

ORIGINAL ARTICLE

Dehydrated egg white: An allergen source for improving efficacy and safety in the diagnosis and treatment for egg allergy

Carmelo Escudero¹, Silvia Sánchez-García¹, Pablo Rodríguez del Río¹, Carlos Pastor-Vargas², Cristina García-Fernández³, Inmaculada Pérez-Rangel¹, Antonio Ramírez-Jiménez¹ & María Dolores Ibáñez¹

¹Allergy Department, Hospital Infantil Universitario Niño Jesús, Madrid, Spain; ²Immunology Department, IIS Fundación Jiménez Díaz, Madrid, Spain; ³Public Health Department, Hospital Infanta Sofía, San Sebastian de los Reyes, Madrid, Spain

To cite this article: Escudero C, Sánchez-García S, Rodríguez del Río P, Pastor-Vargas C, García-Fernández C, Pérez-Rangel I, Ramírez-Jiménez A, Ibáñez MD. Dehydrated egg white: an allergen source for improving efficacy and safety in the diagnosis and treatment for egg allergy. *Pediatr Allergy Immunol* 2013; **00**.

Keywords

dehydrated egg white; desensitization; diagnosis of egg allergy; dried egg; egg oral immunotherapy; oral food challenge; oral tolerance induction; OVO-DES; OVO-PRO

Correspondence

María Dolores Ibáñez, Allergy Department, Hospital Infantil Universitario Niño Jesús, Avenida de Menéndez Pelayo 65, 28009 Madrid, Spain.
Tel.: +34 91 503 5935
Fax: +34 91 503 5935
E-mail: mibanets@salud.madrid.org

Accepted for publication 15 January 2013

DOI:10.1111/pai.12052

Abstract

Background: Raw and cooked eggs are used as allergens in oral food challenge (OFC). Raw egg is the best option, as it keeps proteins intact and retains their allergenicity, albeit microbiologically safe manipulation is difficult. Therefore, the use of dehydrated egg white (DEW) could improve the efficacy and safety profile of OFC. The aim of the study was to compare the allergenicity of DEW, a product that undergoes a double heat treatment (pasteurization and drying), with that of raw egg white (REW) and determine the efficacy of DEW in the diagnosis of egg allergy.

Methods: We conducted a prospective study of 40 egg-allergic patients who visited our outpatient clinic. Each patient underwent OFC with DEW and REW to determine the correlation between the tests. DEW and REW extracts were analyzed using SDS-PAGE. We compared the allergenicity of both extracts using IgE immunoblotting with a serum pool from patients with positive OFC results.

Results: Ten patients (25%) had positive OFC results with both DEW and REW, and the doses that triggered an allergic reaction in each patient were similar ($p > 0.05$). All 30 patients (75%) with a negative OFC result with DEW also had negative OFC results with REW. SDS-PAGE and IgE immunoblotting revealed that the protein composition and IgE-binding capacity of both extracts were virtually identical.

Conclusions: This is the first time that it is shown that the allergenicity of commercially available DEW is equivalent to raw egg whites. *In vivo* and *in vitro* tests showed that processing of DEW does not affect the allergenicity of egg proteins. DEW is an effective and microbiologically safer source of allergen for the diagnosis of egg allergy. Furthermore, DEW can be used in egg oral immunotherapy.

Food allergy is a major public health issue, and its prevalence has increased in the last 15 years (1, 2). Egg allergy is one of the most frequent food allergies in children, with an estimated point prevalence of 1.6% (3). Egg allergy is resolved in most cases by the age of 8 (4, 5), and therefore, appropriate diagnostic procedures are key to revealing the persistence or resolution of the disease. Diagnosis of egg allergy is often determined by skin prick tests (SPT) and/or serum-specific IgE (sIgE) analysis (6), although it needs (OFC) to be confirmed through oral food challenge (7). The allergens generally used for egg OFC are raw, pasteurized, or cooked egg (scrambled egg, omelet, and boiled egg). However, the use of raw egg to perform oral challenge tests in the study of patients allergic to egg ensures stability and allergenicity of

proteins (8). Hence, correct selection of the allergen source is essential. Raw egg is the best material for oral challenge tests as it is not degraded by heat, while protein integrity and allergenicity are preserved. However, use of these products in challenge tests entails a risk of food-borne diseases caused by agents such as *Salmonella* spp (9). Egg products that are processed and pasteurized and available in liquid and dried forms ensure a high level of food safety by preventing the risk of food-borne diseases.

Cooked egg prevents the risk of food poisoning, although allergenicity is diminished (10). In fact, egg is one of the foods whose allergenicity is most altered by cooking or processing. Ovalbumin, lysozyme, and ovotransferrin are heat-labile allergens (11). Ovomucoid, on the other hand, is characterized by

its high heat stability, possibly as a result of its strong disulfide bonds (12). The above observations must be taken into consideration when choosing the allergen source for oral food challenges with egg. Moreover, over the last few years, new treatments have been successfully developed for egg allergy such as oral immunotherapy (13–15). Therefore, these issues must also be considered when choosing the allergenic source for oral immunotherapy with egg.

The aim of this study was to compare the allergenicity and efficacy in clinical practice for the diagnosis of egg allergy of a commercially available Dehydrated egg white (DEW), a product that undergoes a double heat treatment (pasteurization and drying), with that of Raw egg white (REW). This is the first time the allergenicity of DEW has been compared with REW.

Materials and methods

Patients

Inclusion criteria for the selection of patients were a clear history of immediate symptoms after egg intake and positive SPT and/or sIgE to egg white, ovomucoid, and/or ovalbumin and written informed consent obtained in accordance with the institution's ethical guidelines for research involving children. Exclusion criteria for the selection of patients were as follows: anaphylactic shock after the intake of egg, non-IgE-mediated adverse reactions to egg, malignant diseases, immunopathologies and/or severe primary or secondary immunodeficiencies, and associated pathologies contraindicating the use of adrenaline.

The patients were prospectively recruited from the allergy department at Niño Jesús University Children's Hospital, Madrid, Spain. The study and informed consent forms were approved by the institution's independent ethics committee (internal code R-0007/11).

Dehydrated egg white

Commercially available DEW was obtained from the producer (Egg Albumin Powder; OVOSEC S.A., Valladolid, Spain). The product is made from eggs of the species *Gallus gallus domesticus*. Briefly, REW is pasteurized (heated to 59°C for 6 min) to eradicate any microorganism (*Salmonella* spp., *Enterobacteriaceae*, *Staphylococcus aureus*, *Bacillus cereus*, yeast, and molds) according to the process hygiene and food safety criteria of the Commission Regulation of the European Communities No. 2073/2005 of November 15, 2005 on microbiological criteria for foodstuffs (16). After pasteurization, pH is adjusted to 6.5–7.5 by addition of citric acid. Oxidizing glucose, catalase, and hydrogen peroxide are then added to remove glucose and prevent Maillard reaction. Finally, pasteurized liquid egg white is passed through a spray tower where it is dried with hot air at 80°C for 1 min. The resulting DEW can be stored at room temperature in the dark for 18 months after production. The protein content of DEW is 78%. According to the manufacturer, 3600 mg of DEW is the equivalent of the egg white contained in a fresh egg.

Skin tests

Skin prick tests were performed according to standard procedure as shown in the European Academy of Allergology and Clinical Immunology position paper (17) by using commercial extracts of egg white (EW) (1 mg/mL), ovalbumin (OVA) (1 mg/mL), and ovomucoid (OVM) (1 mg/mL) (Leti, Madrid, Spain). In addition, SPT were performed using the prick-to-prick method with DEW and REW (18). The DEW extract was prepared by diluting 3600 mg of DEW in 43 mL of saline solution. SPT were performed on the volar surface of the forearm with ALK Lancets (ALK Abelló, Madrid, Spain). Histamine phosphate at 10 mg/mL and normal saline solution were used as positive and negative controls, respectively. The response was read 15 min after puncture, and the results were expressed as the mean wheal diameter (mm). A wheal diameter of 3 mm or more compared with the saline control was defined as a positive reaction.

IgE determinations

Serum-specific IgE (ImmunoCAP; Phadia, Uppsala, Sweden) were performed with native allergens of EW, OVM (Gal d 1) and OVA (Gal d 2).

Correlation between skin prick tests and egg white, ovalbumin, and ovomucoid-specific IgE

To analyze the correlation between the size of SPT and EW, OVA and OVM-specific IgE levels, the Spearman's rank correlation coefficient (SRCC) statistical test was used.

Oral food challenge

All the patients underwent an open OFC with DEW and REW (19). To administer equivalent doses of egg white in the OFC, 43 mL of REW was compared with 3600 mg of DEW diluted in 43 mL of sweetened orange juice. REW was mixed with sugar to enhance flavor without modifying volume. The average weight and volume of the egg white contained in a large size egg was 43.72 g and 43 mL, respectively (Table 1). DEW is easily diluted in liquid at room temperature to provide an easily administered homogeneous mixture. Challenges with DEW and REW consisted of nine doses—0.1, 0.2, 0.5, 1, 2, 4, 6, 9, and 20.2 mL (cumulative dose of 43 mL)—administered at 30-min intervals (19) to better identify the doses that produced symptoms, both in the REW and in the DEW challenge to compare the results of each challenge on each patient. Challenges were stopped if objective clinical symptoms of allergy were observed (urticaria, rhinitis, vomiting, cough, shortness of breath, and/or hypotension) or when a cumulative dose of 43 mL was tolerated; challenges were classed as positive or negative, respectively. Patients remained hospitalized for observation for 2 h after the administration of the last dose in the case of negative results or 2 h after symptoms resolved in the case of positive results. In positive OFC, doses of DEW and REW eliciting symptoms were compared.

Table 1 Dosing schedule of oral challenges with raw egg white and dehydrated egg white

Dose no.	Raw egg white	Dehydrated egg white		
	Volume (mL)	Volume (mL)	Weight (mg)	Protein content (mg)
1	0.1	0.1	8.3	6.5
2	0.2	0.2	16.6	12.9
3	0.5	0.5	41.5	32
4	1	1	83	65
5	2	2	166	129
6	4	4	332	259
7	6	6	498	388
8	9	9	747	583
9	20.2	20.2	1707	1331
	Total: 43 mL	Total: 43 mL	Total: 3600 mg	Total: 2808 mg

3600 mg of dehydrated egg white is equivalent to a raw egg white.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to Laemmli (20) using the Hoofer SE 600 electrophoresis system (GE Healthcare, Little, Chaffont, Buckinghamshire, UK). Polyacrylamide concentrations of 14% (w/v) and 5% (w/v) were used for separate and stacking gels, respectively. Samples of DEW and REW were mixed with 0.1 M Tris (pH 6.8) containing 4% (w/v) SDS, 20% (w/v) glycerol, 10% (w/v) 2 β -mercaptoethanol, and 0.02% (w/v) bromophenol blue; 20 μ g of protein was loaded in each lane. Electrophoresis was carried out in 0.025 M (w/v) Tris-HCl (pH 8.3), 0.192 M (w/v) glycine, and 0.1% (w/v) SDS. Gels were stained with PageBlue Protein Staining Solution (Fermentas International Inc. Burlington, Canada) to ensure proper protein separation and visualization or used for immunoblotting, as described below.

IgE immunoblotting

DEW and REW protein bands and spots separated by SDS-PAGE were transferred by semi-dry blotting onto nitrocellulose sheets according to the method of Towbin et al. (21). Membranes were blocked with phosphate-buffered saline (PBS) solution containing 0.1% Tween 20 (PBS-T), 3% skimmed milk powder, and 3% bovine serum albumin for 2 h at room temperature and incubated overnight at 4°C with serum diluted by 1/10 in 50 mM Tris-buffered saline solution, 150 mM NaCl, 5 mM EDTA, and 0.05% triton X-100. After five washes with PBS-T, the blots were incubated for 1 h at room temperature with mouse antihuman IgE antibody conjugated with horseradish peroxidase (Nordic Immunological Laboratories, Cultec SL, Madrid, Spain) diluted by 1:5000 in PBS containing 0.05% Tween-20 and 1.5% skimmed milk powder. Finally, after five washes with PBS-T, the protein bands were visualized using enhanced chemiluminescence (GE Healthcare, Little, Chaffont, Buckinghamshire, UK), following the instructions provided by the manufacturer. IgE

immunoblotting was carried out with a serum pool obtained from patients with positive OFC results to DEW and REW.

IgE immunoblotting inhibition

IgE immunoblotting inhibition was performed with both DEW and REW in solid phase and as inhibitors in the liquid phase and a pool of sera obtained from patients with positive OFC results.

Results

Patients

Forty (76.9%) out of the 52 patients who were invited to participate in the study agreed to do so. Patient age ranged from 2 to 17 yr (mean $\pm$ s.d. age, 6.77 ± 3.96 yr); 67.5% of patients were male. The mean duration of allergy to egg was 5.43 ± 0.97 yr. Egg allergy onset with immediate symptoms in all cases: 37.5% with oral-facial hives, 47.5% with generalized hives, 30% with gastrointestinal symptoms, 10% with rhinitis and 5% with asthma (Table 2). The 87.5% of patients had a history of atopic eczema, 35% of rhinitis, 40% of asthma, and 45% of other food allergy.

Skin tests

The results of SPT to EW, OVA, OVM, REW, and DEW are shown in Tables 2 and 3. The positive predictive value of SPT for EW, OVA, and OVM was 25%, 33%, and 38%, respectively. Prick-to-prick results for REW and DEW were positive in all patients. SPT showed no significant differences between REW and DEW extracts in patients with negative OFC (mean, 4.81 and 4.37 mm, respectively; $p > 0.05$) and in patients with positive OFC (mean, 8.4 and 7.1 mm, respectively, $p > 0.05$). No discrepancies were observed in each patient.

IgE determinations

The results of sIgE determination to egg white, ovalbumin, and ovomucoid are shown in Table 2. The positive predictive value of sIgE for EW, OVA, and OVM was 38%, 37% and 39%, respectively.

Correlation between skin prick tests and egg white, ovalbumin, and ovomucoid-specific IgE

SRCC between EW-SPT and EW-specific IgE was 0.7, between OVA-SPT and OVA-specific IgE was 0.27, and between OVM-SPT and OVM-specific IgE was 0.74. These correlations were statistically significant for EW and OVM ($p < 0.05$).

SDS-PAGE

The protein profile of the REW and DEW extracts was very similar. The main proteins were observed at 14, 37, 45, and 77 kDa (Fig. 1).

Table 2 Clinical and immunological characteristics of patients at the time of study

No.	Age (yr)	FAR (symptoms)	SPT (mm) (commercial extracts)			slgE (kU/l)			OFC
			EW	OVA	OVM	EW	OVA	OVM	
1	17	GU + GI	3	3	3	ND	ND	ND	NEG
2	16	OU + GI	5	3	4	1.62	1.75	1.91	NEG
3	15	GU + R + GI	4	5	3	0.5	0.35	0.36	NEG
4	14	GU	6	4	4	3.15	1.8	2.15	NEG
5	13	GI	5	0	0	0.75	1.04	<0.35	NEG
6	12	OU + GI	5	4	6	0.92	1.1	1.09	NEG
7	10	GU + A	7	5	4	0.53	0.35	0.56	NEG
8	9	OU	5	3	4	ND	ND	ND	NEG
9	9	GU	4	0	5	ND	ND	ND	NEG
10	11	OU + GI	4	0	0	ND	ND	ND	NEG
11	8	R	6	0	3	0.69	0.72	0.68	NEG
12	8	OU	5	4	6	0.88	1.11	0.96	NEG
13	6	OU	7	3	4	1.08	1.29	0.65	NEG
14	7	OU	5	3	3	ND	ND	ND	NEG
15	6	GU + A	5	4	3	1.17	1.38	0.91	NEG
16	4	OU	5	3	0	ND	ND	ND	NEG
17	4	OU	3	0	0	ND	ND	ND	NEG
18	2	OU	3	3	0	0.48	0.58	0.71	NEG
19	4	OU	3	0	3	ND	ND	ND	NEG
20	4	OU	3	3	0	ND	ND	ND	NEG
21	5	GU	3	3	0	0.35	0.35	0.35	NEG
22	3	OU	4	0	4	<0.35	2.11	<0.35	NEG
23	5	GI	3	0	0	1.07	0.61	0.75	NEG
24	2	OU	3	3	0	<0.35	<0.35	<0.35	NEG
25	3	OU	4	0	0	<0.35	<0.35	<0.35	NEG
26	5	GU	4	0	0	0.41	0.45	<0.35	NEG
27	2	OU	3	3	0	<0.35	<0.35	<0.35	NEG
28	3	GU	0	3	0	ND	ND	ND	NEG
29	3	OU + GI	3	3	4	0.59	0.68	0.4	NEG
30	3	OU	3	3	3	0.45	0.69	0.48	NEG
31	7	GU	5	3	6	1.42	1.2	1.76	POS
32	7	OU + GI	6	5	4	398	260	256	POS
33	6	OU + R + GI	6	3	5	6.53	8.49	2.23	POS
34	6	GU	6	5	6	9.29	12.5	9.84	POS
35	5	GI	7	3	5	15	4.21	8.84	POS
36	5	GU + R	6	4	0	0.98	1.38	<0.35	POS
37	6	GI	6	2	5	13.7	11.3	2.46	POS
38	5	GU	5	3	4	0.63	0.83	0.4	POS
39	6	OU	5	3	4	1.1	1.01	0.9	POS
40	5	GI	5	5	5	5.79	4.77	6.57	POS

No., patient number; FAR, first allergic reaction after egg ingestion; OU, oro-facial urticaria; GU, generalized urticaria; GI, gastrointestinal symptoms; R, rhinitis; A, asthma; SPT, skin prick tests; EW, egg white; OVA, ovalbumin; OVM, ovomucoid; slgE, specific IgE; ND, not performed; OFC, oral food challenges; NEG, negative; POS, positive.

IgE immunoblotting and IgE immunoblotting inhibition

IgE immunoblotting showed that sera recognized the same bands in both extracts. The molecular weights of the recognized bands were, approximately, 14, 37, 45, 55, 66, and 77 kDa (Fig. 1). In addition, immunoblotting inhibition showed that the behavior of DEW and REW was identical (Fig. 2).

Challenge tests

The time between challenges ranged from 1 to 17 days. Ten patients (25%) had a positive result in both oral challenges, and 30 patients (75%) had a negative result in both oral challenges (Table 2). In all cases, positivity and negativity were consistent for both types of egg white. In the case of a positive OFC result, no significant differences were observed between

Table 3 Patients with positive oral food challenges: Results of prick-to-prick tests and doses that triggered symptoms and symptoms observed during the challenge

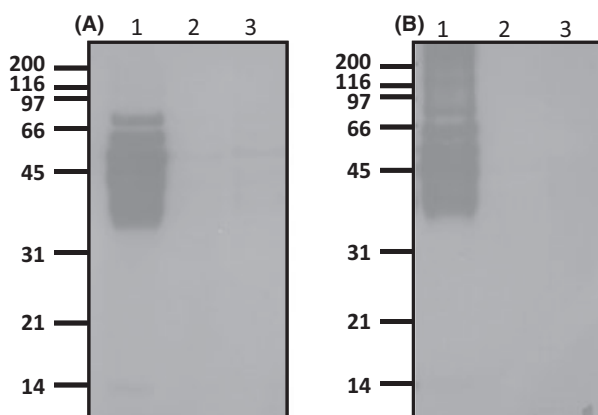
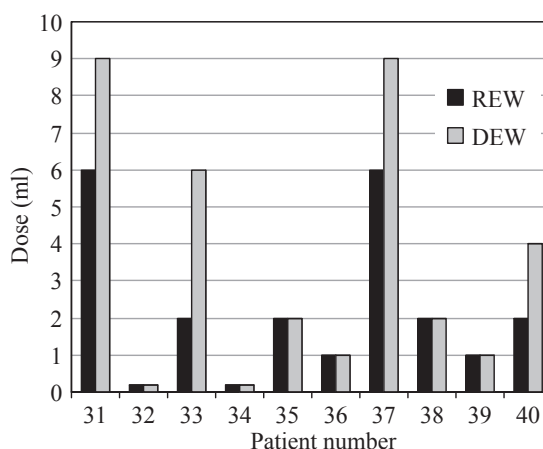
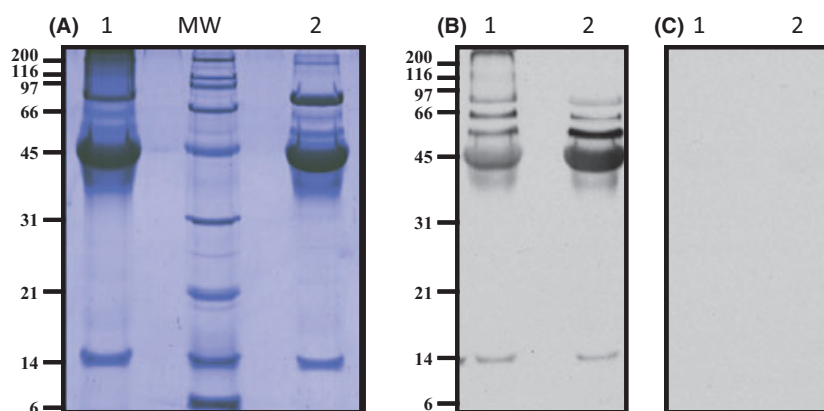
	Prick-to-prick (mm)		OFC			
			OFC with REW		OFC with DEW	
No.	REW	DEW	Dose (mL)	Symptoms	Dose (mL)	Symptoms
31	6.5	5.5	6	OU	9	GI
32	9.5	7.0	0.2	GI	0.2	OU + GI
33	7.0	7.0	2	R + GI	6	OU + GI
34	9.5	8.0	0.2	OU	0.2	OU + GI
35	9.5	6.0	2	GI	2	GI
36	7.5	4.5	1	OU	1	OU + GI
37	9.5	9.5	6	R + GI	9	R + GI
38	7.0	5.5	2	OU + GI	2	OU + GI
39	7.0	8.0	1	GI	1	OU + GI
40	11.0	10.0	2	R + GI	4	R + GI

No., patient number; REW, raw egg white; DEW, dehydrated egg white; OFC, oral food challenge; OU, oro-facial urticaria; GI, gastrointestinal symptoms; R, rhinitis.

the doses that elicited allergic symptoms when using DEW and REW (mean, 3 and 2.1 mL, respectively; $p > 0.05$) (Table 3 and Fig. 3). Six of the 10 patients (60%) with positive challenge results had symptoms with the same dose in both the DEW challenge and in the REW challenge; in three patients, the challenges were positive with a difference of one dose and in one patient with a difference of two doses. No differences in symptoms were observed during the positive challenges with DEW and REW. The clinical manifestations in the 10 positive OFC with DEW were skin symptoms ($n = 6$), gastrointestinal symptoms ($n = 7$), and rhinitis ($n = 2$); the clinical manifestations in the 10 positive OFCs with REW were skin symptoms ($n = 4$), gastrointestinal symptoms ($n = 7$), and rhinitis ($n = 3$) (Table 3).

Discussion

In this study, the results of the skin tests and oral challenges show that DEW maintains the same allergenicity *in vivo* as

**Figure 2** IgE immunoblotting inhibition. Panel A, solid phase with raw egg white; Panel B, solid phase with dehydrated egg white; Lane 1, IgE immunoblotting; Lane 2, inhibition with dehydrated egg white; Lane 3, inhibition with raw egg white.**Figure 3** Patients with positive oral food challenges: doses that elicited symptoms.**Figure 1** Panel A, SDS-PAGE with dehydrated egg white (lane 1) and raw egg white (lane 2): MW, molecular weight (kDa). Panel B, IgE immunoblotting with a serum pool from egg-allergic patients with positive challenge results to DEW and REW. Panel C, IgE immunoblotting negative control with sera from non-atopic patients.

REW, given that we observed no inconsistent results in both tests, no significant differences in the doses that led to symptoms in the positive challenges and no significant differences in symptoms observed during the positive challenges with DEW and REW. The results of IgE immunoblotting and IgE immunoblotting inhibition revealed that DEW maintained heat-sensitive egg white allergens intact and that these were similar to those of REW.

Some foods may contain cooked egg or raw egg among their ingredients. To date, there is no consensus as to whether oral challenge with egg in allergic patients should be performed first, with raw egg or with cooked egg (8). Osborne et al. (22) demonstrated that in a population of patients with raw egg allergy diagnosed by oral challenge, 80.3% of patients tolerated baked egg. Patients with low IgE levels are more likely to tolerate cooked egg in a specific challenge. However, these patients may remain at risk for several years with foods containing raw eggs (e.g. pastry, homemade mayonnaise, sauces, cakes, meringue, ice cream) (23). The allergenicity of egg proteins depends on their resistance to heat (10). Ovomucoid is highly heat-stable (12), whereas ovalbumin is relatively heat-labile. Exposure of ovalbumin to a temperature of 80°C for 3 min decreases its IgE-binding activity by 90% (11, 24). Urisu et al. (25) analyzed OFC performed in patients with high IgE antibody values to egg white to compare the allergenicity of three different egg white preparations. The study showed that heat treatment (90°C for 60 min) reduced the allergenic activity of egg white; in fact, 21 of 38 patients (55.3%) with positive immediate reactions to a laboratory-made freeze-dried egg white had negative responses to heated egg white. Therefore, DEW can be used as an allergen source for oral challenge tests without the risk of false negatives that could occur in patients allergic to heat-labile proteins who tolerate cooked egg, but not foods containing raw eggs.

We observed that the double heat treatment (pasteurization and drying) the egg white undergoes to obtain DEW does not affect the allergenicity of its proteins or its capacity to bind to IgE. Our results are consistent with those of Jurado-Palomo et al. (26) who found no allergenic differences *in vitro* or *in vivo* between raw and pasteurized egg white. The present study also showed that the second heat treatment after pasteurization to obtain DEW – drying with air at 80°C for 1 min – does not affect the allergenicity of the proteins.

Raw eggs may be contaminated by *Salmonella enteritidis*, which is a risk factor for food-borne diseases (27). The European Food Safety Authority has shown that in the European Union, egg is still the most frequently implicated source in food-borne human salmonellosis. *Salmonella enteritidis* is the only human pathogen that contaminates eggs routinely (28). One of the key interventions aimed at preventing contamination and growth of *Salmonella* spp. in eggs is education of consumers and food workers about the risk of consuming raw or undercooked eggs (9). Healthcare

professionals are not certified food handlers and so must handle food as safely as possible to avoid contamination or deterioration. Hence, raw egg should be avoided in OFC. In addition, DEW through the pasteurization and drying processes ensures microbiological safety of the product compared with other egg products such as freeze-dried or lyophilized egg that do not undergo pasteurization (29). Dehydrated egg removes the risk of food poisoning.

DEW has a shelf life of 18 months and does not need refrigeration, as compared to pasteurized egg white that has a shelf life of 3 days after opening the packaging and needs refrigeration. The product is easy to prepare and allows for homogeneous doses, accurate quantification of protein per dose, and blind challenge. These advantages could equally apply to products subjected to the same heat treatments of other DEW manufacturing companies.

Moreover, DEW is an effective allergen for oral immunotherapy treatments in egg-allergic patients (15, 30). In oral immunotherapy with egg, DEW is useful, because treatment starts with very small doses of food during the first few days, and dosing is simple and reproducible.

The DEW analyzed is a commercially available and inexpensive product useful for daily clinical practice in the diagnosis of egg allergy. This product is currently marketed in individual doses with different weights (amounts) that can be used for oral challenge or oral immunotherapy (OVO-DES and OVO-PRO; Nutrición Médica, Madrid, Spain).

We conclude that there are no *in vivo* or *in vitro* allergenic differences between DEW and REW. This is the first time that the allergenicity of commercially available dehydrated egg white is demonstrated as equivalent to that of raw egg. DEW is useful, as it can be used as an allergen source in oral challenge tests for diagnosis of any patient with egg allergy, including patients who are allergic to heat-labile egg proteins. Furthermore, DEW eliminates the danger of *Salmonella* spp., has advantages over pasteurized egg White, such as long shelf life without refrigeration, and ensures homogeneity of the prepared doses. Finally, DEW is a suitable source of egg protein for oral immunotherapy, because it enables small doses to be prepared and is therefore easier to manipulate than raw egg.

Acknowledgments

The authors are most grateful for the extensive work of our nurses and nurse assistants in our daily practice and especially for their contribution to the clinical work of this manuscript: Catalina Quiñero, Inmaculada Sanz, Ascensión López, Esperanza Doval, Carmen Ballesteros, and María Jesús Jiménez.

Conflict of interest

The authors of this paper declare no conflict of interests with OVOSEC S.A., Spain and Nutrición Médica S.L., Spain.

References

- Prescott S, Allen KJ. Food allergy: riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol* 2011; **22**: 155–60.
- McBride D, Keil T, Grabenhenrich L, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Pediatr Allergy Immunol* 2012; **23**: 230–9.
- Osterballe M, Hansen TK, Mortz CG, Host A, Bindslev-Jensen C. The prevalence of food hypersensitivity in an unselected population of children and adults. *Pediatr Allergy Immunol* 2005; **16**: 67–73.
- Boyano-Martínez T, García-Ara C, Díaz-Pena JM, Martín-Esteban M. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. *J Allergy Clin Immunol* 2002; **110**: 304–9.
- Savage JH, Matsui EC, Skripak JM, Wood R. The natural history of egg allergy. *J Allergy Clin Immunol* 2007; **120**: 1413–7.
- Diéguez MC, Cerecedo I, Muriel A, Zamora J, Abaira V, Camacho E, Antón M, de la Hoz B. Utility of diagnostic tests in the follow-up of egg-allergic children. *Clin Exp Allergy* 2009; **39**: 1575–84.
- Wainstein BK, Studdert J, Ziegler M, Ziegler JB. Prediction of anaphylaxis during peanut food challenge: usefulness of the peanut skin prick test (SPT) and specific IgE level. *Pediatr Allergy Immunol* 2010; **21**(4 Pt 1): 603–11.
- Nowak-Węgrzyn A, Assa'ad AH, Bahna SL, Bock S, Sicherer SH, Teuber SS. Work Group report: oral food challenge testing. *J Allergy Clin Immunol* 2009; **123**: S365–83.
- Braden CR. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. *Clin Infect Dis* 2006; **43**: 512–7.
- Mine Y, Yang M. Recent advances in the understanding of egg allergens: basic, industrial, and clinical perspectives. *J Agric Food Chem* 2008; **56**: 4874–900.
- Mine Y, Zhang JW. Comparative studies on antigenicity and allergenicity of native and denatured egg white proteins. *J Agric Food Chem* 2002; **50**: 2679–83.
- Hirose J, Kitabatake N, Kimura A, Narita H. Recognition of native and/or thermally induced denatured forms of the major food allergen, ovomucoid, by human IgE and mouse monoclonal IgG antibodies. *Biosci Biotechnol Biochem* 2004; **68**: 2490–7.
- Patriarca C, Romano A, Venuti A, Schiavino D, Di Rienzo V, Nucera E, Pellegrino S. Oral specific hyposensitization in the management of patients allergic to food. *Allergol Immunopathol* 1984; **12**: 275–81.
- Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy* 2007; **62**: 1261–9.
- Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* 2012; **367**: 233–43.
- Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union.
- European Academy of Allergology and Clinical Immunology. *Position Paper: Immunotherapy* 1993; **48**(suppl):7–35.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983; **38**: 167–72.
- Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004; **59**: 690–7.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–5.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350–4.
- Osborne NJ, Koplin JJ, Martin PE, et al. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol* 2011; **127**: 668–76.e1–2.
- Eigenmann PA. Anaphylactic reactions to raw eggs after negative challenges with cooked eggs. *J Allergy Clin Immunol* 2000; **105**: 587–8.
- Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994; **93**: 1047–59.
- Urisu A, Ando H, Morita Y, et al. Allergenic activity of heated and ovomucoid-depleted egg white. *J Allergy Clin Immunol* 1997; **100**: 171–6.
- Jurado-Palomo J, Fiandor-Román AM, Bobolea ID, Sánchez-Pastor S, Pascual C, Quirce S. Oral challenge with pasteurized egg white from *Gallus domesticus*. *Int Arch Allergy Immunol* 2010; **151**: 331–5.
- Guard-Petter J. The chicken, the egg and *Salmonella enteritidis*. *Environ Microbiol* 2001; **3**: 421–30.
- Anonymous. Scientific opinion of the panel on biological hazards on a request of the European Commission on a quantitative microbiological assessment on *Salmonella* in meat. *EFSA J* 2008; **625**: 5–32.
- Matsuoka DM, Costa SF, Mangini C, et al. A nosocomial outbreak of *Salmonella enteritidis* associated with lyophilized enteral nutrition. *J Hosp Infect* 2004; **58**: 122–7.
- Itoh N, Itagaki Y, Kurihara K. Rush specific oral tolerance induction in school-age children with severe egg allergy: one year follow up. *Allergol Int* 2010; **59**: 43–51.