Oral immunotherapy in children with IgE-mediated hen's egg allergy: Follow-ups at 2.5 and 7 years

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ABSTRACT

Background: *The present report was a follow-up investigation at 2.5- and 7-year intervals of a previous study of 20 children with moderate-to-severe immunoglobulin E (IgE) mediated hen's egg (HE) allergy who received oral immunotherapy (OIT) with raw HE. The study design of the previous study divided the 20 subjects into two groups of 10 each: (1) group 1, the OIT group (OIT-G), and, (2) group 2, an age-matched control group (C-G). In that study, 8 of 10 of the children in the OIT-G were successfully desensitized, one child was partially desensitized, and desensitization failed in one child. The aims of the present study were to evaluate the long-term effectiveness and safety profile of OIT with raw HE, and to assess the course and prognostic value of skin-prick tests (SPT) and serum-specific HE-IgEs in this study population.*

Methods: *Of the 20 children who were recalled, 2 dropped out, which left 18 to be evaluated. Information on their HE intake was recorded, and SPTs with HE allergen extracts and with raw and hard-boiled HE were performed. Ovomucoid- and ovalbumin-specific IgE levels were also measured.*

Results: *At the first (2.5-year) and second (7-year) follow-ups, 87.5% of the children in the OIT-G who tolerated raw HE were still tolerant, whereas the children in the C-G were significantly less tolerant. Overall, cutaneous sensitivity to HE significantly decreased after the 6-month desensitization period and at both follow-ups with regard to the OIT-G but not with regard to the C-G. A significant reduction in serum ovomucoid- and ovalbumin-specific IgE levels was seen in both the OIT-G and the C-G.*

Conclusion: *Clinical raw HE tolerance induced by OIT persists over time. Negativization of SPTs could be considered a more reliable prognostic indicator of clinical tolerance to raw HE than the reduction in specific-HE IgE levels. Raw-HE OIT would seem to be a promising method to treat HE allergy.*

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The definitive treatment of food allergy is controversial and, even if the practical approach to treating adverse reactions to foods is mainly to avoid the offending item,[1] oral immunotherapy (OIT) for food allergy, otherwise known as oral food desensitization, is a frequently used regimen in experimental settings,[2–4] as in the case of OIT to hen's egg (HE).[5–18] Elimination diets for common foods, however, may pose logistic difficulties, sometimes associated with psychological problems. Moreover, children and their families are often concerned about the possibility of inducing (severe) reactions after consuming, sometimes inadvertently, small amounts of the offending food.[6] Although many children outgrow their food allergies, some children persist with their sensitivity, and the more persistent the food allergy is over time, the smaller the probability of achieving spontaneous tolerance in the short term.[19–21]

For these reasons, we performed two different OIT trials, one with cow's milk[22] and one with raw HE,[14] to attempt to induce oral clinical tolerance in children in whom the occurrence of spontaneous oral tolerance would be unlikely (or, in whom, if it did occur, would have taken a long time to reach), and/or when there was a risk of severe reactions. Our controlled protocol for raw HE OIT[14] directed at desensitizing a group of children with mild-to-severe IgE-mediated HE allergy (HEA) over a period of 6 months by introducing increasing daily doses of raw HE by using a very gradual method of reintroduction,[23] was fully successful in 80% of the cases (8/10 children) and partially successful in 10% of the cases (1/10 children), which was of one child who was able to tolerate 2 mL/day of raw HE, thus reducing the risk of severe reactions after possible inadvertent introduction of HE. The protocol failed in 10% of the children (1/10). This study was the follow-up at 2.5 and 7 years of a this earlier study[14] and the objectives were to investigate the following: (a) the long-term effectiveness of OIT to raw HE, (b) the long-

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term safety of our desensitization protocol, (c) the change in cutaneous sensitivity to HE proteins by means of SPTs, (d) the course of the serum-specific HE IgE levels, and (e) the prognostic value of SPTs and the serum-specific HE IgE levels.

Because at the end of OIT it is virtually impossible from a clinical point of view to distinguish between "desensitization" (the ability to ingest a food without reactions, provided that the food is eaten regularly) from "true food tolerance" (a state of total clinical and immunologic unresponsiveness to a food allergen independently from the rate of administration), we provided the definition of some terms used in this article. We adopted the term "sustained unresponsiveness,"[24] defined as the ability to consume the food without clinical symptoms after a period of OIT and subsequent avoidance of food consumption for a certain time (generally in terms of days or weeks). We used the term "sustained clinical food tolerance" (in contrast to "true food tolerance") at 2.5 years and at 7 years to indicate those children who had eaten *ad libitum* raw and/or cooked HE without symptoms and with various periods of sustained unresponsiveness. Also, we used the terms "to tolerate" and "tolerant" only in the sense "to eat a food without symptoms," so not necessarily implying the concepts of "true tolerance" or "sustained unresponsiveness" or "sustained clinical food tolerance."

METHODS

Clinical Subjects

All children with mild-to-severe IgE-mediated HEA, according to the severity classification of Clark and Ewan,[25] who had participated in the initial study, 10 in the OIT group (OIT-G) and 10 in the control group (C-G), were called back twice: after 2.5 years ± 4 months, and after of 7 years ± 11 months (Tables 1 and 2). At the end of the desensitization protocol (Table 3),[14] the parents were advised not to discontinue the free daily intake of raw HE, cooked HE, and products that contained HE (given *ad libitum*) to maintain the effects of the OIT. Moreover, the importance of always having emergency therapy at hand was advised. During the follow-up visits, a structured interview was used to record the clinical history, which detailed sex, age, family and personal history of allergies, and symptoms over the past years. Moreover, the parents were asked whether the children who were totally or partially desensitized were still consuming raw and/or cooked HE. Data on safety and adverse reactions to HE were also elicited. Our hospital's ethics committee approved the procedure, and informed consent was obtained from parents on behalf of all the study subjects.[14]

Skin-Prick and Prick-by-Prick Tests. At both follow-ups, all the children underwent skin-prick tests (SPT) with common, commercial inhalant allergens and commercial HE allergens (HE white and HE yolk) (Lofarma, Milan, Italy). In addition, SPTs or prick-by-prick tests were performed directly with HE (raw HE white and HE yolk; and HE white and HE yolk that had been boiled at 100°C for 10 minutes). The tests were read as previously described.[14]

Oral Food Challenges. With regard to the OIT-G, at the first and second follow-ups, oral food challenge (OFC) with raw HE was not required because the majority of the children who had undergone oral raw HE desensitization were still consuming raw and cooked HE at least once a week. The two patients for whom OIT failed declined the OFC because of a convincing history (see the Results section). With regard to the C-G, at the first (2.5-year) study follow-up, 5 of 10 children underwent OFC with raw HE. The other 4 of 10 children did not undergo OFC because OIT with raw HE had been performed (the data for these children were removed from statistical analysis). The last child in this group did not undergo OFC because he was still tolerant to raw HE. At the second (7-year) follow-up period, no OFCs were performed because 6 of 10 patients declined to be challenged due to a convincing history or personal reasons, 3 of 10 were already tolerant to raw HE; 1 patient dropped out (see the Results section).

Blood Samples. Venous blood samples were collected and stored at −20°C at the start and the end of the desensitization protocol (or, in the C-G, after 6 months) and after mean period of 2.5 and 7 years (two follow-ups). For each serum sample (including the samples obtained from the original study), ovomucoid (Gal d 1) and ovalbumin (Gal d 2) specific IgE levels were determined with ImmunoCAP (Thermo Scientific, Vienna, Austria) in accordance with the producer's manual. When necessary, a 1:10 serum dilution was performed to assay samples with IgE-specific concentrations >100 kU/L.

Statistical Analysis

Follow-up SPT results and ImmunoCAP values were compared with those at the start of the desensitization protocol, the "pre" data of the previous study,[14] to assess the evolution in an intention-to-treat analysis. Wilcoxon matched pairs signed rank test was used and ImmunoCAP values were log-transformed to reduce the effect(s) of outliers' weight. To determine whether there were significant differences between the expected and the observed frequencies in tolerance induction between the OIT-G and C-G, the χ^2 test was used. In determining

Table 1 **Patients who underwent OIT with raw HE (OIT-G): Symptoms after DBPCFCs with raw HE or after accidental ingestion of HE, and outcome of raw HE desensitization; status of OIT at the first and at the second follow-up***

OIT-G Patients, no.	Sex	Age at the Start of the Raw HE OIT (years, months)	Symptoms after DBPCFC with Raw HE (or convincing history)	Outcome of Raw HE OIT	Symptoms during Raw HE OIT (no. occurrences)	Duration of the First Follow-up (years, months)	Status of OIT at the First Follow-up	Duration of the Second Follow-up (years, months)	Allergy to HE at the Second Follow-up
01	M	8, 4/12	Cough, rhinorrhea, sneezing, palpebral edema, vomiting, abdominal pain	Tolerated raw HE#	Throat pruritus (10)	2, 11/12	Tolerated raw HE	9, 8/12	Tolerated raw HE
02	F	9, 7/12	Tongue pruritus, throat pruritus, lip edema, face urticaria	Tolerated raw HE#	Tongue pruritus (4), throat pruritus (4)	2, 11/12	Tolerated raw HE	8, 0/12	Tolerated raw HE
03	F	6, 8/12	Cough, asthma, vomiting	Tolerated raw HE#	Throat pruritus (15), cough (10)	D.O.	D.O.	D.O.	D.O.
04	M	14	Urticaria, vomiting	Partially tolerated raw HE§	Vomiting (4), abdominal pain (10), diarrhea (1)	2, 3/12	Did not tolerate raw HE (stopped consuming a low quantity of HE 1 y earlier) (convincing history)	8, 6/12	Did not tolerate raw HE (convincing history)
05	M	7, 4/12	Diffuse urticaria, pruritus, lip edema just after eating a mouthful of omelette	Did not tolerate HE (OIT failed)	Diffuse urticaria (4), pruritus (4)	2, 10/12	Did not tolerate HE	6, 9/112	Did not tolerate HE
06	F	5, 4/12	Throat pruritus, tightening of throat, vomiting, abdominal pain	Tolerated raw HE#	Cough (6), perioral dermatitis (2), slight wheezing (1)	2, 7/12	Tolerated raw HE	7, 8/12	Tolerated raw HE

Table 1 **Continued**

OIT-G Patients, no.	Sex	Age at the Start of the Raw HE OIT (years, months)	Symptoms after DBPCFC with Raw HE (or convincing history)	Outcome of Raw HE OIT	Symptoms during Raw HE OIT (no. occurrences)	Duration of the First Follow-up (years, months)	Status of OIT at the First Follow-up	Duration of the Second Follow-up (years, months)	Allergy to HE at the Second Follow-up
07	M	13, 1/12	Throat pruritus, tongue pruritus, vomiting, abdominal pain ~3 min after eating ~1 g of cooked egg	Tolerated raw HE#	Throat pruritus (2), vomiting (1), abdominal pain (1)	1, 11/12	Tolerated raw HE	7, 6/12	Tolerated raw HE
08	M	5, 6/12	Throat pruritus, tightening of throat, lip edema, perioral erythema	Tolerated raw HE¶	None	2, 4/12	Tolerated raw HE	7, 2/12	Tolerated raw HE
09	F	4, 5/12	Perioral erythema, lip edema, face erythema, eyelid edema, eye pruritus	Tolerated raw HE¶	None	2, 3/12	Tolerated raw HE	7, 6/12	Tolerated raw HE
10	F	10, 2/12	Tongue pruritus, lip edema, face urticaria, abdominal pain, diarrhea	Tolerated raw HE¶	None	2, 8/12	Tolerated raw HE	8, 6/12	Tolerated raw HE

OIT = Oral immunotherapy; HE = hen's egg; OIT-G = oral immunotherapy group; DBPCFC = double-blind, placebo controlled food challenge; D.O. = dropped out.

**See Ref. 14, Table 1 for more details about the OIT period.*

#Presented with some symptoms during the desensitization period, which occurred a short time after ingestion of raw HE and that persisted for <2 hr.

§Tolerated up to 2 mL of raw HE, but he was able to eat 15 g of cooked HE.

¶No symptoms during the desensitization period.

Table 2 **Patients of the C-G: Symptoms after DBPCFCs with raw HE at enrollment and after 6 months; status of HE allergy at the first and second follow-ups**

C-G Patients, no.	Sex	Age at the Enrollment (years, months)	Symptoms after DBPCFC with Raw HE (or convincing history)	Symptoms after DBPCFC with Raw HE Performed 6 Months after the Enrollment	Dose that Evoked Symptoms, mL	Duration of the First Follow-up (years, months)	Allergy to HE at the First Follow-up	Duration of the Second Follow-up (years, months)	Allergy to HE at the Second Follow-up
11	M	14, 10/12	Throat pruritus, lips edema, cough, vomiting, abdominal pain, general weakness 5 min after eating ice cream with egg (2 occurrences)	Throat pruritus	25.0	2, 8/12	Did not tolerate raw HE (positive OFC result with raw HE); tolerated cooked HE (negative OFC result with cooked HE)	6, 3/12	Did not know whether he tolerated raw HE (declined OFC with raw HE); tolerated cooked HE
12	F	10, 5/12	Throat pruritus, tightening of throat, vomiting, abdominal pain	Throat pruritus, vomiting, abdominal pain	5.0	2, 9/12	Did not tolerate raw HE (underwent OIT with raw HE and the OIT failed)	6, 4/12	Did not tolerate raw HE (convincing history); tolerated cooked HE
13	M	5, 6/12	Throat pruritus, nasal pruritus, sneezing, rhinorrhea, stomach ache, vomiting	Throat pruritus, sneezing, rhinorrhea, stomach ache, vomiting	3.0	2, 2/12	Tolerated raw HE (underwent, successfully, OIT with raw HE)	6, 9/12	Tolerated raw HE (previous OIT with raw HE)
14	F	5, 9/12	Hoarseness, nasal pruritus, sneezing, rhinorrhea, stomach ache, vomiting	Hoarseness, sneezing, stomach ache, vomiting, abdominal pain	5.0	2, 2/12	Did not tolerate raw HE (underwent OIT with raw HE and the OIT failed)	5, 10/12	Did not tolerate raw HE (convincing history)

Table 2 **Continued**

C-G Patients, no.	Sex	Age at the Enrollment (years, months)	Symptoms after DBPCFC with Raw HE (or convincing history)	Symptoms after DBPCFC with Raw HE Performed 6 Months after the Enrollment	Dose that Evoked Symptoms, mL	Duration of the First Follow-up (years, months)	Allergy to HE at the First Follow-up	Duration of the Second Follow-up (years, months)	Allergy to HE at the Second Follow-up
15	M	9, 0/12	Diffuse urticaria	None	Tolerated 25 mL	2, 6/12	Tolerated raw HE	6, 0/12	Tolerated raw HE
16	M	12, 10/12	Throat pruritus, tightening of throat, lip edema, mouth erythema, abdominal pain	Throat pruritus, lip edema, vomiting, abdominal pain	5.0 mL	2, 8/12	Did not tolerate raw HE (positive OFC result with raw HE)	6, 9/12	Did not tolerate raw HE; did not tolerate cooked HE (convincing history) (declined OFC)
17	M	4, 7/12	Lip erythema and edema, stomach ache, vomiting	Lip erythema and edema, stomach ache, vomiting	5.0	2, 4/12	Tolerated raw HE (underwent, successfully, OIT with raw HE)	6, 0/12	Tolerated raw HE (previous OIT with raw HE)
18	M	7, 6/12	Sneezing, tongue pruritus, stomach ache, vomiting	Tongue pruritus, stomach ache, vomiting	5.0	2, 7/12	Did not tolerate raw HE (positive OFC with raw HE)	D.O.	D.O.
19	M	4, 7/12	Perioral edema, perioral erythema, vomiting	Perioral edema, vomiting	5.0	2, 7/12	Did not tolerate raw HE (positive OFC result with raw HE)	7, 10/12	Did not tolerate raw HE (convincing history) (he declined OFC); tolerated cooked HE

Table 2 Continued

C-G Patients, no.	Sex	Age at the Enrollment (years, months)	Symptoms after DBPCFC with Raw HE (or convincing history)	Symptoms after DBPCFC with Raw HE Performed 6 Months after the Enrollment	Dose that Evoked Symptoms, mL	Duration of the First Follow-up (years, months)	Allergy to HE at the First Follow-up	Duration of the Second Follow-up (years, months)	Allergy to HE at the Second Follow-up
20	F	14,8/12	Throat pruritus, lips edema, vomiting, abdominal pain, hypotensive symptoms after eating a small amount of food that contained cooked egg (4 occurrences)	Throat pruritus, lip edema, vomiting, abdominal pain	3.0	2,2/12	Did not tolerate raw HE (positive OFC with raw HE)	6, 6/12	Did not tolerate raw HE; did not tolerate cooked HE (convincing history) (declined OFC)

C-G = Control group; DBPCFC = double-blind placebo controlled food challenge; HE = hen's egg; OIT = oral immunotherapy; OFC = oral food challenge; D.O. = dropped out.

the fairness of the comparison group of patients examined at various times of follow-up, a power analysis was performed by using the GPower 3.1 software.

RESULTS

At the first (2.5-year) follow-up period, 19 of 20 children who had previously participated in the desensitization protocol were evaluated; 1 child (5%) in the OIT-G had dropped out. At the second (7-year) follow-up, 18 of the 20 children were evaluated; 1 child (5%) in the C-G had dropped out. In both cases of the children who dropped out, it was not possible to contact the families.

Outcome of the OIT Follow-ups

Overall, at the end of the initial study, 8 of 10 children (80%) in the OIT-G tolerated the daily intake of 25 mL of raw HE over a 6-month period; 1 child (10%) tolerated up to 2 mL/day, whereas desensitization failed for another child (10%) (Tables 1 and 2). Six months after enrollment, only two children in the C-G (20%) could tolerate raw HE. At the first and second follow-ups, 7 of 8 children (87.5%) of the OIT-G who tolerated raw HE at the end of the previous study were still tolerant, *i.e.,* raw and/or cooked HE could be eaten at least one time a week without symptoms. The other child who was tolerant at the end of the desensitization protocol dropped out. The child who could tolerate only 2 mL of raw HE at the end of the desensitization protocol (patient 4) stopped consuming this low quantity after ~1 year. As a consequence, he became nontolerant (convincing history) and tested positive to raw HE at both the first and second follow-ups.

All the subjects in the C-G underwent a second double-blind, placebo controlled food challenge 6 months after enrollment, and all but two children (patients 11 and 15) tested positive. At the first follow-up, patient 11 could tolerate cooked HE (negative OFC) but could still not tolerate raw HE (positive OFC). The other patient who was spontaneously tolerant (patient 15) tolerated raw HE both at the first and second follow-ups. Of the subjects in the C-G with HEA, two (patients 13 and 17) were able to tolerate raw HE (eaten at least one time a week) both at the first and second follow-up periods because they had successfully undergone OIT to raw HE. In addition, two subjects in the C-G (patients 12 and 14) could not tolerate raw HE either at the first or second follow-up periods even though they had undergone OIT with raw HE. The other four subjects in the C-G (patients 16, 18, 19, and 20) could not tolerate raw HE at the first and second follow-up periods (with the exception of subject 18, who dropped out at the second follow-up).

As can be seen in Table 4, in comparison with the C-G, subjects in the OIT-G were significantly more

Table 3 **Schedule of oral raw HE desensitization***

Day	Diluted HE Drops, no. (HE dilution)	Day	Diluted HE Drops, no. (HE dilution)	Day	Diluted HE Drops, no. (HE dilution)	Day	Not Diluted HE Drops, no.	Day	Not Diluted HE Drops, no.	Day	Not Diluted HE, mL	Day	Not Diluted HE, mL
Start	1 (:100)	31	15 (:100)	62	22 (:10)	74	6	105	27	127	3.5	158	10.5
1	1 (:100)	32	16 (:100)	63	23 (:10)	75	7	106	28	128	3.5	159	11.0
2	1 (:100)	33	17 (:100)	64	26 (:10)	76	7	107	29	129	3.5	160	11.5
3	1 (:100)	34	19 (:100)	65	28 (:10)	77	8	108	30	130	3.5	161	12.0
4	1 (:100)	35	21 (:100)	66	30 (:10)	78	9	109	31	131	3.5	162	12.5
5	2 (:100)	36	23 (:100)	67	33 (:10)	79	9	110	33	132	4.0	163	13.0
6	2 (:100)	37	25 (:100)	68	36 (:10)	80	10	111	34	133	4.0	164	13.0
7	2 (:100)	38	27 (:100)	69	39 (:10)	81	11	112	35	134	4.0	165	13.5
8	2 (:100)	39	29 (:100)	70	43 (:10)	82	11	113	36	135	4.5	166	14.0
9	2 (:100)	40	3 (:10)	71	47 (:10)	83	11	114	38	136	4.5	167	14.5
10	2 (:100)	41	3 (:10)	72	51 (:10)	84	12	115	39	137	4.5	168	15.0
11	3 (:100)	42	4 (:10)	73	56 (:10)	85	12	116	41	138	5.0	169	16.0
12	3 (:100)	43	4 (:10)			86	13	117	43	139	5.0	170	16.5
13	3 (:100)	44	5 (:10)			87	13	118	44	140	5.0	171	17.0
14	3 (:100)	45	5 (:10)			88	14	119	46	141	5.5	172	18.0
15	4 (:100)	46	5 (:10)			89	14	120	48	142	5.5	173	18.5
16	4 (:100)	47	6 (:10)			90	15	121	50	143	6.0	174	19.0
17	4 (:100)	48	6 (:10)			91	16	122	52	144	6.0	175	20.0
18	5 (:100)	49	7 (:10)			92	16	123	54	145	6.5	176	21.0
19	5 (:100)	50	8 (:10)			93	17	124	56	146	6.5	177	21.5
20	6 (:100)	51	8 (:10)			94	18	125	58	147	7.0	178	22.0
21	6 (:100)	52	9 (:10)			95	18	126	60	148	7.0	179	23.0
22	7 (:100)	53	10 (:10)			96	19			149	7.5	180	24.0
23	7 (:100)	54	11 (:10)			97	20			150	7.5	181	25.0
24	8 (:100)	55	12 (:10)			98	20			151	8.0		
25	9 (:100)	56	13 (:10)			99	21			152	8.0		
26	10 (:100)	57	14 (:10)			100	22			153	8.5		
27	10 (:100)	58	15 (:10)			101	23			154	9.0		
28	11 (:100)	59	17 (:10)			102	24			155	9.0		
29	12 (:100)	60	18 (:10)			103	25			156	9.5		
30	13 (:100)	61	20 (:10)			104	26			157	10.0		

HE = Hen's egg.
**Diluted HE drops, drops, and milliliters refer to mixed raw HE white and yolk.*

Table 4 **Comparison between children who were raw HE tolerant in the OIT-G and the C-G at the end of the desensitization protocol (6 mo), at the first (2.5 y), and the second (7 y) follow-up (χ^2 test)**

	Children Raw HE Tolerant, no./total no.								
	OIT Protocol After 6 mo			At the First Follow-up			At the Second Follow-up		
	OIT-G	C-G	*P*	OIT-G	C-G	*p*	OIT-G	C-G	*p*
A*	8/10	2/10	<0.01	7/9	3/10	<0.05	7/9	3/9	N.S.
B#	8/10	2/10	<0.01	7/9	1/8	<0.01	7/9	1/7	<0.01

HE = Hen's egg; OIT-G = oral immunotherapy group; C-G = control group; OIT = oral immunotherapy; N.S. = not significant.

**For statistical purposes, all the children in the C-G were considered.*

#For statistical purposes, the children in the C-G who became tolerant to raw HE due to subsequent successful OIT with raw HE were not considered.

tolerant at the end of the desensitization period ($p < 0.01$), as previously described,[14] and significantly more tolerant at the first (2.5-year) follow-up period ($p < 0.05$) (Table 4, row A). In contrast, there were no differences between groups at the second (7-year) follow-up period (Table 4, row A). However, if the two subjects in the C-G who were successfully submitted to OIT with raw HE were included in the analysis, then the differences between the OIT-G and the C-G became significant at the second (7-year) follow-up ($p < 0.01$) as well (Table 4, row B).

Safety Data and Adverse Reactions to Raw HE

None of the subjects required the use of adrenaline or emergency care during the follow-up periods.

SPTs and Prick-by-Prick Tests

All the subjects underwent SPTs and Prick-by-Prick Tests. The comparison among the SPTs performed at the beginning, at the end of the study, and at the two follow-up periods did not reveal significant differences between the two groups, with the exception of house-dust mite, for which the OIT-G had a higher positive reading at the first and second follow-up periods ($p = 0.02$), and for cat dander, for which the C-G had a higher positive reading at the second follow-up ($p = 0.04$) (data not shown). SPT results to HE decreased significantly over time only in the OIT-G. In particular, cutaneous positivity significantly decreased for egg white (commercial extract, raw and hard-boiled), and raw yolk but not for commercial and hard-boiled yolk, whereas there was no significant change in cutaneous sensitivity to HE in the C-G (except for two who became tolerant to raw HE because they had been successfully submitted to OIT) (Wilcoxon matched pairs test) (Table 5).

Specific IgE Values (ImmunoCAP) for Gal d 1 and Gal d 2

As previously reported,[14] the differences between specific IgE values for Gal d 1 before and after the 6-month desensitization period in the OIT-G were significant ($p = 0.01$, Wilcoxon matched pairs test), whereas the IgE values for Gal d 2 did not show significant differences (Table 6). The specific IgE values for both Gal d 1 and Gal d 2 decreased at the first and second follow-up periods compared with the values at the start of the protocol, in both the OIT-G and the C-G. For this study, all the samples (including those of the previous study) (14) were retested with ImmunoCAP.

DISCUSSION

This article reported two consecutive follow-ups (at 2.5 and 7 years) to our previous study performed in 20 children with mild-to-severe IgE-mediated HEA.[14] To our knowledge, this was the first long-term follow-up study of subjects with HEA allergy who underwent a controlled protocol for OIT at home. Overall, only two children dropped out. Four children of the C-G underwent OIT with raw HE during the follow-up, but only two of them were able to tolerate raw HE.

Persistence of the Effect of OIT

Overall, at the end of the previous study,[14] 8 of 10 children of the OIT-G tolerated 25 mL of raw HE over a 6-month period, whereas 1 child tolerated only 2 mL of raw HE. The desensitization protocol failed for one child. At both the first and second follow-ups, all but one of the children in the OIT-G (patient 3 [who dropped out]) presented tolerance to raw HE, thus indicating a substantial persistence of the effect of OIT (Tables 1, 2, and 4). This point needs some clarification. Because our subjects were accustomed to eating raw and/or cooked HE at least once a week without symptoms, we could speak of sustained unresponsiveness of

Table 5 **Outcome of the SPTs with HE egg-white and yolk (commercial extracts, raw and hard boiled) at the beginning (pre) and at the end (post) of the desensitization period; after the first and the second follow-ups in the desensitization group; and at the beginning, after 6 months, and after the first and the second follow-ups in the control group**

	OIT-G							C-G						
	Pre, mm	**Post, mm**	**First Follow-up, mm**	**Second Follow-up, mm**	***p*#**	***p*§**	***p*¶**	**Pre, mm**	**Post, mm**	**First Follow-up, mm**	**Second Follow-up, mm**	***p*#**	***p*§**	***p*¶**
Egg white (commercial extract)					0.01	0.02	N.S.					N.S.	N.S.	N.S.
Average ± SD	5.06 ± 1.47	3.31 ± 1.67	3.71 ± 1.58	2.38 ± 2.72				4.30 ± 2.11	5.40 ± 2.17	3.95 ± 1.50	2.72 ± 2.22			
Median (range)	5.5 (2.5–7.0)	3.5 (1.0–5.0)	3.0 (2.0–6.0)	2.0 (0.0–7.0)				4.0 (1.0–7.0)	6.0 (1.0–8.0)	4.0 (1.0–6.0)	3.0 (0.0–6.5)			
Egg yolk (commercial extract)					N.S.	N.S.	N.S.					N.S.	N.S.	N.S.
Average ± SD	3.25 ± 2.05	2.25 ± 1.83	2.43 ± 0.98	2.79 ± 0.95				3.30 ± 1.70	3.70 ± 1.49	3.35 ± 1.18	3.17 ± 1.03			
Median (range)	4.0 (1.0–5.0)	2.5 (1.0–5.0)	2.5 (1.0–4.0)	2.5 (1.5–4.5)				3.5 (1.0–5.0)	4.0 (1.0–5.0)	3.0 (1.5–5.5)	3.0 (1.5–5.0)			
Egg white (raw)					0.01	0.02	N.S.					N.S.	N.S.	N.S.
Average ± SD	8.25 ± 1.16	6.25 ± 1.04	5.86 ± 1.46	4.13 ± 3.75				8.10 ± 3.18	7.60 ± 2.59	6.4 ± 2.27	5.33 ± 3.70			
Median (range)	8.0 (6.0–10.0)	6.5 (4.0–7.0)	6.0 (3.0–7.0)	3.5 (0.0–11.0)				8.5 (1.0–12.0)	7.5 (3.0–12.0)	6.5 (2.0–9.0)	6.0 (0.0–11.5)			
Yolk (raw)					N.S.	0.02	0.02					N.S.	N.S.	N.S.
Average ± SD	4.75 ± 2.06	3.88 ± 1.96	2.50 ± 1.38	1.75 ± 1.75				4.00 ± 2.62	3.80 ± 1.69	2.67 ± 1.58	2.56 ± 2.07			
Median (range)	4.5 (1.0–8.0)	3.5 (2.0–8.0)	2.5 (0.0–4.0)	2.0 (0.0–5.0)				4.0 (1.0–9.0)	3.5 (1.0–6.0)	2.5 (0.0–5.5)	2.0 (0.0–6.0)			
Egg white (hard boiled)					N.S.	N.S.	0.03					N.S.	N.S.	N.S.
Average ± SD	6.63 ± 3.34	5.50 ± 2.67	4.43 ± 1.99	2.75 ± 2.25				5.25 ± 2.46	5.50 ± 2.88	4.38 ± 2.33	2.61 ± 2.15			
Median (range)	7.0 (1.0–10.0)	5.5 (1.0–12.0)	5.0 (1.0–7.0)	3.0 (0.0–7.0)				5.5 (1.0–10.0)	6.0 (1.0–10.0)	5.5 (0.0–4.5)	3.0 (0.0–6.0)			
Yolk (hard boiled)					N.S.	N.S.	N.S.					N.S.	N.S.	N.S.
Average ± SD	3.75 ± 2.71	3.38 ± 1.92	2.00 ± 1.53	1.13 ± 1.25				1.65 ± 1.06	1.60 ± 1.07	1.60 ± 1.05	1.06 ± 1.01			
Median (range)	3.5 (1.0–8.0)	3.0 (1.0–7.0)	2.5 (0.0–4.0)	1.0 (0.0–3.0)				1.0 (1.0–4.0)	1.0 (1.0–4.0)	1.3 (0.0–3.5)	1.0 (0.0–3.0)			

SPT = Skin-prick test; HE = hen's egg; OIT-G = oral immunotherapy group; C-G = control group; N.S. = not significant; SD = standard deviation; OIT = oral immunotherapy. The cutaneous sensitivity significantly decreased for egg white (commercial extract, raw and hard boiled), and raw yolk but not for commercial and hard-boiled yolk; no significant change was observed in the children of the CG (but two who became tolerant to raw HE because they were successfully submitted to OIT) (matched-pairs Wilcoxon test); no significant differences were observed in the C-G.

#Pre vs post.

§Pre vs first follow-up.

¶Pre vs second follow-up.

Table 6 **Outcome of the specific serum IgEs (ImmunoCAP) to ovomucoid (Gal d 1) and ovalbumin (Gal d 2) at the beginning (pre), at the end (post) of the desensitization period, and at the first and second follow-ups#**

	OIT-G						C-G					
	Pre, kUA/L	Post, kUA/L	First Follow-up, kUA/L	Second Follow-up, kUA/L	*p*§	*p*¶	*p*‖	Pre, kUA/L	Post, kUA/L	First Follow-up, kUA/L	Second Follow-up, kUA/L	*p*§	*p*¶	*p*‖
Gal d 1					0.01	0.02	0.01					N.S.	0.01	0.02
Average ± SD	22.64 ± 48.04	13.89 ± 30.62	13.92 ± 33.28	13.30 ± 33.47				5.31 ± 6.31	5.99 ± 7.11	3.14 ± 4.46	2.37 ± 4.34			
Median (range)	7.34 (1.20–150.00)	1.45 (0.57–95.00)	1.22 (0.01–101.00)	0.27 (0.01–101.00)				2.40 (0.53–16.05)	2.61 (0.45–18.45)	1.20 (0.01–13.00)	0.20 (0.02–12.58)			
Gal d 2					N.S.	0.01	0.01					N.S.	0.01	0.02
Average ± SD	28.36 ± 46.25	22.38 ± 28.81	13.05 ± 32.03	12.56 ± 31.08				11.12 ± 11.33	12.30 ± 11.76	1.81 ± 1.89	1.19 ± 1.03			
Median (range)	9.98 (7.08–150.00)	11.25 (5.01–95.15)	0.90 (0.41–97.00)	0.92 (0.10–94.00)				6.90 (1.95–36.57)	8.34 (1.87–38.15)	1.35 (0.01–5.73)	1.11 (0.16–2.80)			

IgE = Immunoglobulin E; OIT-G = oral immunotherapy group; C-G = control group; N.S. = not significant; SD = standard deviation. In the OIT-G, the differences between specific IgE values for Gal d 1 before and after the 6-mo desensitization period were significant (p = 0.01, Wilcoxon matched pairs test), but the IgE values for Gal d 2 did not show any significant difference; with regard to the two follow-ups, both specific IgE values for Gal d 1 and Gal d 2 decreased both in the OIT-G and in the C-G at both the first and second follow-ups compared with the values at the start of the protocol.

#Note: If ImmunoCAP values were >100 kUA/L, then the samples were diluted 1:10 and retested.

§Pre vs post.

¶Pre vs first follow-up.

‖Pre vs second follow-up.

at least 7 days. Even when considering the long time between the first study and the two follow-ups, it was not known whether our subjects reached HE true tolerance (*i.e.*, a permanent and definitive capacity to ingest HE without elicitation of symptoms independent of the rate of ingestion) or whether they were simply long-term desensitized. For this reason, we spoke of sustained clinical food tolerance that, from a practical point of view, resolved the problem for which these patients came to our attention. With regard to the C-G, 2 of 10 patients could spontaneously tolerate raw HE after the 6-month period of the previous study.[14] Four children agreed to be submitted to HE-OIT with raw HE, but only two of the four children could tolerate raw HE. If we added the two children who were not tolerant to the others of the C-G, then this indicated a substantial, significant nontolerance in those who were not submitted to OIT (Table 4).

The natural history of HEA is for the majority of children to undergo spontaneous resolution over time, but only a few studies have reported the long-term course of HEA, and most studies did not use raw HE but only cooked HE.[26–32] In the study of Savage *et al.*,[26] the resolution of HEA was assumed if patients could tolerate one whole cooked HE and did not consider tolerance to raw HE. By using this criterion, the Kaplan-Meier analysis predicted resolution in 4% of patients by the age of 4 years, 12% by the age of 6, 37% by the age of 10 years, and 68% by the age of 16 years. Sicherer *et al.*[27] enrolled 213 children with HEA, ages 3–15 months, and found that HEA resolved in 105 children (49.3%), at a median age of 72 months, but, even in this case, the children were considered HE tolerant if they could ingest scrambled egg or French toast, which thus raised doubts as to whether they could really tolerate raw HE. In a retrospective study, which included 106 children <2 years old with atopic dermatitis and HEA, Kim *et al.*,[28] concluded that 41% of the children had developed tolerance to cooked HE by the age of 3 years, and 60% by the age of 5 years. Ford and Taylor[29] observed 25 children with HEA confirmed by double-blind challenge (probably administering raw HE) and found that HEA was resolved in 44% of children after 2.5 years. Boyano-Martinez *et al.*[30] studied 58 children with HEA, ages <2 years at diagnosis, and established that the average time to tolerance to raw HE was 35 months, whereas tolerance was reached in 66% of the patients after a 5-year follow-up.

In this context, we compared our data only with the studies in which raw HE was considered.[29,30] Roughly, we can say that, in the C-G of our population (not considering the two children who were submitted to OIT during the first follow-up period), only 12.5% could tolerate raw HE after a mean of 2.5 years and 37.5% after a mean of 7 years, which indicated a worse prognosis of raw HEA with respect to previous stud-

ies.[29,30] At any rate, in our opinion, we changed the natural history of HEA in most of our patients both at the first and second follow-ups by reducing the natural time frame for clinical raw HE tolerance, which thus enabled our population to eat cooked HE freely as well. The contrary cannot be validated by those studies in which only cooked HE was considered.[26–28]

Effect of Stopping Consumption of Raw HE After OIT (sustained unresponsiveness)

Overall, during the follow-up time frame, the majority of the children in the OIT-G could interrupt raw HE intake for 7 days without any symptoms once raw HE was consumed again. The possibility that OIT can induce a long-lasting or even permanent tolerance (true tolerance) is still debated, and it is conceivable that not all the patients who submitted to OIT will achieve true tolerance. Indeed, it is probable that some of them maintain tolerance only if the food is consumed on a regular basis.[6,33] In this context, Caminiti *et al*.[17] recently reported that, of 16 children ages 4–11 years who were desensitized to dehydrated HE within 4 months and who then underwent a 3-month period of HE avoidance, only 31% remained tolerant to the oral challenge with raw HE. We do not know what would have happened if our patients had interrupted consuming raw HE for 3 months, but, in our opinion, in real life, it is not usual to interrupt a common, regularly consumed food for ≥3 months. At any rate, as a consequence of our experience, we advise not to stop taking the food for >1 week. Moreover, we empirically maintain that the more severe the case of HEA allergy, the more regular and frequent the food intake should be during the maintenance period.

Long-Term Safety

During the two follow-up periods, none of the children of the OIT-G needed to use adrenaline or presented symptoms that had to be controlled by oral antihistamines. Due to the limited number of children in the OIT-G, we could not extend these positive findings to all children submitted to OIT. In particular, we had to take into account conditions that could reduce oral tolerance at least temporarily, such as hard physical exercise within 2 hours of a dose, respiratory tract febrile infections, gastroenteric infections, gastrolesive drugs, poorly controlled asthma, seasonal pollen allergy, and menses.[34–37]

Course of SPTs, Specific HE IgE Values, and Prognostic Factors

In our population, the cutaneous sensitivity (SPTs) for all the HE allergens tested (except commercial and hard-boiled yolk) significantly decreased over time only in the OIT-G. Indeed, there was a significant decrease from the "presituation" and at the end of the desensitization period ("postsituation") and with respect to the first and second follow-ups (Table 5). However, with regard to the serum-specific IgE levels, apart from the not significant difference during in the initial 6-month observation, we noted a significant decrease both at the first and second follow-ups (Table 6). This was not surprising in that this significant decrease indicated only a minor reactivity with respect to the initial condition and that an ImmunoCAP level of >0.35 kU indicated, in each case, the possibility of a reaction to raw HE. Globally, we can say that if we put the positivity cutoff at 3 mm for SPTs and at 0.35 kU for ImmunoCAP, in our experience, SPTs were more reliable when tracking the status of sensitization to raw HE.

CONCLUSION

OIT is a promising method for treatment of HEA. Follow-up evaluations after 2.5 and 7 years of 20 children previously studied indicated that, overall, 8 of 10 children in the OIT-G were still consuming raw HE and cooked HE freely. However, the subjects in the C-G overall could not tolerate raw HE either at the first or the second follow-up period, with the exception of two children who successfully underwent OIT with raw HE. Our protocol, even if time-consuming, offered the advantage that it could be performed at home. Moreover, it was an overall success and was safe. Nevertheless, due to the small number of children enrolled in the protocol, we maintain that children submitted to OIT need be monitored, even after the end of the protocol, and that only appropriately trained staff should use this methodology.

Although it is not possible to conclude that, in a variable number of cases, tolerance to raw HE is true immunologic tolerance, we maintain that sustained clinical food tolerance lasts as long as the offending food is regularly consumed. In general, it was difficult to evaluate whether the good prognosis of the OIT-G was due to the natural course of HEA alone or was the result of the OIT performed 2.5 to 7 years previously. However, we demonstrated that most of the children in the C-G still did not tolerate raw HE at the follow-ups, and so we believed that, in the case of most of our children in the OIT-G, we at least reduced the natural time frame for clinical tolerance. Conversion of SPTs to negative responses to HE proteins, rather than the reduction of specific serum HE IgE levels, could be considered a better prognostic indicator of the development of sustained clinical food tolerance to raw HE.

REFERENCES

1. Sampson HA, Aceves S, Bock SA, et al. Food allergy: A practice parameter update—2014. J Allergy Clin Immunol 134:1016–1025, 2014.

2. O'Keefe AW, De Schryver S, Mill J, et al. Diagnosis and management of food allergies: New and emerging options: A systematic review. J Asthma Allergy 7:141–164, 2014.
3. McGowan EC, and Wood RA. Sublingual (SLIT) versus oral immunotherapy (OIT) for food allergy. Curr Allergy Asthma Rep 14:486, 2014.
4. Sato S, Yanagida N, Ogura K, et al. Immunotherapy in food allergy: Towards new strategies. Asian Pac J Allergy Immunol 32:195–202, 2014.
5. Morisset M, Moneret-Vautrin DA, Guenard L, et al. Oral desensitization in children with milk and egg allergies obtains recovery in a significant proportion of cases. A randomized study in 60 children with cow's milk allergy and 90 children with egg allergy. Eur Ann Allergy Clin Immunol 39:12–19, 2007.
6. Staden U, Rolinck-Werninghaus C, Brewe F, et al. Specific oral tolerance induction in food allergy in children: Efficacy and clinical patterns of reaction. Allergy 62:1261–1269, 2007.
7. Buchanan AD, Green TD, Jones SM, et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. J Allergy Clin Immunol 119:199–205, 2007.
8. Burks AW, and Jones SM. Egg oral immunotherapy in nonanaphylactic children with egg allergy: Follow-up. J Allergy Clin Immunol 121:270–271, 2008.
9. Itoh N, Itagaki Y, and Kurihara K. Rush specific oral tolerance induction in school-age children with severe egg allergy: One year follow up. Allergol Int 59:43–51, 2010.
10. Vickery BP, Pons L, Kulis M, et al. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. Ann Allergy Asthma Immunol 105:444–450, 2010.
11. García Rodríguez R, Urra JM, Feo-Brito F, et al. Oral rush desensitization to egg: Efficacy and safety. Clin Exp Allergy 41:1289–1296, 2011.
12. Burks AW, Jones SM, Wood RA, et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med 367:233–243, 2012.
13. Ojeda P, Ojeda I, Rubio G, and Pineda F. Home-based oral immunotherapy protocol with pasteurized egg for children allergic to hen's egg. Isr Med Assoc J 14:34–39, 2012.
14. Meglio P, Giampietro PG, Carello R, et al. Oral food desensitization in children with IgE-mediated hen's egg allergy: A new protocol with raw hen's egg. Pediatr Allergy Immunol 24:75–83, 2013.
15. Dello Iacono I, Tripodi S, Calvani M, et al. Specific oral tolerance induction with raw hen's egg in children with very severe egg allergy: A randomized controlled trial. Pediatr Allergy Immunol 24:66–74, 2013.
16. Vazquez-Ortiz M, Alvaro M, Piquer M, et al. Baseline specific IgE levels are useful to predict safety of oral immunotherapy in egg-allergic children. Clin Exp Allergy 44:130–141, 2014.
17. Caminiti L, Pajno GB, Crisafulli G, et al. Oral Immunotherapy for egg allergy: A double-blind placebo-controlled study, with post desensitization follow-up. J Allergy Clin Immunol Pract 3:532–539, 2015.
18. Jones SM, Burks AW, Keet C, et al. Consortium of Food Allergy Research (CoFAR). Long-term treatment with egg oral immunotherapy enhances sustained unresponsiveness that persists after cessation of therapy. J Allergy Clin Immunol 137:1117–1127, 2016.
19. Høst A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. Pediatr Allergy Immunol 5(suppl.):1–36, 1994.
20. Høst A. Clinical course of cow's milk protein allergy and intolerance. Pediatr Allergy Immunol 9:48–52, 1998.
21. Savage J, and Johns CB. Food allergy: Epidemiology and natural history. Immunol Allergy Clin North Am 35:45–59, 2015.
22. Meglio P, Bartone E, Plantamura M, et al. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 59:980–987, 2004.
23. Meglio P. Oral food desensitization: The BACH proposal for the very gradual reintroduction of a food. Curr Opin Allergy Clin Immunol 13:306–314, 2013.
24. Burks AW, Jones SM, Wood RA, et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med 367:233–243, 2012.
25. Clark AT, and Ewan PW. Food allergy in childhood. Arch Dis Child 88:79–81, 2003.
26. Savage JH, Matsui EC, Skripak JM, and Wood RA. The natural history of egg allergy. J Allergy Clin Immunol 120:1413–1417, 2007.
27. Sicherer SH, Wood RA, Vickery BP, et al. The natural history of egg allergy in an observational cohort. J Allergy Clin Immunol 133:492–499, 2014.
28. Kim J, Chung Y, Han Y, et al. The natural history and prognostic factors of egg allergy in Korean infants with atopic dermatitis. Asian Pac J Allergy Immunol 27:107–114, 2009.
29. Ford RP, and Taylor B. Natural history of egg hypersensitivity. Arch Dis Child 57:649–652, 1982.
30. Boyano-Martínez T, García-Ara C, Díaz-Pena JM, and 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 110:304–309, 2002.
31. Caubet JC, and Wang J. Current understanding of egg allergy. Pediatr Clin North Am 58:427–443, xi, 2011.
32. Tey D, and Heine RG. Egg allergy in childhood: An update. Curr Opin Allergy Clin Immunol 9:244–250, 2009.
33. Rolinck-Werninghaus C, Staden U, Mehl A, et al. Specific oral tolerance induction with food in children: Transient or persistent effect on food allergy? Allergy 60:1320–1322, 2005.
34. Caminiti L, Passalacqua G, Vita D, et al. Food-exercise-induced anaphylaxis in a boy successfully desensitized to cow milk. Allergy 62:335–336, 2007.
35. Barbi E, Longo G, Berti I, et al. Adverse effects during specific oral tolerance induction: In home phase. Allergol Immunopathol (Madr) 40:41–50, 2012.
36. Varshney P, Steele PH, Vickery BP, et al. Adverse reactions during peanut oral immunotherapy home dosing. J Allergy Clin Immunol 124:1351–1352, 2009.
37. Pajno GB, Caminiti L, Chiera F, et al. Safety profile of oral immunotherapy with cow's milk and hen egg: A 10-year experience in controlled trials. Allergy Asthma Proc 37:400–403, 2016. □