

## Original Article

# A Randomized, Open-Label Trial of Hen's Egg Oral Immunotherapy: Efficacy and Humoral Immune Responses in 50 Children

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**What is already known about this topic?** Oral immunotherapy is an experimental treatment for food allergy. Oral immunotherapy can successfully desensitize approximately 80% of children with persistent egg allergy.

**What does this article add to our knowledge?** We describe an egg oral immunotherapy protocol able to desensitize up to 88% of children with moderate to severe allergic reactions to heated egg in double-blind, placebo-controlled food challenge. High baseline egg white-specific IgE and polysensitization to Gal d 1-4 relate with impaired response.

**How does this study impact current management guidelines?** Many patients with high egg white-specific IgE levels and sensitization to multiple egg allergens achieve desensitization after prolonged treatment.

**BACKGROUND:** Egg allergy is the second most common food allergy in children. Persistent food allergy increases the risk of anaphylaxis and reduces the quality of life.

**OBJECTIVE:** To determine the efficacy of oral immunotherapy (OIT) with raw egg white powder and study its effects on humoral responses in children with persistent egg allergy.

**METHODS:** Fifty children aged 6 to 17 years with egg allergy, diagnosed by double-blind, placebo-controlled food challenge, were randomized 3:2 to 8 months of OIT with a maintenance dose of 1 g of egg white protein or 6 months of avoidance after which the avoidance group crossed over to OIT. We examined changes in IgE, IgG<sub>4</sub>, and IgA concentrations to Gal d 1-4 during OIT compared with avoidance and assessed clinical reactivity at 8 and 18 months.

**RESULTS:** After 8 months, 22 of 50 children (44%) on OIT and 1 of 21 (4.8%) on egg avoidance were desensitized to the target dose, 23 of 50 (46%) were partially desensitized (dose <1 g), and 5 of 50 (10%) discontinued. IgG<sub>4</sub> concentrations to Gal d 1-4 and IgA to Gal d 1-2 increased significantly, whereas IgE to Gal d 2 decreased. A heatmap analysis of the IgE patterns revealed 3 distinct clusters linked with the clinical outcome. High baseline egg white-specific IgE and polysensitization to Gal d 1-4 related with failure to achieve the maintenance dose at 8 months. After 18 months of treatment, 36 of 50 patients (72%) were desensitized and 8 of 50 (16%) partially desensitized.

**CONCLUSIONS:** OIT with raw egg enables liberation of egg products into the daily diet in most patients. Subjects with high egg white-specific IgE concentrations and sensitization to multiple egg allergen components at baseline benefit from prolonged treatment. © 2021 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>). (J Allergy Clin Immunol Pract 2021;■:■-■)

**Key words:** Desensitization; Egg allergens; Gal d 1; Hen's egg allergy; Oral immunotherapy; Ovalbumin; Ovomucoid; Tolerance

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## INTRODUCTION

Oral tolerance is the normal physiologic response to ingested food proteins, and a breakdown in this process leads to sensitization and the development of food allergy.<sup>1</sup> The prevalence of food allergy ranges from 3% to 8% in children and seems to be increasing.<sup>2</sup> Hen's egg allergy is the second most common food allergy in young children, with an estimated prevalence of 0.5% to 2.5%.<sup>2</sup> Although most food allergies are transient and resolve by school age, egg allergy may persist until adulthood.<sup>3,4</sup> Persistent food allergy is associated with a more severe clinical

**Abbreviations used***DBPCFC*-Double-blind, placebo-controlled food challenge*EW-IgE*-Egg white-specific IgE*IQR*-Interquartile range*OIT*-Oral immunotherapy

course and high specific IgE levels with sensitization to food allergens resistant to heat and enzymatic digestion, and thus, reactivity to the food in the processed or heated form.<sup>3</sup>

The current standard of care for food allergy is avoidance of the offending food and administration of emergency medication on accidental exposure.<sup>4</sup> Food avoidance and unpredictable reactions related to accidental exposures decrease the quality of life of the patients and their families.<sup>4</sup> There is, therefore, an unmet need to develop effective therapies for the treatment of food allergy.

Oral immunotherapy (OIT) is an experimental, therapeutic approach in which gradually increasing doses of food allergens are administered orally.<sup>5</sup> OIT can desensitize up to 80% of children with persistent food allergy, and in a subset lead to long-term immune tolerance.<sup>6</sup> Desensitization is a state of temporary antigen hyporesponsiveness that depends on regular food intake. If dosing is interrupted, the protective effect is lost.<sup>7</sup> Moreover, cofactors, such as physical exercise and infections, may lower the threshold of reactivity and trigger reactions to a previously tolerated dose.<sup>8</sup> The ultimate goal is to develop long-lasting immune tolerance, which is defined as the ability to ingest the food without symptoms regardless of irregular consumption or periods of avoidance. Many studies prefer the term “sustained unresponsiveness,” because there is no consensus on the length of the avoidance needed to define permanent tolerance.<sup>5</sup> OIT inducing successful desensitization has been reported to cow’s milk, hen’s egg, wheat, and peanut.<sup>6</sup> OIT uses the pathways underlying oral tolerance, that is, the physiologic tolerogenic response upon oral administration of food proteins.<sup>1</sup> Current evidence suggests that OIT can desensitize more than 80% of children with egg allergy,<sup>9</sup> and elicit long-term tolerance in 50% after 4 years of treatment.<sup>10,11</sup> The mechanisms behind desensitization and possible long-term effects remain under active investigation.<sup>7</sup> Open questions remain regarding optimal desensitization and maintenance protocols, safety, and selection of patients benefiting from OIT.

We aimed to determine the efficacy of an 8-month egg OIT protocol with raw, pasteurized egg white powder and study its effects on humoral immune responses in children with persistent egg allergy. The maintenance dose was 1 g of egg white protein, which corresponds to approximately one-third of the protein content of an egg white (3 g).

## METHODS

This was a randomized, open, trial investigating the efficacy and immunologic effects of hen’s egg OIT in children. The key study outcome was the proportion of participants desensitized after 8 months of OIT. Secondary outcomes were (1) the proportion of participants partially desensitized after 8 months of OIT, (2) the proportion of participants desensitized after 18 months of OIT, (3) the proportion of participants partially desensitized after 18 months of OIT, (4) the proportion of participants able to consume 1.5 g of heated egg protein after 3 months of maintenance therapy, and (5)

changes in Gal d 1-4-specific IgE, IgA, and IgG<sub>4</sub> antibody levels and their correlation to the tolerated egg dose after 0 and 8 months of OIT.

Desensitization was defined as the ability to consume 1 g of egg white protein without symptoms and partial desensitization as the ability to consume any dose below 1 g of egg white protein without symptoms.

## Study population

The study included 50 children and adolescents, aged 6 to 17 years, referred to the Department of Allergology, Helsinki University Central Hospital, Finland, for evaluation of hen’s egg allergy. The participants were recruited between June 2013 and September 2017. The Helsinki University Hospital of Children and Adolescents Ethics Committee approved the study, and each participant older than 6 years as well as his or her guardian gave written informed consent.

Seventy-six patients underwent double-blind, placebo-controlled food challenges (DBPCFCs) with heated egg white, performed as previously described.<sup>12</sup> The challenges were interpreted positive and dosing was stopped in line with the PRACTALL consensus criteria.<sup>13</sup> We used a modified threshold-adjusted score to evaluate reaction severity: 1 to 5 indicated mild, 6 to 13 moderate, and 14 to 23 severe reactions.<sup>12,14</sup> The inclusion criteria for the study were age 6 to 17 years, a clinical history of hen’s egg allergy, sensitization to egg white (egg white-specific IgE [EW-IgE]  $\geq 0.35$  kU/L), and a moderate to severe reaction in the baseline DBPCFC to heated egg white. Subjects were excluded for any of the following reasons: poor adherence, uncontrolled or severe asthma, severe systemic illness, active autoimmune disease, malignant neoplasia, or pregnancy.<sup>15</sup>

The study subjects were randomized 3:2 to egg OIT or avoidance. The avoidance group underwent an open oral egg rechallenge after 6 months and crossed over to OIT after 8 months (Figure 1, A).

## Oral immunotherapy

The OIT protocol is presented in Table E1 in this article’s Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org). OIT was carried out with pasteurized, spray-dried, raw egg white powder (Dava Foods, Piispanrasti, Finland) with daily dosing at home. The first dose and escalations to 1, 4, 25, and 350 mg were taken at the outpatient clinic. The dose was increased weekly for the first 3 weeks and from then on biweekly. The build-up phase lasted for 8 months. The target maintenance dose was 1 g of egg white protein, corresponding approximately to one-third of the protein content of an egg white. In the maintenance phase, the participants consumed a total amount of one-third of an egg white daily: raw egg white powder at least 3 times a week and boiled or fried egg or foods containing heated egg on the remaining days. Egg yolk and different degrees of heating were allowed. The participants who were unable to ingest 1 g at 8 months continued up dosing with raw egg powder, and in the case of continuous dosing symptoms, they switched to heated egg. After 3 months of maintenance therapy on the target dose, an open oral egg challenge with 1.5 g of heated egg protein, corresponding to one-half of a boiled egg white, was performed. If passed, the patients were allowed to consume up to one-half of an egg in the heated or one-third in the raw form. Daily consumption was continued. They were advised to incorporate heated egg products (eg, meatballs, pasta, bread, pancakes, and pastries) into their daily diet and liberate dietary restrictions of heated egg at home and in the school diet. Exercise restrictions were removed at this point.

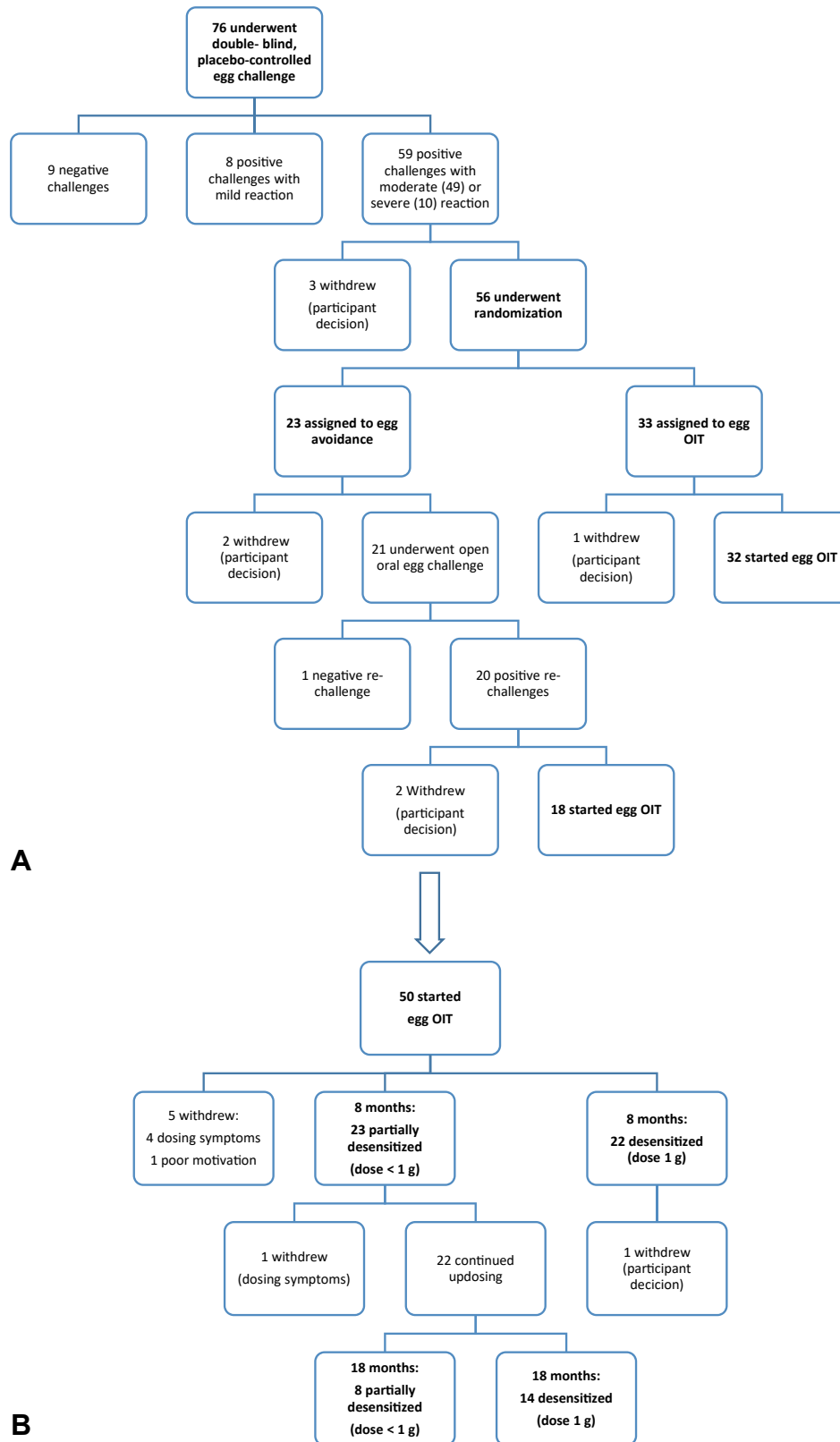


FIGURE 1. (A) Study enrollment and randomization and (B) OIT outcome in 50 patients.

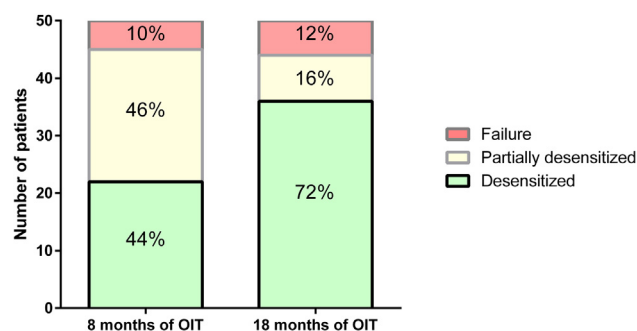
**TABLE I.** The baseline characteristics of the study population

Characteristic	Value
Sex, n (%)	
Male	23 (46)
Female	27 (54)
Age (y)	
Range	6-17
Mean	11.2
Median	11.0
Age at diagnosis (y)	
Range	0.2-6
Mean	1.2
Median	1.0
Additional food allergy, n (%)	34 (68)
Milk	12 (24)
Wheat	6 (12)
Soy	9 (18)
Fish	5 (10)
Peanut	17 (34)
Treenut	17 (34)
Other	7 (14)
Previous OIT, n (%)	7 (14)
Milk	5 (10)
Milk and wheat	2 (4)
Asthma, n (%)	30 (60)
Persistent	21 (42)
Intermittent	9 (18)
Allergic rhinitis, n (%)	39 (78)
Atopic dermatitis, n (%)	37 (74)
Baseline DBPCFC, n (%)	
Moderate	41 (82)
Severe	9 (18)

A standard dose of antihistamine was taken daily at least 1 hour before the egg dose to reduce possible dose-related oropharyngeal symptoms.<sup>15</sup> Dosing symptom severity was assessed by the CoFAR Grading System for Allergic Reactions.<sup>10</sup> Mild to moderate dosing symptoms were treated with an additional antihistamine dose and with oral prednisolone if the symptoms persisted for more than 30 minutes. For treatment of severe systemic reactions, the patients had an adrenaline autoinjector. In the case of mild dosing symptoms, the patient continued with the same dose until resolution of symptoms before dose escalation. If the symptoms were moderate or severe, the patient reduced dosing to the previously tolerated dose, after which dose escalation was adjusted individually. Physical exercise was forbidden 1 hour before and after each dose, and on days of escalation. The dose was not escalated in the case of an infectious disease.

### Blood samples and immunologic parameters

Venous blood samples were collected before OIT and avoidance, and after 3 and 8 months of OIT. Serum and plasma were separated, aliquoted, and stored at  $-20^{\circ}\text{C}$ . EW-IgE, serum IgE, and plasma IgA and IgG<sub>4</sub> antibody concentrations to Gal d 1 (ovo-mucoid), Gal d 2 (ovalbumin), Gal d 3 (conalbumin), and Gal d 4 (lysozyme) were measured by ImmunoCAP (Thermo Fisher, Uppsala, Sweden).

**HE OIT outcome in 50 patients after 8 and 18 months****FIGURE 2.** Hen's egg OIT outcome at 8 and 18 months. HE, Hen's egg.

### Statistics, correlations, and hierarchical clustering

For analysis of data, we used Graph Pad Prism 8 for Windows (GraphPad Software, Inc, La Jolla, Calif). The antibody data were expressed as medians with 95% CI, and differences between medians were analyzed with the Student *t* test with a 2-tailed test of significance. The Mann-Whitney *U* test or the Kruskal-Wallis test was used when variances were different between groups for unpaired comparisons. The association between clinical parameters, antibody concentrations, and the OIT dose was examined by Spearman Rank and Pearson correlation tests. Differences at *P* less than .05 were considered statistically significant. K-means-based hierarchical clustering was carried out with Perseus omics analysis and visualization software.<sup>16</sup>

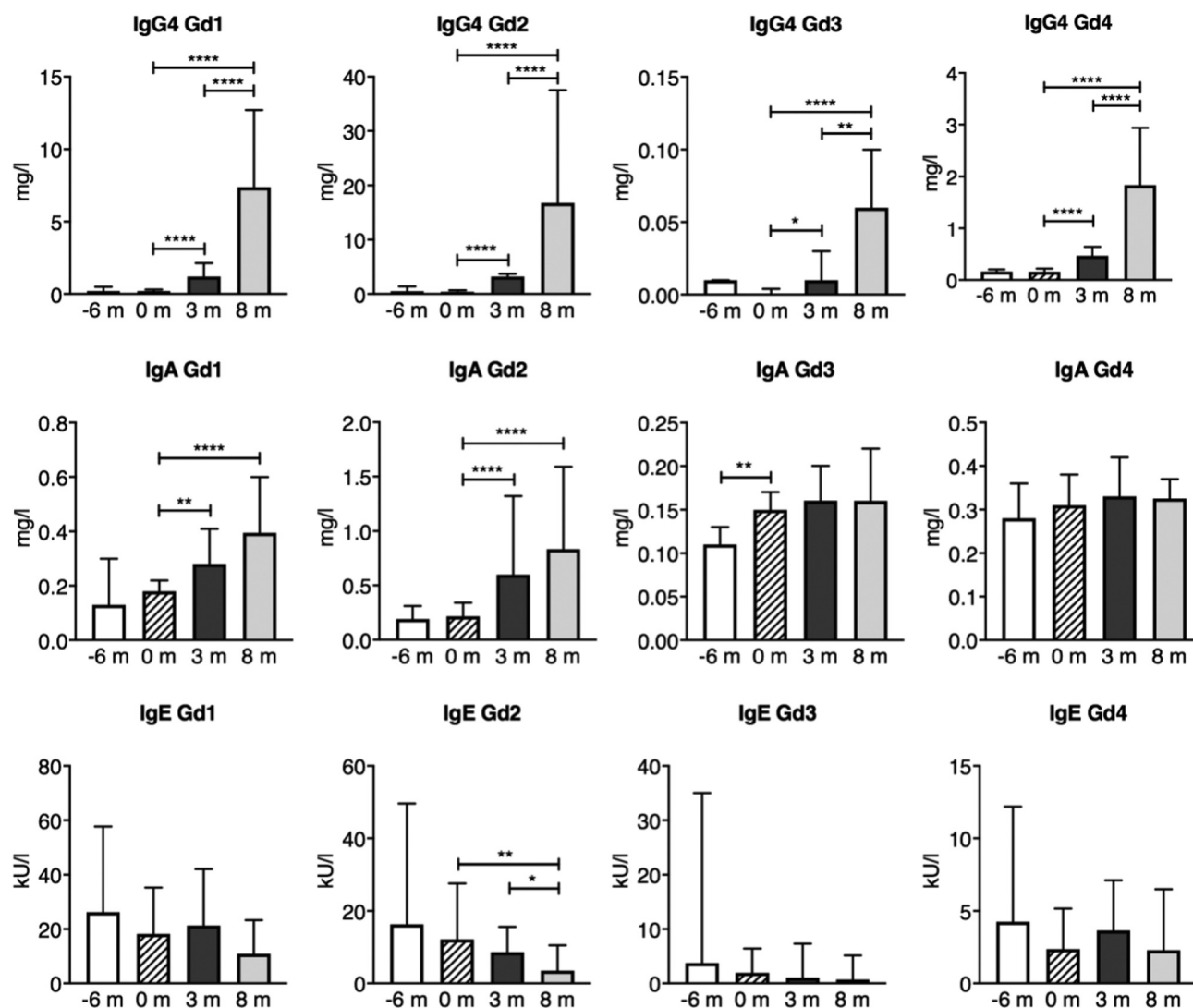
### RESULTS

The study enrollment is shown in Figure 1, A. Of the 76 patients who underwent DBPCFC, 59 were eligible for the study. Fifty-six patients were randomized: 33 to start OIT and 23 to continue avoidance. Five withdrew and 1 rechallenged was negative. Altogether 50 patients started the OIT protocol (Table E1; Figure 1, B). The baseline characteristics of these 50 patients are presented in Table I.

### Desensitization

In the baseline DBPCFC, the median (interquartile range [IQR]) heated egg white protein dose successfully consumed without symptoms was 5 (5-50) mg. One (4.8%) of the 21 controls passed the rechallenge after 6 months. In the remaining 20 patients, the rechallenge was positive with no significant difference in the successfully consumed dose compared with the baseline challenge. After 8 months of OIT, 22 of 50 patients (44%) reached the maintenance dose of 1 g of raw egg protein (desensitized) and 23 of 50 (46%) a dose less than 1 g (partially desensitized), with a median (IQR) dose of 300 (175-700) mg and a range of 20 to 700 mg. Five patients (10%) discontinued during the build-up phase, 4 because of continuous gastrointestinal dosing symptoms and 1 because of nonadherence (Figure 1, B). The median (IQR) successfully consumed dose increased from 5 (5-50) mg of heated egg protein to 700 (175-1000) mg of raw egg protein (*P* < .0001).

During the build-up phase, 41 of 50 patients (82%) experienced dosing symptoms. Gastrointestinal symptoms (abdominal



**FIGURE 3.** Antibody responses at -6, 0, 3, and 8 months of OIT. Concentrations of Gal d 1-4-specific IgG<sub>4</sub>, IgA, and IgE antibodies were measured by ImmunoCAP. Results are expressed as medians with 95% CI. Significance is indicated by \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$  by Mann-Whitney  $t$  test.

discomfort, nausea, vomiting) were observed in 34 of 50 (68%), oral pruritus in 12 of 50 (24%), and skin symptoms (erythema, urticaria, flare-up of eczema) in 3 of 50 patients (6%). No respiratory symptoms, severe reactions requiring adrenaline, or anaphylaxes<sup>17</sup> were observed. Of the 23 of 50 patients partially desensitized at 8 months, 22 of 23 continued up dosing and 1 withdrew because of dosing symptoms (Figure 1, B). At this point, 8 of 22 switched to heated egg because of dosing symptoms caused by raw egg but tolerated egg that had been heated for at least 10 minutes (boiled egg or egg-containing foods). After 18 months of OIT, 14 of 22 of the partially desensitized subjects reached the target dose of 1 g and 8 of 22 continued regular egg consumption at a median (IQR) maintenance dose of 350 (350-550) mg and a range of 100 to 700 mg. After 18 months of OIT, altogether 44 of 50 patients (88%) were consuming egg; 36 of 50 (72%) were considered desensitized and 8 of 50 (16%) partially desensitized (Figure 2). More than half the patients, that is, 27 of

50 (54%), had liberated dietary restrictions of egg at home and in the school diet.

To assess tolerance to heated egg, a post-OIT open oral egg challenge with one-half of a cooked egg white, corresponding to 1.5 g of protein, was performed to the 36 fully desensitized patients who reached the target dose of 1 g egg protein. All 36 passed the challenge after 3 months of maintenance therapy on the target dose.

### Changes in concentrations of Gal d 1-4-specific IgE, IgG<sub>4</sub>, and IgA during OIT

Serum IgE and plasma IgA and IgG<sub>4</sub> levels to Gal d 1 (ovo-mucoid), Gal d 2 (ovalbumin), Gal d 3 (conalbumin), and Gal d 4 (lysozyme) and EW-IgE were measured at different time points: at the beginning of the avoidance period (-6 m) or OIT (0 m), and after 3 months (3 m) and 8 months (8 m) of OIT (Figure 3).



**TABLE II.** Baseline EW-IgE and Gal d 1-4–specific IgE levels in the different patient groups according to OIT outcome after 8 mo of treatment

Baseline IgE levels	Desensitized (dose 1 g) (n = 22)	Partially desensitized (dose 20-700 mg) (n = 23)	Failure (n = 5)
Egg white IgE (kU/L)*			
Median	9.8	53	137
Mean	21.6	197	246
Gal d 1 IgE (kU/L)*			
Median	8.2	36	39.3
Egg white IgE (kU/L)*			
Mean	21.9	73.4	92.2
Gal d 2 IgE (kU/L)*			
Median	4.8	34.1	39.1
Mean	8.8	127	185
Gal d 3 IgE (kU/L)*			
Median	0.18	6.42	29.5
Mean	1.77	83.8	54.7
Gal d 4 IgE (kU/L)†			
Median	0.71	2.61	19.6
Mean	2.28	23.7	33.0
Polysensitization to Gal d 1-4, n (%)			
No. of patients	6 (27)	16 (70)	5 (100)

The median and mean IgE levels differed significantly between the subjects desensitized at 8 mo compared with the partially desensitized or failed subjects. Polysensitization to all 4 allergens was frequent in the partially desensitized and failed groups.

\* $P < .005$ , Kruskal-Wallis test.

† $P < .01$ , Kruskal-Wallis test.

IgG<sub>4</sub>, IgA, and IgE antibody concentrations to Gal d 1-4 showed no statistically significant differences between the beginning of avoidance (–6 m) and OIT (0 m) except for increased IgA to Gal d 3. This is probably explained by technical variation in the measurements because IgA concentrations at all time points were close to or below the detection level. The concentration of IgG<sub>4</sub> antibodies to Gal d 1-4 and IgA antibodies to Gal d 1 and 2 increased linearly and significantly throughout the OIT period ( $P < .001$ ). The concentration of IgE to Gal d 1-4 increased during the first 3 months followed by a decrease at 8 months, but the decrease was significant only for Gal d 2 ( $P < .01$ ) (Figure 3).

### Correlation of clinical characteristics and antibody levels with OIT clinical outcome

There was a moderate correlation between the tolerated and cumulative DBPCFC doses ( $r = 0.53$  and  $0.56$ , respectively) and the OIT dose at 8 months ( $P < .0001$ ). Age, reaction severity, or the presence of comorbidities did not associate with the OIT outcome. The baseline median and mean EW-IgE and Gal d 1-4–specific IgE levels differed significantly in the subjects desensitized at 8 months compared with the partially desensitized or failed subjects (Table II). Because of large variability, we could not determine threshold IgE levels predictive of desensitization. However, 21 of 22 (95%) subjects desensitized at 8 months had IgE levels below 57 kU/L for egg white, 44 kU/L for Gal d 1, and 30 kU/L for Gal d 2 at baseline. Approximately half, that is,

12 of 23 (48%), of the partially desensitized subjects had baseline IgE levels below these cutoffs.

Gal d 1-4–specific IgE concentrations correlated at all time points (apart from Gal d 4 at 8 months) inversely with the tolerated egg dose at 8 months of OIT ( $r = -0.51$  to  $-0.61$ ;  $P < .0001$ ). Similarly, IgA antibody concentrations to Gal d 1 and Gal d 2 at 8 months correlated inversely with the tolerated egg dose at 8 months. Figure 4 shows the correlation of the OIT dose at 8 months with Gal d 1– and Gal d 2–specific IgE (logarithmic) concentrations at 0 month and IgA (logarithmic) concentrations at 8 months.

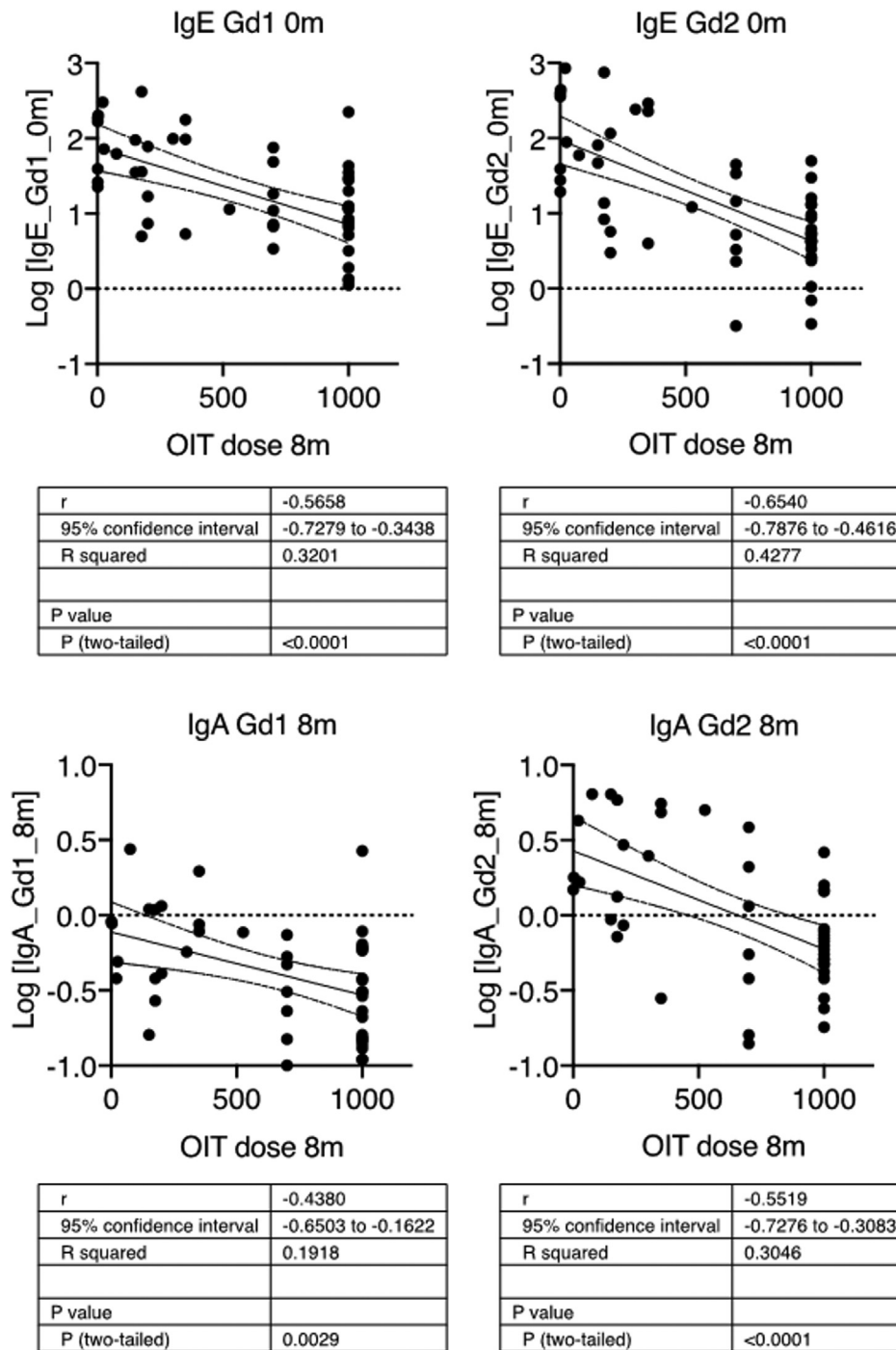
A heatmap analysis of the logarithmic ( $\log_{10}$ ) IgE concentrations at all time points, based on the egg dose tolerated at 8 months, formed 3 distinct clusters (Figure 5, A). Cluster 1 (bottom) includes subjects with the highest log-transformed IgE concentrations (mean,  $2.02 \pm 0.60$ ) to Gal d 1-4 throughout the OIT period. None of the patients in this cluster were fully desensitized at 8 months. Cluster 2 (middle) includes the subjects with the lowest Gal d 1-4–specific IgE concentrations (mean,  $-0.14 \pm 0.96$ ) throughout the OIT period. Of these patients, 71% (15 of 21) were fully desensitized by 8 months. Cluster 3 (top) includes polysensitized patients with intermediate IgE concentrations (mean,  $0.85 \pm 0.70$ ) to Gal d 1-4; in this cluster, 39% (7 of 18) were desensitized after 8 months of OIT. Some patients showed increased production of IgA and IgG<sub>4</sub> mostly to Gal d 2 at 3 and 8 months, but associations of clusters and the OIT dose were not formed (Figure 5, B and C).

At baseline, 54% (27 of 50) of the subjects were sensitized to all 4 allergen molecules Gal d 1-4 (Table II). This polysensitization correlated significantly with the dose tolerated at 8 months ( $r = -0.477$ ;  $P < .001$ ) and was associated with OIT discontinuation and failure to achieve the maintenance dose of 1 g. All 5 OIT failures and 70% (16 of 23) of the partially desensitized patients were polysensitized to Gal d 1-4, whereas only 22% (6 of 27) of the polysensitized patients were fully desensitized at 8 months.

### DISCUSSION

We describe an effective hen's egg OIT protocol using raw, pasteurized egg white powder in 50 children with moderate to severe allergic reactions to heated egg diagnosed by DBPCFC. In line with previous studies, allergen-specific IgE levels increased slightly during the first 3 months followed by a decrease at 8 months, whereas allergen-specific IgA and IgG<sub>4</sub> antibodies gradually increased throughout the OIT build-up phase. A heatmap analysis of the antibody responses revealed distinct clusters linked to the clinical outcome. High baseline EW-IgE and especially polysensitization to Gal d 1-4 related with OIT discontinuation and failure to achieve the maintenance dose at 8 months. However, after 18 months of OIT, up to 88% were consuming egg. Most children were able to incorporate egg products into their daily diet and more than half were able to liberate dietary restrictions at home and in the school diet.

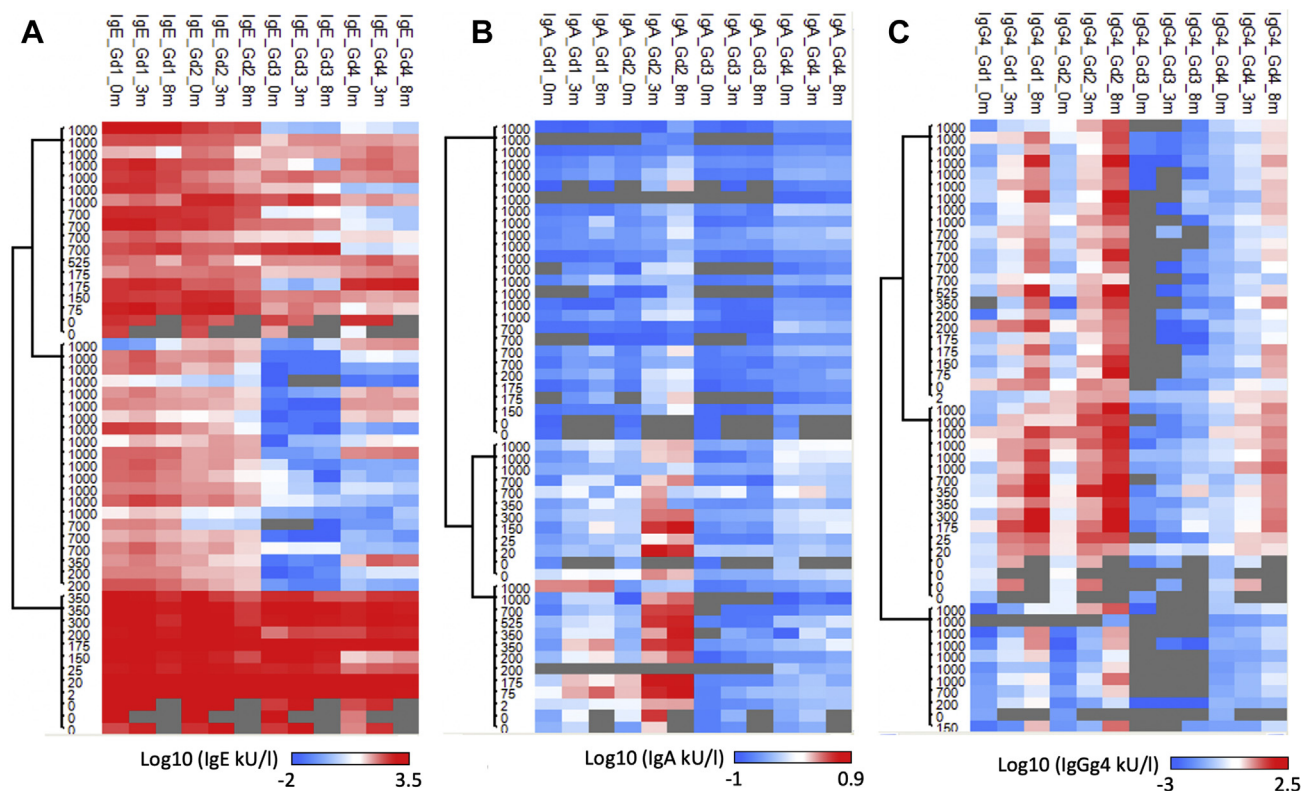
Hen's egg allergy resolves spontaneously in most children,<sup>18</sup> and approximately 70% of children reacting to raw egg tolerate baked egg.<sup>19</sup> The most important egg allergen, Gal d 1 (ovomucoid), retains its allergenicity even after extensive heating and is stable against enzymatic digestion, in contrast to the unstable egg allergens Gal d 2 (ovalbumin), Gal d 3 (conalbumin), and Gal d 4 (lysozyme). Sensitization to Gal d 1 and its sequential



**FIGURE 4.** Pearson correlation of the OIT dose at 8 months to Gal d 1– and Gal d 2–specific IgE and IgA antibody (logarithmic) concentrations after 0 and 8 months of OIT.

IgE-binding epitopes is associated with an increased risk of persistent egg allergy.<sup>12,20,21</sup> An inclusion criterion for our study was a moderate to severe reaction to heated egg in DBPCFC. Consequently, children with mild symptoms or allergy to raw egg only were excluded. In the OIT build-up phase, we used raw egg white to ensure optimal desensitization to all egg allergens. In the maintenance phase, the children were advised to consume both raw and heated egg to maintain successful desensitization.

During the OIT build-up phase, 82% of the children experienced dosing symptoms, mainly mild to moderate gastrointestinal symptoms. No severe reactions were seen. In the case of continuous symptoms, we adjusted the protocol individually by returning to a dose tolerated without symptoms and delayed dose escalation. These children benefited from a longer build-up phase with individually adjusted, slower dose increments. After 18 months of OIT, 60% of these partially desensitized subjects were



**FIGURE 5.** Hierarchical Euclidean clustering of the OIT dose at 8 months (y-axis) in relation to logarithmic (log<sub>10</sub>) allergen-specific (A) IgE, (B) IgA (B), and (C) IgG<sub>4</sub> antibody concentrations at 0, 3, and 8 months. Each row represents 1 patient, and each column the log-transformed antibody concentrations, with blue color for the lowest and red for the highest log<sub>10</sub> concentrations. Gray is NA. NA, Not applicable/available.

successfully desensitized to the target dose. Eight children with symptoms from raw egg powder tolerated the equivalent dose in the heated form, although they reacted to heated egg in the baseline DBPCFC. These children were able to continue up dosing with heated egg without symptoms. Our results are consistent with previous studies suggesting that continued allergen administration over a prolonged period improves desensitization rates and promotes a shift toward long-lasting tolerance.<sup>11,22</sup> We did not discontinue egg ingestion to evaluate sustained unresponsiveness, so we could not distinguish patients with long-lasting tolerance from those with transient desensitization.

In accordance with previous studies, OIT failure correlated with high baseline EW-IgE levels.<sup>1,23,24</sup> In 95% of the successfully desensitized subjects, the baseline IgE levels were below 57 kU/L for egg white, 44 kU/L for Gal d 1, and 30 kU/L for Gal d 2. The higher the IgE concentrations of Gal d 1 and Gal d 2 were at baseline, the lower was the tolerated egg dose at 8 months. Our study showed for the first time that polysensitization to all 4 egg allergen molecules Gal d 1-4 is associated with poor desensitization responses. In an Australian HealthNuts cohort,<sup>21</sup> sensitization to multiple egg white allergens (Gal d 1, 2, 3) and egg yolk Gal d 5 at the age of 12 months increased the risk of persistent egg allergy 4-fold at the age of 4 years, whereas 93% of children sensitized to only 1 egg allergen outgrew their allergy. This suggests that IgE epitope diversity plays an

important role both in natural tolerance formation and in desensitization induced by OIT. Most of our patients with high baseline EW-IgE and sensitization to multiple egg allergens were, nevertheless, able to achieve at least partial desensitization, but they required individually adjusted prolonged treatment. These partially desensitized patients continued regular ingestion of a smaller egg portion, which was beneficial in protecting them from unpredictable reactions related to accidental egg exposures.

IgA antibody concentrations to all studied allergens were very low in all patients. Concentrations of IgA to Gal d 1 and 2 increased significantly at 8 months, predominantly in the group of partially desensitized patients. Wright et al,<sup>24</sup> on the contrary, demonstrated increases in egg white-specific IgA in subjects with sustained unresponsiveness evaluated after 2 to 4 years of therapy. Our IgA measurements were from an earlier time point in OIT and only to the specific allergen molecules, which might explain the difference. Our data suggest, however, that patients with elevated allergen-specific IgE at baseline and increased IgA production during the OIT build-up phase may benefit from prolonged and individually adjusted OIT. IgG<sub>4</sub> antibodies increased in all patients throughout the OIT build-up phase, but neither this IgG<sub>4</sub> increase nor the modest decrease in IgE antibodies associated with the clinical outcome of OIT.

The efficacy of egg OIT in our study was similar to previously published studies. Comparisons are, though, difficult to perform, because the studies are methodologically very heterogeneous



regarding inclusion criteria, age groups, egg preparations, build-up protocols, duration of OIT, target doses, and maintenance dosing.<sup>10,23,25-29</sup> The first randomized trial on egg OIT was published in 2012 by Burks et al<sup>10</sup> with desensitization rates comparable to our study. In this placebo-controlled, multicenter study, 55% of 40 egg-allergic children aged 5 to 11 years were desensitized after 10 months, and 75% after 22 months of OIT with raw egg powder. Sustained unresponsiveness, evaluated by passing an oral food challenge after discontinuing egg intake, was seen in 28% after 2 years and in 50% after 4 years of OIT.<sup>11</sup> The largest randomized study so far is a multicenter study from Spain including 101 children, aged 6 to 9 years, receiving OIT with pasteurized raw egg white.<sup>30</sup> They also included 16 children tolerant to heated egg. The desensitization rate was 84% after 1 year of egg OIT, compared with 16% in children on an egg-free diet. A daily 5% dose increment pattern seemed more efficient than weekly dose escalations. A systematic Cochrane review on egg OIT<sup>9</sup> including 10 randomized trials summarized that 82% of 249 egg-allergic children receiving OIT were desensitized, defined as being able to consume a partial egg serving (1-7.5 g), and 45% were able to consume a full serving, compared with 10% of 190 control children. All studies reported mild to severe adverse effects, seen in altogether 75% of children receiving OIT compared with 6.8% in the control group. All studies used different methods and the sample sizes were small, which decreases the quality of the evidence.

There are several limitations to our study. This was not a randomized controlled trial, and we had no placebo group. The design was open-label and only the avoidance group crossed over to OIT after 8 months. The rechallenge was performed after 6 months of avoidance as an open food challenge, which may have caused misclassification bias in the challenge outcome. Some children could have outgrown their allergy during the 2 months before starting OIT, although they were above the typical age of natural egg allergy resolution. Safety assessment was not included as a study outcome, which is a clear limitation. We monitored dosing symptoms by a questionnaire filled during each visit instead of a daily diary, which may have underestimated the occurrence of dosing symptoms. Unfortunately, we did not collect blood samples for antibody analysis at 18 months.

## CONCLUSIONS

Our study shows that OIT with raw egg enables liberation of egg products into the daily diet of most children with persistent allergy to heated egg. Clustering of the protein-specific antibody responses by heatmap analysis reveals different clinical patterns that may help in designing the clinical treatment protocol. High baseline EW-IgE and polysensitization to Gal d 1-4 relate with discontinuation and the need for individually adjusted, prolonged treatment protocols.

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## ONLINE REPOSITORY

**TABLE E1.** Hen's egg OIT build-up phase protocol and timing of venous blood samples

Product	Week	Dose	Dose (mg protein)	Blood samples
Egg white powder 1 mg/mL solution	1	0.1 mL	0.1 mg*	0 mo
	2	0.2 mL	0.2 mg	
	3	0.4 mL	0.4 mg	
	4	1 mL	1 mg*	
	6	2 mL	2 mg	
Egg white powder 10 mg/mL solution	8	0.4 mL	4 mg	
	10	0.8 mL	8 mg	
	12	1.2 mL	12 mg	
	14	2.5 mL	25 mg*	3 mo
	16	5 mL	50 mg	
	18	10 mL	100 mg	
	20	20 mL	200 mg	
Egg white powder	22	3 g	350 mg*	
	24	6 g	700 mg	
	26	12 g	1 g	
	32	12 g	1 g	8 mo

The protocol for OIT consisted of an 8-mo build-up phase and a maintenance phase. During the build-up phase, the participants ingested a daily dose of spray-dried, pasteurized egg white powder dissolved in water (1 and 10 mg/mL solutions) and after reaching the 350-mg dose, the powder was taken as such. The dose was increased weekly for the first 3 weeks and from then on biweekly according to the protocol above. The maintenance dose (1 g egg white protein) corresponded approximately to one-third of an egg white. Antihistamine premedication was taken daily at least 1 h before the OIT dose and was discontinued 2 wk after reaching the maintenance dose. During the maintenance phase, the participants ingested a total amount of one-third of an egg white daily: raw egg white powder at least 3 times a week and boiled or fried egg or foods containing heated egg on the remaining days.

\*Dose taken at the outpatient clinic.