

ORIGINAL ARTICLE

Specific oral tolerance induction with raw hen's egg in children with very severe egg allergy: A randomized controlled trial

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Keyword

food allergy; hen's egg; anaphylaxis; skin prick test; end-point skin prick test; IgE; double-blind placebo-controlled food challenge; specific oral tolerance induction.

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Abstract

Background: Treatment of severe egg allergy is avoidance of hen's egg (HE) and carrying self-injectable epinephrine. Specific oral tolerance induction (SOTI) seems a promising alternative treatment. However, some aspects of SOTI are still considered experimental.

Methods: We evaluated the efficacy and safety of an original 6-month SOTI protocol in children with very severe HE allergy using raw HE emulsion. Twenty children (age range: 5–11 yr) were randomized equally into a SOTI treatment group and a control group. The treatment group started SOTI and underwent a second challenge 6 months later. Control children were kept on an egg-free diet for 6 months and then underwent a second challenge.

Results: After 6 months, 9/10 children of the SOTI group (90%) achieved partial tolerance (at least 10 ml, but <40 ml of raw HE emulsion, in a single dose) and 1 (10%) was able to tolerate only 5 ml (no tolerance). After 6 months, nine control children tested positive to the second challenge at a dose ≤ 0.9 ml of raw HE emulsion, and one reacted to 1.8 ml (SOTI vs. control group $p < 0.0001$). All children in the SOTI group had side effects, but no child had a grade 5 reaction according to the Sampson grading.

Conclusion: Six months of SOTI with raw HE emulsion resulted in partial tolerance, with regular intake, in a significant percentage of children with severe egg allergy.

Food allergy is the primary cause of anaphylaxis in children (1, 2). The current strategy for the management of IgE-mediated food allergy is avoidance of the causative food and self-injectable epinephrine (3, 4). Accidental exposure is frequent especially in the case of common foods (4). Moreover, elimination diets can lead to psychological problems, particularly in children with potentially life-threatening reactions (5). Cow's milk and hen's egg (HE) proteins are the main offenders in Europe (1, 2). In recent studies, only 11% of children with HE allergy recovered by the age of 4 yr and about 80% recovered by the age of 16 yr (6–8).

Many allergic patients can tolerate gradually increasing amounts of known allergens, including HE (9–24). This strategy, called 'specific oral tolerance induction' (SOTI), seems to be a promising approach to food allergy. Introduction of

baked egg may increase tolerance in egg-allergic patients (19), but tolerance to extensively heated egg is not predictive of clinical reactions to regularly cooked or raw egg (20). In fact, patients could remain allergic to raw HE and hence risk an allergic reaction if they ingest foods containing raw HE or food in which heating was not sufficient to denature allergenic proteins (25–27).

Rush SOTI protocols have been associated with more frequent and more severe adverse effects than slower protocols (18,23,24). However, SOTI with a slow dose escalation of HE might increase safety but would lengthen the duration and thus compromise adherence to treatment. Therefore, we devised a new SOTI protocol with slowly escalating doses of raw HE at home alternating with dosage doubling in hospital. The aim was to reduce the risk of adverse reactions at

home, while achieving tolerance to raw HE within 6 months.

Thus far, no SOTI for raw HE allergy has been conducted in children with previous anaphylaxis. Here we report the outcome of a randomized controlled study of a new 6-month SOTI protocol with raw HE in children with a history of anaphylaxis.

Materials and methods

Twenty-six children (14 boys, median age: 7 yr 7 months; range 5–11 yr), with severe IgE-mediated HE allergy, were recruited from the Paediatric and Allergology Unit of the Fatebenefratelli Hospital in Benevento, Italy, from January 2008 to December 2009 (Fig. 1). Inclusion criteria were as follows: (i) at least 1 anaphylactic reaction (grade 3, 4 or 5 according to Sampson's grading) (28) (Table 1) after accidental exposure to trace amounts of HE or egg-derived products, within 12 months of pre-enrolment; (ii) previous SPT/IgE positive for HE; and (iii) a positive double-blind placebo-controlled food challenge (DBPCFC) at a dose of ≤ 0.9 ml of raw HE emulsion. Exclusion criteria were poorly controlled asthma, namely step 3 or more of pharmacological management according to international guidelines (29), and parents with history of unreliable management of complications and treatment. No patient had a history of adverse reactions, or a positive SPT or prick-by-prick (PP) to cacao. We recorded gender, age, family and personal history of allergies, symptoms after previous ingestion of HE and coexistent allergic clinical manifestations.

Skin prick test protocol

Skin prick tests were performed as previously described (30) with commercial common inhalant allergens and with commercial food allergens (Lofarma, Milan, Italy). Prick-by-prick tests were carried out with natural foods: raw emulsion HE and cacao (Perugina, Perugia, Italy) (milk-free and diluted with water at 10%). Cacao was used for DBPCFC. Skin reactions were considered positive if the mean weal diameter was at least 3 mm larger than the negative control (30). For end-point (EP) SPT, 12 serial dilutions of HE white extract (Lofarma, Milan, Italy) were prepared in sterile saline as described elsewhere (31) and then used only the even dilutions of the extract (1:4, 1:16, 1:64, 1:256, 1:1024, 1:4096). The highest dilution with a weal of at least 3 mm was considered the EP SPT threshold. Results were calculated as the mean of the orthogonal diameters of the wheal. Skin prick tests, EP SPTs with HE white extract, PP with raw HE emulsion and IgEs (Phadia Cap System FEIA, Uppsala, Sweden) were performed upon enrolment and 6 months later.

Double-blind placebo-controlled food challenge

Double-blind placebo-controlled food challenge was performed as previously described (32). The challenge started with 1 drop of a blended emulsion of 45 ml of raw HE and 150 ml of amino acid-based infant formula (Neocate,

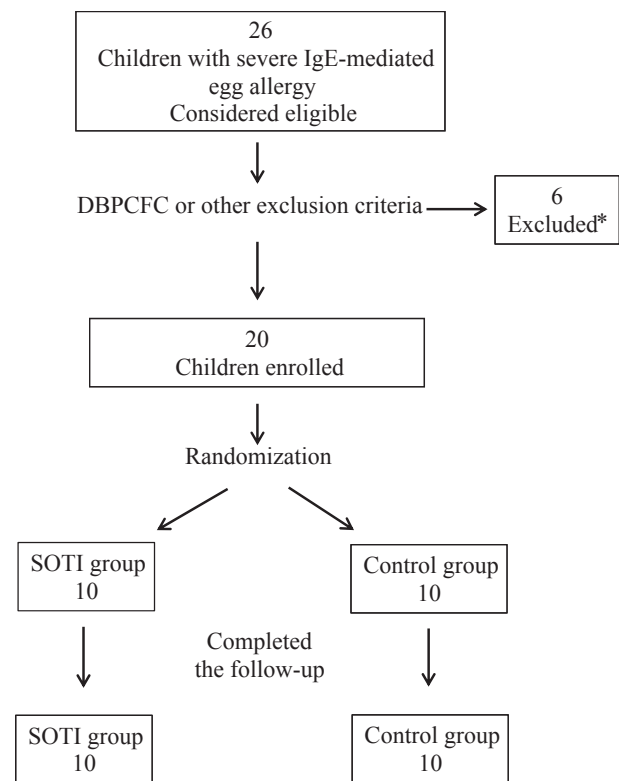


Figure 1 Flowchart of the enrolled population. *Six children (four boys and two girls) were excluded from the study: one child because the parents had a history of unreliable management of complications and treatments; two children because of poorly controlled asthma; and three children because of a positive Double-blind placebo-controlled food challenge at a dose of raw egg emulsion higher than 0.9 ml.

Nutricia), corresponding to about 0.015 ml of HE emulsion, flavoured with vanilla and cacao to make the taste palatable. The placebo was the amino acid-based infant formula alone (Neocate, Nutricia) also flavoured with vanilla and cacao. Masking was checked in three volunteers who were unable to distinguish between verum and placebo. The doses (ml of HE emulsion) were increased every 20 min as follows: 0.015 → 0.030 → 0.060 → 0.120 → 0.225 → 0.45 → 0.9 → 1.8 ml → 3 ml → 5 ml and so on until the highest single dose tolerated was reached or a reaction appeared. Based on Sampson's criteria for grade 3 food anaphylaxis (28; Table 1), the DBPCFC was considered positive when an immediate reaction involved at least two of the following organs: skin (generalized pruritus, flushing, urticaria or angio-oedema), gastrointestinal tract (repetitive vomiting), respiratory tract (rhinorrhoea, marked congestion, sensation of throat pruritus or tightness), cardiovascular (tachycardia = increase > 15 beats/min) or neurological system (change in activity level plus anxiety). In case of subjective or mild objective symptoms corresponding to Sampson's grade 1–2 (28; Table 1), the dose previous to the symptom-inducing dose was re-administered, the next dose was increased by 20%, and the interval between doses was prolonged to 30 min.

Table 1 Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms

Reaction grade	Skin	GI Tract	Respiratory Tract	Cardiovascular	Neurological
1	Localized pruritus, flushing, urticaria, angioedema	Oral pruritus, oral 'tingling,' mild lip swelling			
2	Generalized pruritus, flushing, urticaria, angioedema	Any of the above nausea and/or emesis x's 1	Nasal congestion and/or sneezing		Change in activity level
3	Any of the above	Any of the above plus repetitive vomiting	Rhinorrhea, marked congestion, sensation of throat pruritus or tightness	Tachycardia (increase 15 beats/min)	Change in activity level plus anxiety
4	Any of the above	Any of the above plus diarrhoea	Any of the above hoarseness, 'barky' cough, difficulty swallowing, dyspnea, wheezing, cyanosis	Any of the above dysrhythmia and/or mild hypotension	'Light headedness,' feeling of 'pending doom'
5	Any of the above	Any of the above loss of bowel control	Any of the above respiratory arrest	Severe bradycardia and/or hypotension or cardiac arrest	Loss of consciousness

All symptoms are not mandatory. The severity score should be based on the organ system most affected, for example, if grade 3 respiratory symptoms are present but only grade 1 GI symptoms, then the anaphylaxis severity score would be 'grade 3.' **Boldface symptoms** are absolute indications for the use of epinephrine; use of epinephrine with other symptoms will depend on patient's history (28).

The SOTI protocol (Table 2) started within 15 days of the DBPCFC. The dosage increase was delayed in case of side effects, intercurrent illness or asthma worsening. Six months later, the children underwent a second DBPCFC to verify the maximum dose of HE emulsion they could tolerate, and patients continued the SOTI protocol until the day before challenge. SOTI children underwent challenge with only two doses: half the dose tolerated at home and the whole dose. Control group children remained on an egg-free diet for 6 months and then underwent a second DBPCFC.

In this study, we used only premium quality eggs and observed established sanitary regulations to minimize the risk of bacterial infection. Parents received training about managing adverse reactions, including the use of self-injectable epinephrine. They gave their informed consent to the study, which was approved by the ethics committee of the Fatebene-fratelli Hospital, Benevento.

Randomization and blinding

A computer scientist not involved in the study was responsible for the computerized randomization of the children to each group. Healthcare staff and parents were aware of the treatment. The children in SOTI group knew they were ingesting raw HE, but were blind to dosage increases.

Specific oral tolerance induction protocol

Specific oral tolerance induction protocol (SOTI) consisted in the administration of increasing amounts of raw HE emulsion. As shown in Table 2, children started with 1 drop of

undiluted raw HE emulsion (0.015 ml) flavoured with vanilla and cacao in day hospital then continued at home with gradually increasing doses mixed by the parents in the child's breakfast (cow's milk, soymilk, fruit juice or other) and 5 doubling doses in day hospital. Dose increases were customized based on the frequency and severity of side effects, and in case of intercurrent illness or asthma worsening. Children were required to refrain from physical activity for 4 h after dosing. Children were observed for 2 h after receiving doses and were considered to have an adverse reaction if one or more symptoms appeared. If symptoms were subjective or mild (grade 1 according to Sampson) (28), no action was taken, and the protocol was continued. When objective symptoms seemed more important (grade 2, 3 and 4 reactions), medical treatment was given, and instead of increasing the dosage, the previous tolerated dose was administered. Subjects were withdrawn from the study in case of severe adverse reactions (grade 5). Written instructions about the treatment of adverse events were given to parents. They were also advised they could telephone the physician 24 h a day. Control children underwent a clinical visit each month, and their parents also received instructions how to treat reactions after accidental HE ingestion.

Outcome measures

In the SOTI group, we determined the number of children who could tolerate:

1 40 ml of raw HE emulsion in a single dose ('maximum tolerance'), which roughly corresponds to a small egg or

Table 2 Specific oral tolerance induction protocol

Day	Place of administration	Volume
1	Start in DH	1 drop
2–7	At home	1 drop
8	Doubling in DH	2 drops
9–14	At home	2 drops
15–21	At home	3 drops
22–28	At home	4 drops
29	Doubling in DH	8 drops
30–35	At home	8 drops
36–42	At home	9 drops
43–49	At home	10 drops
50–56	At home	12 drops
57–63	At home	14 drops
64	Doubling in DH	1.5 ml (30 drops)
65–70	At home	1.5 ml (30 drops)
71–77	At home	1.8 ml (36 drops)
78–84	At home	2.1 ml (42 drops)
85–91	At home	2.5 ml
92	Doubling in DH	5 ml
93–98	At home	5 ml
99–105	At home	6 ml
106–112	At home	7 ml
113–119	At home	8 ml
120–126	At home	9 ml
127–133	At home	10 ml
134	Doubling in DH	20 ml
135–140	At home	20 ml
141–147	At home	22 ml
148–154	At home	24 ml
155–161	At home	26 ml
162–167	At home	28 ml
168–175	At home	30 ml
176	Increasing	40 ml

DH, day hospital.

2 less than 40 ml but at least 10 ml in a single dose ('partial tolerance') or

3 less than 10 ml in a single dose ('persistence of HE allergy').

Before and after SOTI, we evaluated the change in the mean weal diameter in millimetres after HE white SPTs, EP SPT, PP and HE white IgE levels. Lastly, we determined the number and severity of adverse events in both groups.

Statistical analysis

Based on previous trials in children with very severe food allergy (9, 18, 24), the expected success is 0% and about 90%, respectively, in the control and treatment group. Nevertheless, we assumed success in 80% and 10% of treated and control subjects, respectively. With a statistical power of 80% and a probability of α error of 0.05, 20 patients (10 for group) are required for the final analysis. In our study, EP SPT was considered a categorical variable, and differences were calculated as ratio between HE white EP SPT threshold dilutions after 6 months and at baseline. Median and range were calculated for quantitative variables, and the distribution of the two groups was compared with the Kolmogorov–

Smirnov non-parametric test. Frequencies were calculated for categorical variables, and the Fisher's exact test was calculated for between-group comparisons. The paired non-parametric Wilcoxon test was used to evaluate differences between pre- and post-study values in the SOTI and control groups separately. Spearman's correlation was used to evaluate the relationship between the tolerated dose, adverse reactions, SPT results, HE white PP, EP SPT results and HE white IgE levels. A p value <0.05 was considered statistically significant. The Stata 10.1 software package (StataCorp LP, College Station, TX, USA) was used for all analyses.

Results

Clinical results

Twenty children (10 boys and 10 girls) with a positive DBPCFC at ≤ 0.9 ml of raw HE emulsion were enrolled in the study and were equally randomized to the SOTI or control group. The two groups did not differ in terms of age, gender, clinical symptoms, specific IgE levels, the results of SPT, EP SPT and PP or the DBPCFC threshold of reactivity (Table 3). Nor were there significant differences regarding inhalant sensitization and other food sensitization. All randomized children completed the follow-up and were included in the final data analysis (Fig. 1).

After 6 months of SOTI, no child could tolerate 40 ml of raw HE emulsion in a single dose (maximum tolerance) because none reached the final dose of the protocol; 9/10 (90%) achieved partial tolerance (<40 ml but ≥ 10 ml), and 1/10 (10%) was able to ingest only 5 ml (no tolerance) (Table 4). A second DBPCFC confirmed the tolerated dose reached in 6 months. Nine control children had a positive DBPCFC after 6 months of an egg-free diet to ≤ 0.9 ml of raw HE emulsion, and one reacted to 1.8 ml (Table 4), which demonstrates the failure to achieve spontaneous tolerance, even partial, within 6 months.

To achieve partial tolerance, the absolute increment of benefit would be 0.9, and the number needed to treat (NNT) was 1.1. The median maximal tolerated dose was 20 ml (range: 5–30 ml) in SOTI children and 0.45 (range: 0.225–1.8) in controls. After 6 months, the differences vs. baseline in the tolerated dose (median and range) were 19.1 (4.8–29.8) in SOTI children and 0 (–0.45–0.9) in controls ($p < 0.001$) (Table 3). SOTI outcomes were unrelated to age, gender, HE white SPT weal diameter, EP SPT results and HE white-specific serum IgE level (Table S1).

Safety data

As shown in Table 5, all children who received SOTI experienced adverse events with a total of 53 events; none resulted from accidental HE exposure. According to Sampson's classification (28), 3 (5.6%) were grade 1, 10 (18.9%) were grade 2, 35 (66%) were grade 3 and 5 (9%) were grade 4. No child in the SOTI group had a grade 5 reaction, needed oral or intramuscular steroids or epinephrine or had access to emergency services. Eighty-one per cent of adverse reactions

Table 3 Demographic data, allergic characteristics, SPT with egg white, egg white end-point SPT, PP with raw emulsion egg, egg white-specific IgE, threshold of reactivity in the Double-blind placebo-controlled food challenge in the SOTI and control groups

	SOTI group	Control group	p
No. of children	10	10	
Median age in years (range)	6.6 (5–10.2)	8.6 (4.2–11)	0.294
Female (%)	5 (50)	5 (50)	1.000
Atopic dermatitis (%)	2 (20)	0 (0)	0.474
Rhinoconjunctivitis (%)	4 (40)	3 (30)	1.000
Asthma (%)	4 (40)	4 (40)	1.000
Inhalant sensitization (%)	7 (70)	7 (70)	1.000
Other food sensitization (%)	7 (70)	7 (70)	1.000
Median wheal diameter of egg white SPT (mm) (range)	10 (7–15)	9.25 (5.5–15)	0.976
Median egg white end-point SPT (dilution) (range)	1:256 (1:256–1:1024)	1:256 (1:64–1:4096)	0.976
Median wheal diameter of egg white PP (mm) (range)	15 (10–25)	17.5 (8.5–25)	0.976
Median egg white-specific IgE (kU/l) (range)	23.30 (8.9–35.5)	19.1 (8.5–39.9)	0.66
Median threshold of reactivity in the DBPCFC (ml) (range)	0.45 (0.225–0.9)	0.45 (0.225–0.9)	1.000

DBPCFC, double-blind placebo-controlled food challenge; PP, prick-by-prick; SOTI, specific oral tolerance induction; SPT, skin prick test.

Table 4 Maximal tolerated dose for each patient at baseline and after either SOTI or an egg-free diet

SOTI Group				Control Group			
	Maximal tolerated dose at first DBPCFC (ml)	Maximal tolerated dose post-SOTI (ml)	Difference		Maximal tolerated dose at first DBPCFC (ml)	Maximal tolerated dose after 6 months (ml)	Difference
Patients				Patients			
1	0.45	30	29.55	1	0.9	0.45	–0.45
2	0.45	30	29.55	2	0.45	0.9	0.45
3	0.9	20	19.1	3	0.45	0.9	0.45
4	0.45	10	9.55	4	0.225	0.225	0
5	0.225	5	4.775	5	0.45	0.225	–0.255
6	0.9	20	19.1	6	0.45	0.45	0
7	0.225	30	29.775	7	0.9	1.8	0.9
8	0.45	10	9.55	8	0.9	0.45	–0.45
9	0.9	10	9.1	9	0.225	0.45	0.225
10	0.9	30	29.1	10	0.9	0.9	0
Median (range)	0.45 (0.225–0.9)	20 (5–30)	19.1 (4.8–29.8)*	Median (range)	0.45 (0.225–0.9)	0.45 (0.225–1.8)	0 (–0.45–0.9)

DBPCFC, double-blind placebo-controlled food challenge; SOTI, specific oral tolerance induction.

* $p < 0.001$.

occurred during administration of the first 6 ml, and 100% occurred within administration of 20 ml HE (Fig. 2). Twenty-five adverse reactions were related to the 36 doubling doses administered in day hospital (69%). This is significantly higher than the number of adverse events related to doses administered at home (28/200 administered doses; 14%) $p < 0.001$. The children in the SOTI group had a relative risk of 4.96 (95% CI = 3.30–7.45) of incurring an adverse event, but there were no significant differences in the severity of reactions. No child discontinued treatment because of adverse reactions. In the SOTI group, there were no correlations between adverse reactions and age, HE white SPT wheal diameter or EP SPT (Table S1).

Three (30%) control children had a total of 5 adverse reactions because of accidental ingestion of trace amounts

of HE. Severity grade was 3 or 4; none required im epinephrine. However, they accessed the emergency services four times in 6 months, and two children needed oral steroids.

Immunological variables

Table 6 lists the immunological variables evaluated in the SOTI and control groups at baseline and 6 months later. SOTI caused a significant decrease in HE white-specific serum IgE levels ($p = 0.007$) and median wheal diameters after SPT ($p = 0.006$), EP SPT ($p < 0.001$) and PP ($p = 0.005$). The immunological variables remained essentially unchanged in the control group after 6 months of an egg-free diet. The differences in the reductions of immunolog-

Table 5 Adverse reactions, treatment administered and related doses of the raw egg emulsion during the 6-month study in the control and SOTI groups

SOTI group	N	Reaction grade*	Treatment	Doses of the raw egg emulsion associated with the adverse reaction†
Urticaria and rhinitis	5	3	Antihistamine	Drops: 2-9-20 ml: 2.5-10
Abdominal pain	10	3	No therapy or antihistamine only administered depending on severity	Drops: 8-9-20 ml: 1.5-2.1-2.5-5-6
Urticaria and oral pruritus	10	2	No therapy or antihistamine only administered depending on severity	Drops: 9 ml: 2.5-6-9-10- 20
Urticaria and abdominal pain	5	3	No therapy or antihistamine only administered depending on severity	Drops: 2-10 ml: 1.5-2.5-5
Mild asthma	3	4	β2 agonist	Drops: 14 ml: 1.5-5
Rhinitis and oral pruritus	8	3	No therapy or antihistamine only administered depending on severity	Drops: 2-14 ml: 2.5- 5-10-20
Mild laryngospasm	2	4	Nebulized epinephrine	ml: 2.5-6
Localized urticaria	3	1	Antihistamine	Drops: 8 ml: 2.5- 5
Rhinitis and conjunctivitis	4	3	No therapy or antihistamine only administered depending on severity	Drops: 8 ml: 2.5- 5
Oral pruritus and gastrointestinal symptoms	3	3	No therapy or antihistamine only administered depending on severity	Drops: 8 ml: 5
	0		• Oral steroids • i.m. epinephrine • Access to emergency services	
	30		Telephone calls received from patients because of adverse reactions	
Control group	N	Reaction grade*	Treatment	Doses of the raw egg emulsion associated with the adverse reaction
Vomiting and abdominal pain	1	3	Antihistamine	Trace amounts of egg
Cough and tightening of throat	2	4	Nebulized epinephrine	Trace amounts of egg
Rhinitis and conjunctivitis, lip angioedema and abdominal pain	2	3	Antihistamine and oral steroids	Trace amounts of egg
	0		im epinephrine	
	4		Access to emergency services	

SOTI, specific oral tolerance induction.

*According to Sampson (28).

†Dose doubling in DH in bold type.

ical variables were significantly higher in the SOTI group vs. controls ($p < 0.001$).

Discussion

Some aspects of SOTI are still considered experimental (33), as witnessed by the diversity of protocols used. Ours is the first randomized controlled trial performed with raw egg in children with egg anaphylaxis. We used raw egg because if raw egg is tolerated, the diet can be safely liberalized. In fact, anaphylactic reactions to raw egg can occur after negative challenge to cooked egg (27). Furthermore, raw egg is present in many foodstuffs consumed by children and in many traditional Italian dishes (e.g. tiramisù, mayonnaise, homemade ice cream, zabaglione).

Based on earlier SOTI studies (9, 13, 14, 16, 23, 24), we selected 6 months as the time sufficient to achieve a good degree of tolerance. Children ingested raw HE emulsion (the most allergenic form of this food) daily. Because our goal was to reduce the risk of reactions because of accidental ingestion of HE, we set the target for tolerance at 10 ml raw HE emulsion, which is higher than the amounts that could be ingested accidentally given the dietary habits in Italy.

One of our children did not achieve tolerance to more than 5 ml of HE, which is still 22-fold the maximum dose he tolerated before SOTI. This patient, sensitized to house dust mites, often interrupted the protocol because of intercurrent febrile illness. In no other case of either group did sensitization to environmental allergens affect the patient's adherence to the SOTI protocol. None of our SOTI patients managed

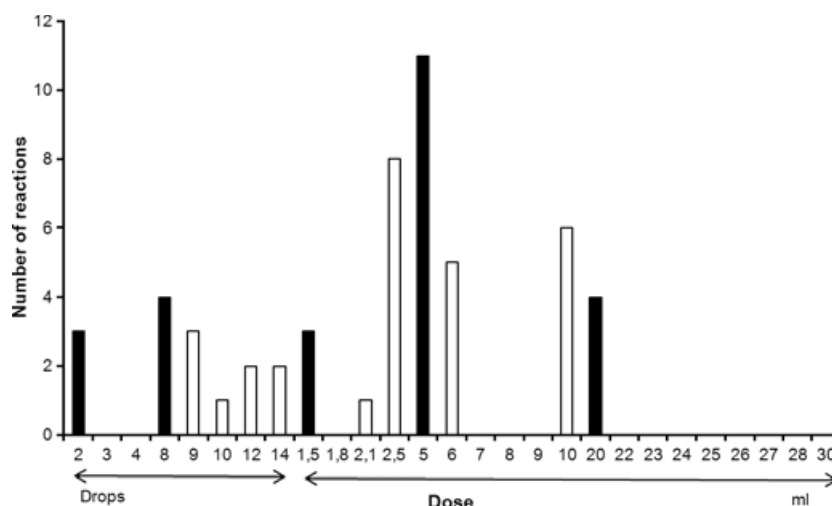


Figure 2 Total number of adverse reactions in relation to SOTI dose increase. Black bars indicate the doubling doses.

Table 6 Immunological variables in the SOTI and control groups at the end of the study and their differences in the reductions

	SOTI group			Control group			
	N = 10			N = 10			
	At baseline	After 6 months	Differences	At baseline	After 6 months	Differences	p*
Median wheal diameter of SPT (range)	10 (7–15)	5 (4–13)	–3.5 (–10–0)	9.25 (5.5–15)	10 (5.5–15)	0 (–2–2)	0.007
Median egg white end-point SPT dilution (range)	1:256 (1:256–1:1024)	1:4 (1:1–1:16)	–64† (–256–64)	1:256 (1:64–1:4096)	1:256 (1:256–1:1024)	0† (+4–4)	<0.001
Median wheal diameter of PP (range)	15 (10–25)	9 (10–12)	–7.5 (–20–2)	17.5 (8.5–25)	18 (8.5–24)	–0.25 (–3–2)	<0.001
Median egg white specific IgE (kUa/l) (range)	23.30 (8.9–35.5)	8.7 (3.3–17)	–14.4 (–27.6–0.1)	23.30 (8.9–35.5)	13.5 (10.5–5.4)	2 (–8.9–13.8)	<0.001

SOTI, specific oral tolerance induction; SPT, skin prick test; PP, prick-by-prick.

*Kolmogorov–Smirnov test to compare the differences in distribution pre- and post-study between the SOTI and control groups.

†Ratio between egg white EP SPT threshold dilutions after 6 months and at baseline.

to ingest 40 ml of HE because of the frequency and severity of side effects, or because of intercurrent illness. However, the minimum increase in tolerance threshold was 11-fold, and the maximum was 133-fold. The increase in tolerance threshold was not correlated with the patient's history, age, level of serum-specific IgE, SPT wheal diameter or the cut-off value of the EP test. The fact that no control child improved their tolerance to HE in 6 months indicates that the positive results of the treated group can be ascribed to SOTI.

Table S2 shows the outcome of the randomized trials of SOTI conducted in children with HE allergy (9, 13, 14, 16,

19, 23, 24). It is difficult to compare the results of these trials because some used boiled (14) or lyophilized (13, 16, 24) HE, some excluded patients with severe HE allergy (13, 14, 16, 19) and one (16) reported the results of SOTI for HE and cow's milk combined. However, the NNT was about 1 in all instances, which indicates a similar efficacy of the protocols used. Adverse events were frequent in all these trials, especially those based on a rush protocol (23, 24). The significant decrease in HE white IgEs and SPT, EP SPTs and PP median wheal diameters in all children in the SOTI group was in accordance with previous studies on SOTI for HE allergy (9, 14, 16). All patients experienced adverse reactions, although

none reached grade 5 of the Sampson classification (28), and adrenaline was not used by any child. The frequency but not the severity of adverse reactions was significantly correlated with the magnitude of the dose increase: dose increments were greater in day hospital, and therefore, adverse reactions were more frequent in this setting. This could have been avoided by not doubling the dose, which for safety's sake must be done in day hospital, and carrying out the entire procedure at home. However, this would have inordinately prolonged the duration of the procedure, and, moreover, the medical supervision would have been lost. Four children of the control group accessed emergency services and none of the SOTI group. This may reflect the fact that the parents of these children could access a physician at any time rather than the severity of adverse events.

A limit of our study is the lack of double blinding. To our knowledge, the only SOTI study conducted in children with double blinding concerns milk allergy (15). We chose SOTI with the unmodified natural food. The parents measured, at home, the amount to be administered, thereby overcoming the delivery of pre-packaged doses; consequently, double blinding was not possible. The children were blind only to the dose increase. The fact that the control group remained positive to the second DBPCFC indicates that our results are reliable, despite the absence of double blinding. To minimize bias resulting from the fact that healthcare staff was aware of the treatment that each child received, the DBPCFC was considered positive when an immediate objective reaction involved at least two target organs (Table S3).

Conclusions

Six-month SOTI with raw HE in children with a history of HE-induced anaphylaxis resulted in partial tolerance to HE. None of our patients reached maximum tolerance. However, tolerance was achieved at doses higher than those associated with anaphylaxis from accidentally ingested HE. Although the procedure is not devoid of adverse events, the safety of our protocol was acceptable. It remains to be established whether to prolong the time required to reach tolerance or to risk more reactions in order to achieve tolerance, albeit partial, sooner. Follow-up will show whether tolerance acquirement is permanent.

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Conflict of interest

Authors declare not to have any conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation between maximal tolerated dose post-SOTI (ml) and age, gender, egg white SPT wheal diameter, EP SPT results and egg white-specific serum IgE level at baseline.

Table S2. Comparison of SOTI trials conducted in children affected by hen's egg allergy.

Table S3. Symptoms and corresponding eliciting dose (ml) in control group during end-of-study DBPCFC.

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Table e1. Correlation between maximal tolerated dose post-SOTI (ml) and age, gender, egg white SPT wheal diameter, EP SPT results and egg white-specific serum IgE level at baseline. Correlation between number of the adverse reactions in the SOTI group and age, egg white SPT wheal diameter, EP SPT results at baseline.

Correlation		p*	Correlation		p*
Maximal tolerated dose post-SOTI (ml)	age	0.589	N. adverse reactions	age	0.226
	gender	0.749		egg white SPT wheal diameter	0.293
	egg white SPT wheal diameter	0.568		end-point SPT results	0.156
	end-point SPT results	0.548			
	egg white PP (mm)	0.226			
	egg white-specific serum IgE level	0.857			

* Spearman Rank Correlation

Table e2– Comparison of SOTI trials conducted in children affected by hen’s egg allergy

<u>Parameter/Reference</u>	<u>Patriarca (9)</u>	<u>Buchanan (13)</u>	<u>Morisset (14)</u>	<u>Staden (16)</u>	<u>Konstantinou (19)</u>	<u>Itoh (23)</u>	<u>Garcia Rodriguez (24)</u>	<u>Dello Iacono</u>
<u>N. children enrolled</u>	<u>21</u>	<u>7</u>	<u>90</u>	<u>47 → 45</u>	<u>51</u>	<u>6</u>	<u>23</u>	<u>20</u>
<u>SOTI</u>	<u>17</u>	<u>7</u>	<u>51</u>	<u>25 (Milk or egg)</u>	<u>51</u>	<u>6</u>	<u>23</u>	<u>10</u>
<u>Controls</u>	<u>4</u>	<u>0</u>	<u>39</u>	<u>20 (Milk or egg-free diet)</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>10</u>
<u>Age (range)</u>	<u>3-16 years</u>	<u>1-7 years</u>	<u>3.5 +/- 1.7 years (1-8 years)</u>	<u>median age 2.5 years (range 0.6–12.9 years)</u>	<u>median age, 24 months (range 12-48 months)</u>	<u>9.7 years (7-12 years)</u>	<u>8.1 years (5-17 years)</u>	<u>SOTI group 6.6 years (5-10.2) Control group 8.6 years (4.2-11)</u>
<u>History of anaphylaxis</u>	<u>4/21</u>	<u>0/7</u>	<u>0/90</u>	<u>0/45</u>	<u>not reported</u>	<u>6/6</u>	<u>2/23</u>	<u>20/20</u>
<u>Cooking grade</u>	<u>raw shaken egg</u>	<u>powdered egg white in distilled water</u>	<u>hard boiled hen’s egg</u>	<u>lyophilized HE protein</u>	<u>cake baked with 1 egg</u>	<u>powdered egg white in distilled water</u>	<u>pasteurized egg heated to 57. 1°C for 4.25min</u>	<u>Raw hen’s egg emulsion in food</u>
<u>SOTI length</u>	<u>168 days</u>	<u>24 months</u>	<u>6 months</u>	<u>21 months</u>	<u>6 months</u>	<u>9-12 months</u>	<u>< 10 days tolerance to a whole cooked egg (omelette)</u>	<u>6 months</u>
<u>Adverse reactions in SOTI group</u>	<u>30% (in SOTI and control group)</u>	<u>100% in the rush fase 15% in the build up fase</u>	<u>missing</u>	<u>100% (0% i.m. epinephrine)</u>	<u>missing</u>	<u>100% (0% i.m. epinephrine)</u>	<u>78.3% (0% i.m. epinephrine)</u>	<u>100% (0% i.m. epinephrine)</u>
<u>Adverse reactions in Control group</u>	<u>not reported</u>	<u>--</u>	<u>--</u>	<u>5/20 (25%)</u>	<u>--</u>	<u>--</u>	<u>--</u>	<u>100% (0% i.m. epinephrine)</u>
<u>Maximum tolerance in SOTI group</u>	<u>12 (70%) Raw hen’s egg</u>	<u>57% scrambled egg</u>	<u>34/49 (69%)</u>	<u>48% Milk and HE protein</u>	<u>95% open food challenges to whole egg</u>	<u>100%</u>	<u>23/23 (86.9%)</u>	<u>0% with raw hen’s egg emulsion</u>
<u>Partial tolerance in SOTI group</u>	<u>2 (12%) Raw hen’s egg</u>	<u>33% scrambled egg</u>	<u>15/49 (31%)</u>	<u>16%</u>	<u>missing</u>	<u>0 (0%)</u>	<u>0 (0%)</u>	<u>90% with raw hen’s egg emulsion</u>

<u>No tolerance in SOTI group</u>	<u>3 (18%)</u> (2 per no compliance)	<u>0% one scrambled egg</u>	<u>0 (0%)</u>	<u>36%</u>	<u>5 (10%) open food challenges to whole egg</u>	<u>0 (0%)</u>	<u>3/23</u>	<u>10% with raw hen's egg emulsion</u>
<u>Tolerance in control group</u>	<u>0 (0%)</u>	<u>inapplicable</u>	<u>18/35 (51%)</u>	<u>35%</u>	<u>inapplicable</u>	<u>inapplicable</u>	<u>inapplicable</u>	<u>0% with raw hen's egg emulsion</u>
<u>Number Needed to Treat</u>	<u>1.2</u>	<u>1.1</u>	<u>1.02</u>	<u>1.56</u>	<u>1.1</u>	<u>1</u>	<u>1.15</u>	<u>1.11</u>

Table e3. Symptoms and corresponding eliciting dose (ml) in control group during end-of-study DBPCFC

<u>Control group</u> <u>(patient n°)</u>	<u>Eliciting dose during</u> <u>end-of-study DBPCFC</u> <u>(ml)</u>	<u>Symptoms</u>
<u>1</u>	<u>0.45</u>	<u>urticaria, angio-oedema, repetitive vomiting, abdominal pain</u>
<u>2</u>	<u>0.9</u>	<u>urticaria, angio-oedema, hypotension</u>
<u>3</u>	<u>0.9</u>	<u>urticaria, angio-oedema, sensation of throat tightness</u>
<u>4</u>	<u>0.225</u>	<u>urticaria, asthma</u>
<u>5</u>	<u>0.225</u>	<u>urticaria, angio-oedema, repetitive vomiting</u>
<u>6</u>	<u>0.45</u>	<u>urticaria, rhinorrhoea and conjunctivitis</u>
<u>7</u>	<u>1.8</u>	<u>urticaria, barking cough</u>
<u>8</u>	<u>0.45</u>	<u>urticaria, angio-oedema, lipothymia</u>
<u>9</u>	<u>0.45</u>	<u>urticaria, angio-oedema, tachycardia, mild asthma</u>
<u>10</u>	<u>0.9</u>	<u>urticaria, angio-oedema, repetitive vomiting</u>