

Skin testing with raw egg does not predict tolerance to baked egg in egg-allergic children

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Abstract

Background: Most children with egg allergy tolerate egg in baked foods, such as cake, but tolerance cannot be predicted with conventional allergy testing. We hypothesized that the skin prick test (SPT) wheal to unprocessed raw egg might predict tolerance of baked egg at formal oral food challenge (OFC).

Methods: We conducted a retrospective chart review to assess the utility of SPT wheal to egg extract (EE), raw egg (RE), and the ratio of EE:RE in predicting outcome of baked-egg OFC in children presenting to our tertiary referral centers with a physician diagnosis of egg allergy and following complete egg avoidance in their diet, between 2009 and 2013. OFC were performed following a standardized protocol using baked egg in cake, to a total dose equivalent to 3g egg protein.

Results: Data were analyzed from 186 completed challenges: OFC was positive in 64 (34%) children and negative in 122 (66%). Six children experienced anaphylaxis at OFC. Children tolerant to baked egg were more likely to have a lower SPT to egg extract/raw egg and EE:RE (median 0.56) than their allergic counterparts (0.70, $p < 0.05$). However, ROC curve analysis demonstrated poor predictivity of challenge outcome, with AUC for SPT to egg extract, raw egg and EE:ER equal to 0.71, 0.63 and 0.60, respectively.

Conclusion: EE:RE was not helpful in predicting outcome of baked-egg OFC. Indeed, SPT to egg extract was slightly better at predicting outcome than either SPT to raw egg or EE:RE. Unfortunately, tolerance to baked egg can only be predicted from previous history or through controlled exposure.

Egg allergy is the most common food allergy in early childhood (1). Up to two-thirds of egg-allergic children are able to tolerate extensively heated egg in baked products (termed baked egg) such as cakes. (1–5). These children also appear to outgrow their egg allergy faster (6), although whether this is due to regular consumption of baked egg or an inherent feature of those children who are able to tolerate baked egg (irrespective of consumption) is unclear. Irrespective, the ability to consume baked egg significantly liberalizes the diet and may help to reduce anxiety over accidental ingestions (7).

The mechanism by which egg-allergic children may tolerate extensively heated egg (EH-egg), but not other forms of egg, remains unclear. Certainly, tolerance is not the result of exposure to a reduced amount of allergen (5). One possibility is that the baking process results in conformational changes in the major allergenic epitopes (8). Egg contains in excess of 20 different

proteins, including ovalbumin and ovomucoid (OVM). Ovalbumin is heat-labile and readily undergoes conformational change during heating (2, 8) while OVM is heat-stable; children with higher levels of IgE to OVM (either on blood testing or at skin testing) are less likely to tolerate EH-egg (2, 9); however, the utility of including testing for OVM to predict tolerance to EH-egg has not been proven to be useful in routine clinical practice (10). The apparent inability of current *in vivo* or *in vitro* tests to reliably differentiate between egg-allergic children who can or cannot tolerate EH-egg, together with the small risk of severe reactions to EH-egg, have resulted in a reliance on supervised oral food challenges. This has a significant resource and time implication.

There are data to suggest that the production of commercial food allergen extracts used in skin testing may render the allergen less potent (11, 12), which for egg protein might be

similar to the consequences of the baking process. In children who are tolerant of EH-egg, a less vigorous immune response may thus be expected to commercial egg extract than to raw egg while those whose clinical allergy is predominantly the result of IgE binding to heat-stable egg proteins, will produce a similar response to both commercial egg extract and raw egg. Given this, we hypothesized that comparing the size of the wheal induced by skin prick testing (SPT) to raw egg with the SPT wheal to commercial egg extract might predict tolerance of baked egg at formal oral food challenge (OFC).

Methods

We conducted a retrospective chart review to assess the utility of SPT wheal to egg extract (EE), raw egg (RE), and the ratio of EE:RE in predicting outcome of baked egg OFC in children presenting to our tertiary referral centers with a physician diagnosis of egg allergy from 2009–2013. We have routinely offered baked-egg OFC to egg-allergic children presenting to our clinics, without a clear history of tolerance or allergic reaction to EH-egg.

Both centers perform SPT to both raw egg and commercial egg extract for routine testing. SPT was performed according to standard guidelines, with histamine 10 mg/mL (Stallergenes, UK) as a positive control (13). A positive SPT was defined as a wheal size of at least 3 mm greater than a saline control read at 15 min.

Following testing, families were offered a baked-egg OFC following a standardized protocol. Exclusion criteria were recent allergic reaction to baked egg within the past 12 months or refusal of consent; children with prior anaphylaxis to egg were not excluded. A standard recipe was used for the muffin baked at 180°C for 20 min. Children were fed incremental doses of muffin, up to a total of 3-g egg protein, at 30-min interval. The challenge was halted at onset of objective clinical symptoms, in line with PRACTALL criteria (14). Children were observed for a further 2 hr following last muffin dose. Anaphylaxis was defined as per WAO criteria (15). Children with reactions involving skin and/or gastrointestinal symptoms alone were classified as mild-moderate reactors. Patients were instructed to avoid all egg for 48 hr in order to detect possible delayed symptoms and then to introduce baked egg (using cake containing a similar amount of egg) two to three times per week. Patients were subsequently followed-up by telephone 1 wk later to confirm tolerance.

Data analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc, La Jolla, CA, USA). Normality of data was assessed using D'Agostino's K-squared test, which failed to demonstrate a normal distribution of data. Statistical analysis was therefore performed using nonparametric tests; further subgroup analysis was undertaken after excluding children who did not have a history of convincing clinical reaction to egg within the previous 12 months (together with evidence of current sensitization) or who did not meet published criteria for >95% likelihood of clinical egg reactivity (ssIgE ≥ 6 kUa/l in children over 2 yr, ≥ 2 kUa/l in those under 2 yr; SPT to egg white > 7 mm in children over 2 yr, > 5 mm in those under 2 yr) (16, 17). Receiver operating characteristic (ROC) curves were generated in order to compare the diagnostic utility

of SPT and compared using the method of DeLong et al. (18). A *p* value of < 0.05 was considered significant. The study was registered as a service evaluation project by the respective hospital ethics committees.

Results

A total of 186 children (median age 5.3 yr, interquartile range 3.3–9.8 yrs) with a physician diagnosis of egg allergy and following strict dietary avoidance of egg completed a baked-egg OFC during the study period. Ninety-five children (51%) met criteria for a >95% likelihood of clinical egg allergy, either on the basis of a recent clinical reaction to egg (within the previous 12 months) together with current sensitization on SPT or serum-specific IgE testing, or meeting published 95% PPV criteria for clinical reactivity (15, 16). Children in this subgroup had a slightly larger SPT wheal to both commercial extract and raw egg, compared to the overall cohort ($p < 0.001$, Mann–Whitney test), but there were otherwise no significant differences in the demographics of these groups (Table 1).

Challenge outcome

Sixty-four children (34%) reacted to their challenge, six of whom experienced anaphylaxis (four with respiratory symptoms and two with persistent abdominal pain and vomiting). None of these six children had experienced prior anaphylaxis to egg. Two children passed their initial OFC but subsequently reacted to ongoing exposure with gastrointestinal symptoms within 30 min of consumption of baked egg at home; these children were classified as reactors.

Children who reacted to baked egg had a larger SPT wheal to both commercial extract and raw egg, compared to baked-egg-tolerant children ($p < 0.01$, Mann–Whitney test) and were more likely to have other food allergies ($p < 0.02$, chi-squared test), but there were otherwise no significant differences in the demographics of these groups (Fig. 1 and Table 2). Baked-egg-tolerant children were more likely to have a lower EE:RE ratio (median 0.56, IQR 0.44–0.75) than their EH-egg-allergic counterparts (0.71, IQR 0.50–0.86; $p < 0.05$, Mann–Whitney test). However, there was significant overlap between values (Fig. 1 and Table 3), and ROC curve analysis demonstrated poor predictive ability of challenge outcome, with AUC for SPT to egg extract, raw egg, and EE:RE equal to 0.71, 0.63 and 0.60, respectively. ROC curve analysis did not demonstrate significant differences when comparing test performance characteristics in those children who met criteria for >95% likelihood of clinical egg allergy to the overall cohort (Fig. 2; $p > 0.05$). Children who experienced anaphylaxis at challenge to EH-egg were not more likely to have a higher SPT wheal to egg extract, raw egg, or EE:RE ratio ($p > 0.05$, Mann–Whitney test).

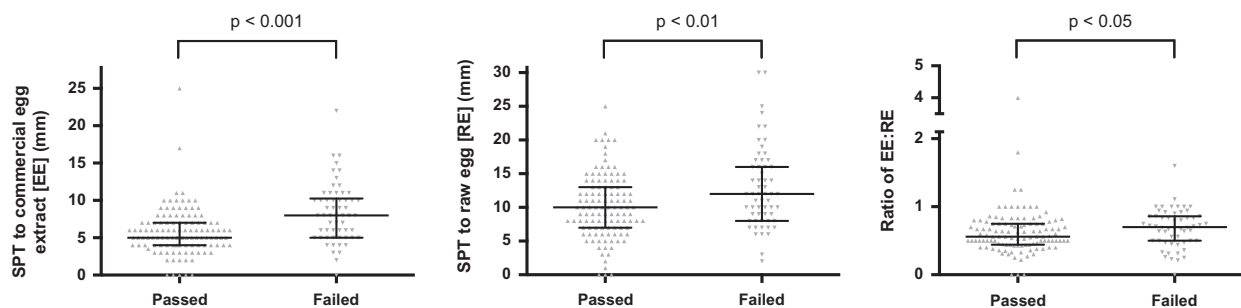
Discussion

The ability to incorporate baked egg into the diet of otherwise egg-allergic children has the potential to significantly reduce the impact of the allergy on a child and has been found to relieve parental concern (7). Furthermore, it may also hasten

Table 1 Patient characteristics

Characteristic	Total cohort	Recent reaction or >95% PPV for egg allergy	p value
Total no. of patients	186	95	
Age at challenge	5.3 yrs	4.6 yrs	ns
median (interquartile range)	(3.3–9.8 yrs)	(2.6–9 yrs)	
SPT to commercial extract,	6 mm	8 mm	p < 0.001
median (interquartile range)	(4–8 mm)	(7–10 mm)	
SPT to raw egg,	10 mm	12 mm	p < 0.001
median (interquartile range)	(8–14 mm)	(10–16 mm)	
Gender, no. (%)			
Male	111 (60%)	57 (60%)	ns
Female	75 (40%)	38 (40%)	ns
Other atopy, no. (%)			
Asthma, on preventer therapy	42 (23%)	21 (22%)	ns
Allergic rhinitis	77 (41%)	36 (38%)	ns
Eczema	129 (69%)	70 (74%)	ns
Other food allergy	147 (79%)	75 (79%)	ns
Previous reaction to egg, no. (%)			
Reaction to any egg	92 (49%)	50 (52%)	ns
Reaction to baked egg	10 (5.4%)	6 (6.3%)	ns
Anaphylaxis	18 (9.6%)	9 (9.5%)	ns
Outcome of OFC to baked egg, no. (%)			
Passed challenge	122 (66%)	53 (56%)	ns
Failed challenge: – mild-mod reaction	64 (34%)	42 (44%)	ns
Failed challenge: – anaphylaxis	6 (3%)	4 (4%)	ns

NB, Data not shown for ssIgE values as these were only performed in the minority of patients enrolled. SPT, skin prick test; OFC, oral food challenge; PPV, positive predictive value; ns, not significant.

**Figure 1** SPT wheal size to commercial egg extract (EE), raw egg (RE), and the ratio of EE:RE, by challenge outcome.

resolution of the allergy to the native antigen (6). Up to 70% of egg-allergic children are able to tolerate baked egg (1–6, 10, 19), consistent with the findings of this study. However, one-third of children will react to baked egg, and this can manifest as anaphylaxis (4, 5), an event which occurred in almost 10% of reactors in this study. It is for this reason that we and others have recommended that baked-egg tolerance should be determined at OFC under medical supervision (2, 5, 20). It would therefore be useful to be able to predict which children are likely to tolerate baked egg.

Unfortunately, the only reliable means by which tolerance can be determined is through oral exposure: to date, routine allergy testing (either SPT or blood tests) has not been found to predict tolerance (or not) with sufficient discrimination for the test to be

clinically useful in most studies (2, 5, 10). Cortot et al. reported that an SPT wheal to commercial egg white extract of <10 mm had a 100% negative predictive value, although this has not been found in other cohorts (1, 2, 5), as well as the cohort reported here. The aim of this study was to test our hypothesis that children who tolerate baked egg may have a relatively higher SPT to raw egg compared to the (processed) commercial egg extract. Unfortunately, adding SPT to raw egg did not improve diagnostic accuracy – in fact, SPT to commercial extract alone was found to have the best predictive utility at ROC analysis, although a cutoff with sufficient sensitivity and specificity could not be determined.

It has been proposed that reactivity to baked egg may reflect the presence of IgE to specific heat-stable epitopes,

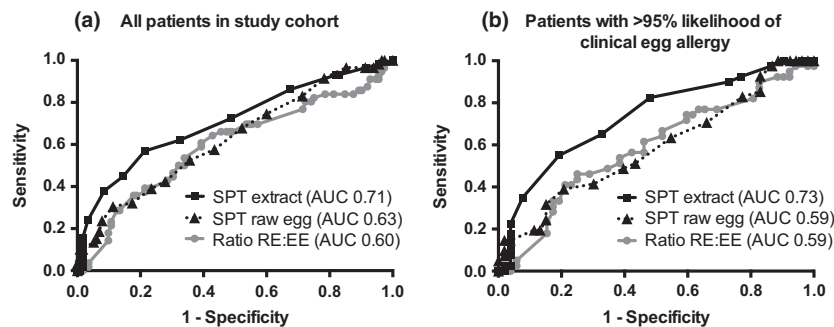


Figure 2 Receiver operating characteristic (ROC) curves (AUC in parentheses within figure legends) for skin prick test (SPT) wheal to commercial egg extract (EE), raw egg, and the ratio of EE:RE in predicting tolerance to baked egg at OFC in (a) the study cohort and (b) a subgroup of children included on the basis of recent clinical history or sensitization meeting >95% predictive criteria for clinical egg allergy.

Table 2 Challenge outcome

Characteristic	Negative OFC: Tolerates baked egg	Positive OFC: Allergic to baked egg	p value
Total no. of patients	122 (66%)	64 (34%)	
Age at challenge, median (interquartile range)	5.1 yrs (3.8–8.8 yrs)	5.6 yrs (2.7–10.6 yrs)	ns
SPT to commercial egg extract at OFC, median (interquartile range)	5 mm (4–7 mm)	8 mm (5–10 mm)	p < 0.001
SPT to raw egg at OFC, median (interquartile range)	10 mm (7–13 mm)	12 mm (8–16 mm)	p < 0.01
Gender, no. (%)			
Male	78 (64%)	33 (52%)	ns
Female	44 (36%)	31 (48%)	ns
Other atopy, no. (%)			
Asthma, on preventer therapy	24 (20%)	18 (28%)	ns
Allergic rhinitis	53 (43%)	24 (38%)	ns
Eczema	83 (68%)	46 (72%)	ns
Other food allergy	90 (74%)	57 (89%)	p = 0.02
Previous reaction to egg, no. (%)			
Anaphylaxis	13 (11%)	5 (8%)	ns
Non-anaphylactic reaction	47 (39%)	27 (42%)	ns
Prior reaction to baked egg	5 (4%)	5 (8%)	ns
No previous reaction to egg	62 (51%)	32 (50%)	ns

SPT, skin prick test; OFC, oral food challenge.

Table 3 Test performance characteristics for SPT to commercial egg extract and raw egg

Cutoff	SPT to Commercial Egg Extract			SPT to Raw Egg		
	Sensitivity%	Specificity%	LR	Sensitivity%	Specificity%	LR
≥3	97	9	1.1	98	4	1.0
≥5	86	32	1.3	97	9	1.1
≥8	57	79	2.7	83	29	1.2
≥10	38	91	4.4	68	48	1.3
≥12	16	98	9.1	53	64	1.5
≥14	10	98	6.1	39	77	1.7
≥16	5	98	3.0	31	89	2.7
≥20	2	99	2.0	14	95	2.6
≥25	0	99	0.0	5	99	5.8

LR, likelihood ratio.

including OVM (2, 8, 21). The measurement of serum-specific IgE to OVM has been found to be predictive of tolerance to hard-boiled egg (22). However, detection of OVM, either through SPT or specific IgE measurement in serum, has not been found to be predictive of reactivity to baked egg. Lemon-Mule et al. found that a very high cutoff of IgE to OVM >50 kUA/l was predictive of allergy to baked egg (2). Tan et al. found that an SPT of >11 mm to OVM had a 100% positive predictive value to clinical reactivity (9). However, most studies have not found a cutoff with sufficient sensitivity and specificity to be of clinical utility in routine practice (2, 5, 10).

The strength of this study is that it is formed of a large cohort of children with a physician diagnosis of egg allergy seen in our tertiary allergy services, undergoing prospective OFC to baked egg. This situation is thus representative of the patient mix seen by clinicians specializing in routine clinical allergy practice. Just under half of our cohort did not meet criteria predicting >95% likelihood of clinical reactivity at the

time of challenge (although they would have done so previously in order for them to be diagnosed with egg allergy). In order to confirm that their inclusion had not confounded our data, we conducted subgroup analysis on those children who met >95% predictive criteria, and we found the same conclusions. Furthermore, the demographics of our cohort are similar to that which has been previously reported in a large prospective cohort of egg-allergic children elsewhere (5).

Finally, 72% of children in this cohort with a *prior* history of anaphylaxis to egg were able to tolerate baked egg, a finding consistent with previous studies (2, 5). Furthermore, the observation that 50% of those with a history of prior reaction to baked egg were able to tolerate baked egg after at least 1 yr indicates that prior severe reaction to egg or reaction to baked egg over 12 months prior should not be used as a contraindication to introduction of baked egg, although our recommendation would be for this to be assessed at in-hospital challenge.

References

- 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.
- Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Węgrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. *J Allergy Clin Immunol* 2008; **122**: 977–83 e1.
- Clark A, Islam S, King Y, et al. A longitudinal study of resolution of allergy to well-cooked and uncooked egg. *Clin Exp Allergy* 2011; **41**: 706–12.
- Lieberman JA, Huang FR, Sampson HA, Nowak-Węgrzyn A. Outcomes of 100 consecutive open, baked-egg oral food challenges in the allergy office. *J Allergy Clin Immunol* 2012; **129**: 1682–4.
- Turner PJ, Mehr S, Joshi P, et al. Safety of food challenges to extensively heated egg in egg-allergic children: a prospective cohort study. *Pediatr Allergy Immunol* 2013; **24**: 450–5.
- Leonard SA, Sampson HA, Sicherer SH, et al. Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol* 2012; **130**: 473–80.
- Lee E, Mehr S, Turner PJ, Joshi P, Campbell DE. Adherence to extensively heated egg and cow's milk following successful oral food challenge. *J Allergy Clin Immunol Pract* 2014; accepted for publication.
- Martos G, Lopez-Exposito I, Bencharitiwong R, Berin MC, Nowak-Węgrzyn A. Mechanisms underlying differential food allergy response to heated egg. *J Allergy Clin Immunol* 2011; **127**: 990–7.
- Tan JW, Campbell DE, Turner PJ, et al. Baked egg food challenges - clinical utility of skin test to baked egg and ovomucoid in children with egg allergy. *Clin Exp Allergy* 2013; **43**: 1189–95.
- Bartnikas LM, Sheehan WJ, Larabee KS, Petty C, Schneider LC, Phipatanakul W. Ovomucoid is not superior to egg white testing in predicting tolerance to baked egg. *J Allergy Clin Immunol Pract* 2013; **1**: 354–60.
- Rance F, Juchet A, Bremont F, Dutau G. Correlations between skin prick tests using commercial extracts and fresh foods, specific IgE, and food challenges. *Allergy* 1997; **52**: 1031–5.
- Norgaard A, Skov PS, Bindslev-Jensen C. Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils. *Clin Exp Allergy* 1992; **22**: 940–7.
- King R on behalf of the British Society for Allergy and Clinical Immunology (BSACI). Paediatric Skin Prick Testing - Standard Operating Procedure. Available at: http://www.bsaci.org/_literature_121183/Paediatric_skin_prick_testing_guideline
- Sampson HA, van Gerth Wijk R, Bindslev-Jensen C, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012; **130**: 1260–74.
- Simons FE, Arduoso LR, Bilò MB, et al. World Allergy Organization Guidelines for the Assessment and Management of Anaphylaxis. *World Allergy Organ J* 2011; **4**: 13–37.
- Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy* 2000; **30**: 1540–6.
- Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; **107**: 891–6.
- DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837–45.
- Cortot CF, Sheehan WJ, Permaul P, et al. Role of specific IgE and skin-prick testing in predicting food challenge results to baked egg. *Allergy Asthma Proc* 2012; **33**: 275–81.
- Leonard SA, Nowak-Węgrzyn A. Re-defining food allergy phenotypes and management paradigm: is it time for individualized egg allergy management? *Clin Exp Allergy* 2011; **41**: 609–11.
- Ando H, Moverare R, Kondo Y, et al. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol* 2008; **122**: 583–8.
- Haneda Y, Kando N, Yasui M, et al. Ovomucoids IgE is a better marker than egg white-specific IgE to diagnose boiled egg allergy. *J Allergy Clin Immunol* 2012; **129**: 1681–2.