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No matrix effect in double-blind, placebo-controlled egg challenges in egg allergic children

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Summary

Background Diagnostic and accidental food allergic reactions may be modified by the matrix containing the allergenic food. Previous studies of double-blind, placebo-controlled food challenges (DBPCFCs) with peanut found an effect of the fat content of the challenge matrix on the severity of the challenge reactions.

Objective The aim of this study was to examine whether the fat content of the food matrix is related to eliciting dose and reaction severity in DBPCFCs with heated hen's egg. **Methods** Sensitized egg allergic children ($n = 59$) undergoing DBPCFCs with egg as a routine diagnostic procedure in our tertiary care centre were evaluated retrospectively. Three different recipes were used for the food matrix: vanilla pudding, pancake and minced meat, containing 22.8%, 31.9% and 52.7% fat (weighted average), respectively. The eliciting dose (i.e. the highest cumulative dose to which the child reacted) was analyzed by Kaplan–Meier log-rank statistic and by Cox regression. Reaction severity was quantified by using an index (range 1–12) and was analysed by multiple linear regression analysis.

Results The overall influence of type of recipe on eliciting dose was not significant ($P = 0.12$). The rate of response to minced meat (with the highest fat content) was not significantly different from pudding [HR = 0.61 (0.26–1.45, $P = 0.26$) or pancake (HR = 1.41 (0.50–3.99), $P = 0.52$] after adjustment for confounders. The type of recipe did not influence the severity of the challenge reaction. The severity of the challenge reaction for minced meat compared to pudding and pancake was 1.06 (0.52–2.16), $P = 0.87$ and 0.81 (0.32–2.01), $P = 0.64$, respectively, after correction for confounders.

Conclusion and Clinical Relevance In contrast to similar research with peanut, no significant influence of the fat content of the matrix was found on the eliciting dose or severity of the reaction in 59 DBPCFCs with hen's egg. Matrix fat content differences comparable to those reported here may not be an important co-determinant of reaction severity for all allergenic foods.

Keywords children, double-blind placebo-controlled food challenge (DBPCFC), egg allergy, matrix effect

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Introduction

Hen's egg allergy is one of the most common food allergies in infants and young children, with an estimated prevalence of between 0.5% and 2.5% [1]. Severe reactions do occur in children with hen's egg allergy,

but anaphylactic reactions are rare [2]. It has previously been suggested that the severity of both diagnostic and accidental food allergic reactions may be modified by the matrix containing the allergenic food. Grimshaw et al. showed in double-blind, placebo-controlled food challenges (DBPCFCs) with peanut that a matrix with a

higher fat content was associated with reactions of greater severity and higher eliciting doses than a matrix with a lower fat content [3]. The aim of this study was to examine whether the fat content of the food matrix is related to eliciting dose and reaction severity in DBPCFCs with heated hen's egg.

Methods

Participants and procedures

Children undergoing DBPCFCs with egg as a routine diagnostic procedure in our tertiary care centre were evaluated retrospectively. Children were offered DBPCFCs if they had had symptomatic accidental ingestions of foods known or likely to contain egg or had had improvement of chronic symptoms such as atopic dermatitis following elimination of egg from the diet. No children were excluded from the challenge for any reason other than unwillingness to undergo the challenge or (unstable) comorbidity compromising challenge safety, which was the case in less than 2% of subjects. Anaphylaxis was not an exclusion criterion for the test. Between 2001 and 2010, 229 egg challenges were performed in 210 consecutive children. Whole egg-specific IgE was measured by CAP-FEIA (Phadia, Uppsala, Sweden). For this analysis, challenges were excluded because they were negative or occurred in children not sensitized to egg (145 challenges), or data were incomplete (1 challenge). In children challenged repeatedly, only the first challenge result was included, resulting in exclusion of 24 challenges. The analysis was thus carried out on the results of 59 challenges. Data were censored for 12 children, because the challenge resulted in subjective symptoms only. This study was exempt from medical ethical approval, as the DBPCFCs were performed as a routine diagnostic test.

DBPCFCs

For the DBPCFCs, three different recipes were used for the food matrix: vanilla pudding, pancake and minced meat, containing 22.8%, 31.9% and 52.7% fat (weighted average), respectively [4]. The choice of recipe was based on the patient's preference. All recipes involved heating of fresh, whole egg by boiling or frying, and none of the recipes involved extensive heating or baking. The starting dose for all challenges was 1.75 mg of the egg protein and followed the schedule: 1.75 mg – 3.50 mg – 14 mg – 70 mg – 350 mg – 1750 mg. Placebo and active challenge foods were administered on separate days. Both the patient and the physician were blinded to the presence of the challenge food (egg) until the code was broken 48 h after the second challenge day. Challenges were assessed as positive when objective allergic symptoms (i.e. angioedema, urticaria, rash/

dermatitis, rhinitis, conjunctivitis, coughing, wheezing, vomiting, diarrhoea or collapse) or repeated subjective symptoms (i.e. generalized itch, nausea, abdominal cramping/pain, throat itch or tightness, itch of the mouth or tongue, irritability, drowsiness) occurred during and/or after the challenge on the active food day, but not on the placebo day according to previously published criteria [5]. Data from children with subjective symptoms only were included and censored in Kaplan–Meier survival analysis. The eliciting dose was the highest cumulative dose to which the child reacted which thus resulted in termination of the challenge. Reaction severity was quantified by using a scoring system which classified all symptoms into five categories: skin (scored as 1), gastrointestinal symptoms (scored as 2), upper airway (eyes, nose, throat), lower airway (lungs), cardiovascular and/or neurological symptoms (each scored as 3). To compute the severity-index, the highest scores for each category were summed. This index ranges from 1 to 12 [6].

Statistical analysis

Data were analyzed using SPSS software for Windows (Version 18.0, SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was considered to be significant. For the analysis of the descriptive characteristics, the children were grouped according the recipe of the DBPCFC (pudding, pancake or minced meat) and compared using the Chi-square test (nominal data) or Kruskal–Wallis test. Furthermore, analyses were carried out to ascertain whether the type of recipe (matrix) influenced two outcome variables: (i) eliciting dose was analyzed by a Kaplan–Meier log-rank statistic and by Cox regression, and (ii) severity of the reaction was analyzed by (multiple) linear regression analysis after a logarithmic transformation to normalize the residuals. The coefficients were then back-transformed to provide geometric mean ratios as effect estimates.

For both regression analyses, consideration of potential confounders was based on a difference greater than 10% in coefficient between crude and adjusted model and/or theoretical considerations. The following confounders were considered: sex, age, specific IgE level, the presence of rhino conjunctivitis and severity of the home reaction [no, mild (score 1 or 2), severe (score > 2)].

Results

The reasons for challenges of the 59 children included in our study were Anaphylactic reaction ($n = 2$), Eczema ($n = 16$), Confirmation of current elimination diet ($n = 47$) and Other ($n = 9$) (some children fitted more than one category). The category Other included 'never eaten before' and 'to confirm or reject food allergy'.

Table 1 shows the descriptive characteristics of the included children who underwent a DBPCFC for egg as well as the outcome of the DBPCFC divided over the three different recipes (pudding, pancake or minced meat). Significant differences were found between the three recipes groups for age ($P = 0.009$), distribution of rhino conjunctivitis ($P = 0.018$) and severity of the reaction at home ($P = 0.027$). No significant differences were found between the three recipes groups for the other descriptive factors and the outcome of the DBPCFC (eliciting dose and reaction severity).

Figure 1 shows the Kaplan–Meier survival plot for the influence of the type of recipe on the rate at which children react during the challenge. The vertical cross-lines indicate 12 patients whose data were censored. The Kaplan–Meier log rank statistic for the overall influence of type of recipe on eliciting dose was not significant ($P = 0.12$). The rate of response to minced meat (with the highest fat content) was not significantly different from pudding [HR (95% confidence interval) = 0.61 (0.26–1.45), $P = 0.26$] or pancake [HR = 1.41 (0.50–3.99), $P = 0.52$] after adjustment for confounders. Including or excluding the subjective reactors did not alter these results. Also the rate of response to pudding was not significantly different from that to pancake [HR = 2.30 (0.83–6.39), $P = 0.109$].

The type of recipe did not influence the severity of the challenge reaction. The geometric mean ratios with 95% confidence intervals of the severity of the challenge reaction for minced meat compared to pudding and pancake were 1.06 (0.52–2.16), $P = 0.87$ and 0.81 (0.32–2.01), $P = 0.64$ respectively after correction for potential confounders. There was also no difference in the severity of the challenge reaction between pudding and pancake (geometric mean ratio for pudding to pancake = 0.76 (0.32–1.83), $P = 0.536$ (Table 2).

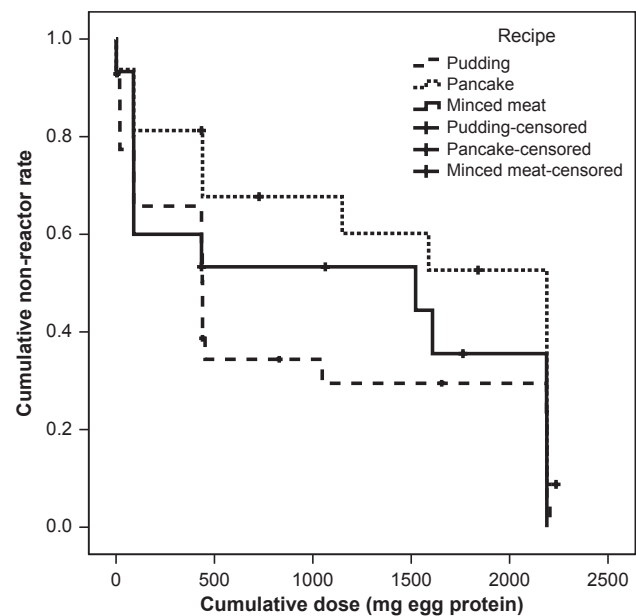


Fig. 1. Kaplan–Meier curve of the reaction rate to DBPCFCs with the three different recipes (pudding, pancake and minced meat). The Kaplan–Meier log rank statistic for the overall influence of the type of recipe on eliciting dose was not significant ($P = 0.12$).

Discussion

Our data did not show a significant influence of the amount of fat in the matrix used in DBPCFCs with hen's egg on the eliciting dose or severity of the reaction during the DBPCFC, and this did not change after correction for possible confounders, including sex, age, specific IgE level, presence of rhino conjunctivitis and severity of the home reaction. This result, which is at variance to that of Grimshaw [3], is unlikely to be due to lack of power, as our study included the results of 59 DBPCFCs as compared with 2 DBPCFCs in each of four patients in the study by Grimshaw. Furthermore,

Table 1. Descriptive characteristics of children who underwent a DBPCFC for egg and information about the eliciting dose (mg protein) and severity of reactions during the DBPCFC

	Pudding, 22.8% fat ($n = 28$)	Pancake, 31.9% fat ($n = 16$)	Minced meat, 52.7% fat ($n = 15$)
Sex, n (m/f)	18/10	10/6	14/1
Age, months	35 (21–58)*	71 (50–91)*	46 (28–108)*
Egg-specific IgE, kU/L	15.0 (3.4–33.5)	3.3 (0.3–18.8)	11.4 (2.8–45.5)
Atopic dermatitis, n (%)	23 (92.0)	12 (85.7)	13 (100)
Asthma, n (%)	10 (37.0)	8 (50.0)	7 (50.0)
Rhino conjunctivitis, n (%)	6 (21.4)*	10 (62.5)*	4 (26.7)*
Reaction severity [†] at home	2 (1–2.8)*	4 (3–8)*	2 (2–3)*
Outcome of DBPCFC			
Eliciting dose, mg egg protein	438 (20–993)	1369 (436–2189)	434 (90–1763)
Reaction severity [†]	2.0 (1.0–4.0)	1.0 (0.3–3.8)	1.0 (0.0–3.0)

* $P \leq 0.05$.

[†]Severity index with range 0 to 12.

Results are shown as median (Inter Quartile Range), unless otherwise indicated.

Table 2. Effects of recipe on the severity of the challenge reaction (expressed as geometric mean ratios with 95% confidence intervals) adjusted for confounders ($n = 47$)

	Geometric mean ratio (95% confidence interval)	P-value
Minced meat vs. pudding	1.06 (0.52–2.16)	0.87
Minced meat vs. pancake	0.81 (0.32–2.01)	0.64
Pudding vs. pancake	0.76 (0.32–1.83)	0.54
Age (in years)	0.88 (0.79–0.99)	0.03
Current rhino conjunctivitis	1.85 (0.86–3.98)	0.11
Specific IgE (natural log)	1.03 (0.86–1.24)	0.73
Home reaction		
No	Reference	
Mild (1 or 2)	0.78 (0.36–1.68)	0.51
Severe (> 2)	1.14 (0.45–2.89)	0.77

the difference between the lowest and highest amount of fat in the matrix in our study was 33.1%, as compared to 8.6% in the study by Grimshaw. Assuming that the effect size on eliciting dose and severity are related to the range of fat content, it is thus unlikely that effects on eliciting dose or severity were missed because of a limited range of fat content in the recipes used.

A possible explanation of the differences between our results and those of Grimshaw is the fat content of the allergenic food itself, i.e. differences in fat content of peanut and egg. It has been shown that matrices with a higher fat content give better blinding because the fat content of a food delays the onset of taste perception and reduces its intensity, particularly when the flavour molecule is lipophilic [7]. This effect is thought to be due to the fact that lipophilic flavours are released more slowly from high-fat oils than from water [8]. Allergens found in the lipid phase, as has been described for oleosins in peanut [9], may also contribute to delay of reactivity. Furthermore, high fat matrices also cause inhibition of binding of IgE antibodies to the allergen. Thus, high fat content of the matrix may delay allergic reactions and ultimately result in more severe reactions because ingestion

of the allergenic food continues [8]. However, these effects may be dependent on an interaction between the allergenic food and the matrix, and may only become clinically meaningful when the allergenic food itself has a relatively high fat content. This fat content is likely to affect the rate at which the allergenic food is assimilated into the matrix and may therefore influence the rate at which allergens may be released and become available to the immune system.

A possible limitation may be that the power of this study to find putative statistically significant differences between the recipes is relatively low. However, the differences that we found between the three recipes are so small that one would need a very large number of study subjects to have enough power to reach statistical significance. More importantly, such differences are unlikely to be clinically relevant based on the data we obtained. For example, the median eliciting dose (See Table 1) of the pudding recipe (22.8% fat) is almost the same as for the minced meat recipe (52.7% fat). Even the difference between these two recipes (with the greatest difference in fat content) as well as the difference with the pancake recipe (31.9% fat) all lie within one dose step of the DBPCFC. Thus, our data do not suggest that there is an important matrix effect for egg recipes, and it is unlikely that small effects beyond the power of this study are of any clinical importance.

In summary, this study found no influence of the amount of fat in matrices for DBPCFC recipes for hen's egg on eliciting dose or severity of reactions during the challenge. This may be due to the relatively low fat content of egg in comparison with peanut. Therefore, matrix fat content differences comparable to those reported here may not be an important co-determinant of reaction severity for all allergenic foods.

Conflict of interest

The authors have no conflict of interest to declare.

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