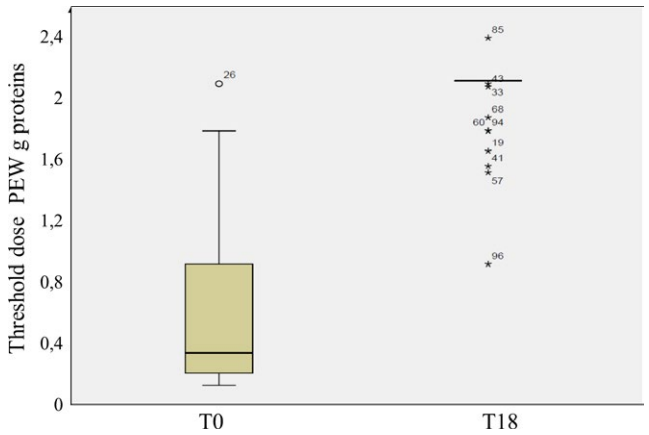

FIGURE 3 Patients who reached total desensitization and failed the challenge at T18: threshold response dose at T0 inclusion and at T18 (4-6 months after ending OIT). All patients who failed the challenge at T18 had increased their threshold dose from T0 to T18 ($P = 0.000$) (Wilcoxon signed-rank test). PEW: pasteurized egg white double-blind, placebo-controlled food challenge. T0: start of OIT; T18: 10 months after start OIT (4-6 months after ending OIT) [Colour figure can be viewed at wileyonlinelibrary.com]

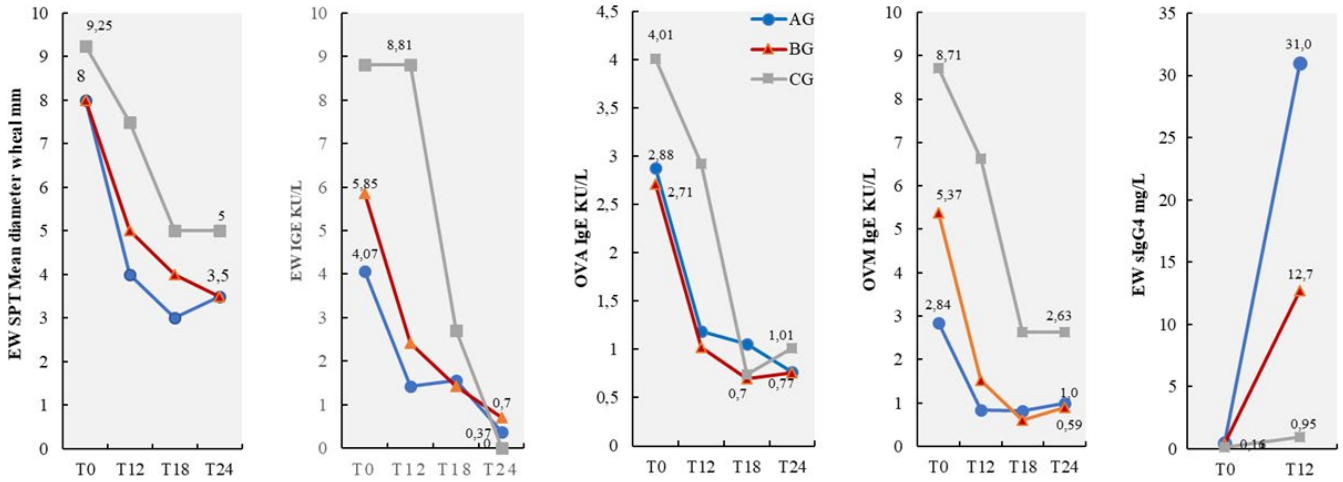

FIGURE 4 Evolution of immunologic markers (EW SPT wheal; egg (EW, OVA, and OVM) sIgE from T0 to T24, in patients who reached and maintained total desensitization; EW sIgG4 evolution in OIT (A and B) and CG patients from T0 to T12). A mixed-model regression analysis showed significant and similar decreased of skin prick test wheal and (EW, OVA, and OVM) sIgE antibody serum levels in OIT patients (AG, BG, and CG) ($P < 0.000$); no differences were observed between AG and BG maintenance patients. EW sIgG4 antibody serum levels increased from T0 to T12 in OIT patients ($P < 0.001$); this increment was higher in patients with daily maintenance ($P = 0.032$); no significant changes were observed in control patients on an egg-free diet from T0 to T12. EW: egg white; SPT wheal: skin prick test wheal (D[mm] + d[mm])/2; OVA: ovalbumin; OVM: ovomucoid [Colour figure can be viewed at wileyonlinelibrary.com]

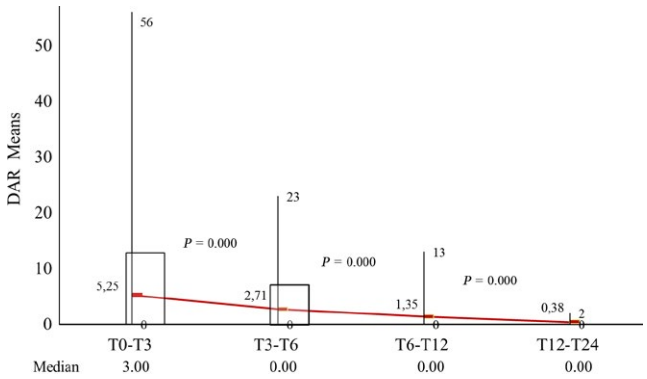

FIGURE 5 Evolution of adverse reactions throughout the study: from T0 to T24. Linear regression analysis (generalized linear model). T0-T3: The first 3 months of the study; T3-T6: from 3rd to 6th month of the study; T6-T12: from 6th to 12th month of the study; T12-T18: from 12th to 18th month of the study; T18-T24: from 18th to 24th month of the study [Colour figure can be viewed at wileyonlinelibrary.com]

(A or B) and the effect of the ad libitum egg consumption on the desensitization stage. The randomization of the patients to OIT (AG, BG) or to an egg-free diet (CG) was homogeneous in terms of sex, history of allergic asthma, threshold dose, and immunologic markers. Although the patients in the CG were younger, only 4 of 22 (18.18%) who completed one year of follow-up reached natural tolerance vs 64/76 (84.21%) of OIT patients who reached total desensitization. This desensitization rate is higher than that reported by previous randomized studies with similar egg sIgE and equivalent materials but lower target doses,[7,14] and the rate is similar to other studies with similar target doses and egg sIgE.[4,5,16]

Patients in our study randomized to OIT who reached total desensitization started ad libitum egg consumption completing an OIT year with the assigned maintenance. AG and BG had a similar maintenance period. Nevertheless, those patients on daily maintenance (AG) had better compliance than BG patients on every two days of maintenance.

TABLE 5 DARs throughout the study (during OIT and after ending it on ad libitum egg consumption)

	Mean ± SD	Median (Max-Min)
Induction period	5.32 ± 7.91	3.00 (0-56)
Grade 1-2	3.97 ± 6.49	2.00 (0-46)
Grade 3	1.17 ± 2.44	0.00 (0-14)
Grade 4	0.10 ± 0.377	0.00 (0-2)
Maintenance phase	1.34 ± 2.55	0.00 (0-10)
Grade 1-2	0.86 ± 1.93	0.00 (0-10)
Grade 3	0.25 ± 0.77	0.00 (0-4)
Grade 4	0.03 ± 0.175	0.00 (0-1)
On ad libitum egg consumption From T12 to T24 Grade 1-2	0.10 ± 0.384	0.00 (0-2)

DARs: dose adverse reactions and grading (Sampson's grading[17]).

We compared evolution of the desensitization state of patients who completed an OIT year with different maintenance patterns vs those who did not complete a year (8 of 12 patients from CG who started OIT at T12 and reached total desensitization) and those patients (2 of 32 from AG and 6 of 32 from BG) who discontinued maintenance and continued in the study on ad libitum egg consumption.

Our results show that most of patients who completed one year of OIT kept total desensitization at T18, and after six months on ad libitum egg consumption (98.85% on daily and 95.00% on every two days maintenance), this rate was higher than in patients who did not complete one year of OIT (93.75% vs 58.33%). Egg consumption at T18 had been higher in patients who passed the PEW DBPCFC, at least two egg servings in patients who completed an OIT year and three those patients who did not complete it.

Previous studies propose various strategies for the egg OIT, with maintenance doses equivalent to one or less than one raw, undercooked, cooked, or hard-boiled egg, at various intervals and periods of OIT.[5,6,10-16,22-25] Most provided protection to a majority of children with egg allergy by raising the threshold dose reaction and allowing them to ingest one egg serving safely.[12-16] Other studies reported lower maintained desensitization rates after a period on ad libitum egg consumption. Fuentes-Aparicio et al[16] observed that only 54% (20/37) of patients desensitized to one dehydrated whole egg passed the raw EW challenge after 12 months eating 2 cooked eggs per week. Itoh et al[11] observed that only 3 of 6 (50%) of patients who reached desensitization to 1 scrambled egg tolerated 1 g of dehydrated EW after 9 months of consuming two cooked eggs twice a week. It is unknown whether even extended periods of OIT are long enough to guarantee lifelong desensitization.

In our study, all patients who performed the PEW DBPFC at T24, except two, maintained total desensitization, 39 of 40 (95.5%) who completed a year of OIT after 12 months on ad libitum egg consumption vs 7 of 8 (87.5%) who did not complete it, after 8-18 months eating egg ad libitum.

Finally, 72 of 88 (81.81%) children reached total desensitization and 63 of 66 (95.45%) passed a challenge with 3.3 g PEW protein at least 6 months after finishing the OIT at T18 and/or T24.

Four factors could have influenced our results: (a) a higher target dose (3.3 g EW protein), equivalent to one raw white egg; (b) a longer OIT period, 1 year; (c) most patients, at least 6 months on maintenance; and (d) the egg consumption after stopping OIT (two or more cooked eggs per week).

Studies that have analyzed maintained tolerance after OIT by following an egg exclusion diet period for 4-6 weeks found loss of desensitization in 25%-72% of patients,[6,10,12,15] and these patients needed to restart OIT to regain desensitization. In clinical practice, parents of children with egg allergy often request OIT in the hope that their child can have a normalized diet without the risk of allergic reactions. An OIT year with a target dose equivalent to a medium-sized egg could improve the effectiveness of the egg OIT by maintaining a consumption of at least 2 whole egg servings per week for one year.

Similar to previous OIT assays,[5,11-14] we observed that EW SPT wheal and EW, OVA, and OVM sIgE antibody serum levels decreased and EW IgG4 serum levels increased during OIT; egg sIgE continued decreasing after stopping OIT, eating egg ad libitum. We observed that those patients who failed the PEW DBPCFC after stopping OIT had greater EW SPT wheal and higher egg sIgE serum levels at the beginning of OIT, confirming the findings of other studies.[4-8,15,16]

Adverse reactions decreased in number and intensity during the study. They were more frequent during the induction period and less frequent and milder in the maintenance phase, although more numerous in patients on every 2 days maintenance pattern. Early discontinuation of maintenance was associated with a grade 4 reaction after gastroenteritis, probably because of an early restoration of the OIT. Only grade 1-2 reactions were observed after discontinuing a year of OIT, and these decreased and practically disappeared 6-12 months after ending it. Very few studies report reactions in the maintenance phase, and none provide these data once the ITO has finished. Both Escudero et al and Burcks[5,14] reported similar data, with fewer reactions and no severe reactions in the maintenance phase; nevertheless, Vazquez-Ortiz et al[22] observed the same frequency of reactions during the induction and maintenance phases with the same maintenance dose, but they require epinephrine treatment only during induction phase. Finally, unlike other studies,[26] we did not observe development of esophagitis throughout the assay, despite the prolonged period of immunotherapy with high doses of the allergen, but esophagitis symptoms were an exclusion criteria in our study and other studies reporting these findings did not evaluate these symptoms at the start of the OIT.

We conclude that 1 year of OIT with at least 6 months' maintenance and dose equivalent to one pasteurized EW improves the effectiveness of the egg OIT and allows ad libitum egg consumption. Daily maintenance pattern appears to have better adherence and fewer dosing adverse reactions. Two egg servings per week ensure the persistence of total desensitization in most patients 6 months after ending a year of OIT.

ACKNOWLEDGMENTS

This study was conducted by the Spanish Society of Pediatric Allergy, Asthma, and Clinical Immunology (SEICAP).

The study protocol and the consent forms were approved by the institutional review board of the Spanish public healthcare system (HULP: 3250) and then at each hospital.

We thank the patients and their families who kindly participated and the staff of the clinical research unit at each institution and the Statistical and Clinical Coordinating Center (La Paz University Hospital), without whose participation the study could not have been performed.

We acknowledge the technical assistance provided by DIATER Laboratories during the process of this study. We are grateful to LETI Laboratories for their support in preparing the English version of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Conception and design: MMMF, PA, MP, EA, MA, and NS; Acquisition of data: MMMF, BMT, EA, ZL, FV, PM, PA, MC, MA, MCC, BC, VB, GC, NS, GJM, and EL; Analysis of "in vitro" tests: MCC; Statistical analysis and interpretation of data: MMMF and MR; drafting of the manuscript: MMMF and EL; and Critical revision and supervision: PA, MP, EA, MA, NS, MMMF, and EL. All the authors have read and approved the final manuscript.

The authors attest to the veracity and completeness of the data and analysis as well as to the adherence of the study to the protocol.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Martín-Muñoz MF, Alonso Lebrero E, Zapatero L, et al. Egg OIT in clinical practice (SEICAP II): Maintenance patterns and desensitization state after normalizing the diet. *Pediatr Allergy Immunol*. 2019;30:214–224. https://doi.org/10.1111/pai.13002