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Presentation of Allergen in Different Food Preparations Affects the Nature of the Allergic Reaction—a Case Series

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Abstract

Background: Characterization of fatal and nonfatal reactions to food indicates that the majority of reactions are due to the ingestion of prepared foods rather than the nonprocessed allergen. In an ongoing study that used a double-blind placebo-controlled food challenge to investigate peanut allergy and clinical symptoms, the observed reaction severity in four of the first six subjects was greater than anticipated. We hypothesized that this was due to differences in the composition of the challenge vehicle. *Objective:* The aim was to investigate whether the severity of observed challenge reactions would be repeated on rechallenge with a lower fat challenge vehicle. *Methods:* Peanut-allergic subjects were rechallenged with a lower fat recipe after reacting more severely than was anticipated to an initial peanut challenge. Similar challenge vehicle recipes were used, the only difference being the lower fat content (22.9% compared with 31.5%). The peanut content of the two recipes was analyzed using RAST inhibition studies and ELISA tests. *Results:* Three of four subjects reacted to much smaller doses of peanut protein on rechallenge (mean dose equivalence –23 times less peanut) with the lower fat recipe. RAST inhibition showed that neither recipe altered epitope recognition. The higher fat recipe required twice as much peanut to cause 50% inhibition. ELISA detected far lower levels of peanut in the higher fat recipe (220 000 parts per million [ppm]) than in the lower fat recipe

(990 000 ppm). *Conclusion:* The fat content of a challenge vehicle has a profound effect on the reaction experienced after allergen ingestion. This is another factor to be considered in assessing the risk of certain foods to food-allergic consumers and adds another dimension to clinical, research, and regulatory practice.

Keywords: anaphylaxis, food allergy, food challenge, food matrix, IgE, peanut

Introduction

The incidence of anaphylaxis as a result of food allergen ingestion is increasing [1]. Peanuts and tree nuts cause most food-related anaphylactic reactions [2, 3]. Retrospective analysis and characterization of fatal allergic reactions have shown that the majority of reactions were due to the ingestion of peanut as an ingredient in a prepared food or dish rather than to unadulterated peanuts [2, 4].

The risk of suffering an allergic reaction of any description is dependent on many factors, some of which may be responsible for the variation of low-dose reactivity (threshold dose) observed in food-allergic individuals [5]. Different forms of cooking appear to affect in vitro assessments of the allergenicity of peanut [6]. Another factor may be the presentation of the allergen in different food preparations or matrices.

Peanuts are eaten in many different forms: as peanut butter, as roasted kernels, or as ingredients in other foods either as flour, a paste, or as peanut pieces of varying size. Although most first reactions to peanut are seen in the home, subsequent reactions increasingly occur outside the home [7], with Asian-style food restaurants, ice-cream shops, and bakeries commonly cited as being where serious reactions most often occur [8]. Desserts and confectionery are also often highlighted as common causes of accidental allergen exposure [7].

Double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for diagnosing food allergy [9]. A challenge consists of an allergenic test food and a vehicle, which is an inert food or food mix in which the test food is delivered. Various strategies are employed to ensure blinding [9–11]. Our experience with peanut DBPCFC led us to try and improve the blinding of our own and other groups' recipes to optimize peanut masking. Fat is known to affect both the physical properties and the flavor of a food [12]; consequently, the fat content of a well-established chocolate recipe to mask peanut was increased from 22.9% fat to 31.5%.

In the course of an ongoing study into threshold doses of peanut in allergic subjects, we observed unexpectedly severe reactions to peanut in challenges using a recipe that had been modified by having its fat content increased. Here, we report the less severe reactions induced by repeat challenge with the lower-fat peanut recipe.

Materials and methods

Ethical approval for a study looking at the relationship between peanut allergens and clinical symptoms was obtained from the Southampton and SW Hampshire Local Research Ethics Committee. All subjects gave written informed consent. All DBPCFC took place in the Wellcome Trust Clinical Research Facility, which is fully equipped for physiological

monitoring and resuscitation. All subjects were well on the day of the challenge, and intravenous access was established prior to the challenge commencing.

The challenge protocol consisted of 11 active doses of increasing magnitude randomly interspersed with four placebo doses. The adequacy of blinding had been determined by tasting sessions that mimicked the challenge procedure using volunteers who were not food-allergic. Each active dose delivered 1, 2, 5, 10, 20, 50, 100, 250, 500 mg, and 1, 2, 4 g of peanut protein, respectively. Doses were given between 15 and 30 min apart depending on clinical history [9].

The first six challenges administered the 31.5% fat recipe, but the study was suspended because four of the subjects progressed further through the challenge and reacted more severely than had been anticipated from their clinical history and from their previous low-dose challenges [13]. Ethical approval was obtained to rechallenge these subjects with the 22.9% fat recipe. All rechallenges took place between 3 and 6 months after the initial challenge.

Both peanut recipes were made using the same method: cooking chocolate (32.9% fat), vanilla essence, mint essence, icing sugar, salt, commercial vegetable fat (Trex), and commercial roasted and partially defatted peanut flour (12% fat, 50% protein; Golden Peanut Company, Alphretta, Georgia, USA) were melted and mixed together for 30 min to form a homogeneous mix of ingredients. No cooking was involved. The mixture was allowed to set and then kept refrigerated until use (maximum storage 4 weeks). The only difference between the two recipes was the amount of fat used in the final formulation (22.9% fat compared with 31.5% fat), which led to a very small difference in the percentage peanut protein of the recipes (7.96% compared with 7.25%).

In vitro evaluation of the peanut challenge recipes was carried out to determine whether levels of detectable peanut differed between the two recipes. The first was a commercial sandwich ELISA (Veratox for peanut, Neogen Corporation, Lansing, Michigan, USA), which utilizes polyclonal rabbit antibodies specific for peanut and has a detection limit of 2.5 parts per million (ppm). Premarketing assessment of this commercial assay included validation in a large variety of chocolate matrices with differing amounts of fat in them (personal communication, Neogen Corporation). ELISA tests were performed according to the manufacturer's instructions.

The second system was RAST inhibition, which utilizes the serum of peanut-allergic subjects. RAST inhibition was generally performed by the method described in Adolphson et al. [14], where the solid phase was prepared by attaching roasted peanut protein to cyanogen bromide-activated microcrystalline cellulose. A standard curve was generated using an extract of partially defatted peanut flour and mixing various dilutions with the solid phase. Various dilutions of the peanut-in-chocolate challenge material extracts were also mixed with solid phase support. Pooled sera from six individuals with documented peanut allergy were added to each set of tubes. Tubes were incubated with serum overnight and washed three times with buffer. Radioactive iodine-125-labeled antihuman IgE was added and incubated overnight, washed three times with buffer, and the amount of IgE bound to the solid phase was determined by counting in a solid scintillation counter [15].

Results

In contrast to the challenge results seen with the 31.5% fat, no challenge subject reacted at a higher dose than was anticipated and all reactions were mild/moderate with the 22.9% fat recipe. Table 1 shows the reaction details of the four peanut challenges and rechallenges, and details of their most severe community reaction.

When comparing the challenge results of subjects 1, 2 and 4, it can be seen that they ate far more peanut protein in the first challenge before experiencing any symptoms than they did in the second challenge. Also, the symptoms experienced were more severe in the first challenge compared with the second. Subject 3 consumed the same dose of peanut in both challenges.

Table 1. Reactions during first and second peanut challenges

Study number	Age (years)	SPT weal (mm)	Symptoms on history	First challenge symptoms	First challenge cumulative dose (mg peanut protein)	Length of first challenge (min)	First challenge treatment	Second challenge symptoms	Second challenge cumulative dose (mg peanut protein)	Length of second challenge (min)	Second challenge treatment	Dose equivalence (31.5% fat chocolate vs. 22.9% fat chocolate)
1	32	14	Peri-oral angio-oedema, urticaria	Generalized angio-oedema, rhinitis	936	263	i.m. epi, i.v. antihista-mine, i.v. steroid	Peri-oral angio-oedema and pruritus	86	125	i.v. antihis-tamine, i.v. steroid	26 times
2	20	12	Peri-oral angio-oedema, urticaria, vomiting	Generalized urticaria, vomiting	186	170	Oral antihis-tamine, i.v. steroid, i.m. epi	Peri-oral angio-oedema and pru-ritus, vomiting	16	60	Oral antihis-tamine, i.v. antihis-tamine, oxygen	12 times
3	28	17	Abdominal pain, vomiting	Abdominal pain, nausea	36	150	Oral antihis-tamine	Abdominal pain, nausea	36	100	i.v. antihis-tamine, i.v. steroid	0
4	25	7	Peri-oral pruritus and urticaria	Nausea, ↓ peak flow	186	175	i.v. antihis-tamine	Peri-oral angio-oedema and pru-ritus, nausea	6	35	Oral antihis-tamine, i.v. antihis-tamine, i.v. steroid	31 times

RAST inhibition showed that the amount of peanut-specific IgE bound to the solid phase is inversely proportional to the amount of peanut IgE-binding sites in the extracts (Fig. 1). Similarity in the slopes of the curves generated to the slope of the standard suggests that these products contain similar allergenic epitopes [16]. It can be seen that twice as much of the higher-fat recipe was needed to cause 50% inhibition of binding, compared with the lower-fat recipe; in other words, half the allergen is available in the high-fat recipe. This indicates that in vitro, the amount of fat present has a negative effect on the inhibitory action of the extract. The mode of action may be on biological activity or may be purely due to the physical properties of the fat. Whatever the mechanism, these in vitro observations agree with the in vivo observations.

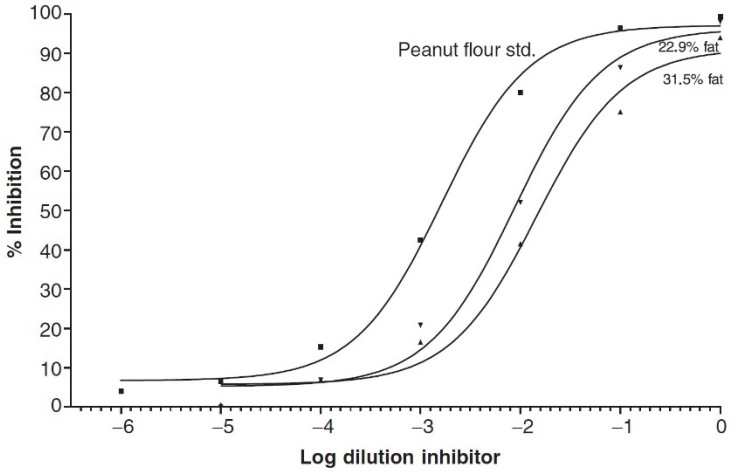

Figure 1. Comparison of peanut in chocolate challenges: 31.5% and 22.9% fat.

The ELISA results showed that there were 990 000 ppm of peanut detected in the 22.9% fat recipe but only 220 000 ppm in the 31.5% fat recipe. This result cannot be due to the nonspecific binding of fat to the plate surface, as the plate comes precoated with an anti-peanut antibody.

Discussion

As well as having physiological effects on the body such as delaying stomach emptying and increasing the release of cholecystokinin (CCK) [17], the presence of fat in food is known to influence taste perception [18]. Advances have been made into improving the acceptability of low-fat foods, but producing low-fat foods with flavors similar to their high-fat equivalent has been hard to achieve [19]. The fat content of a food has been shown to both delay the onset of taste perception and to reduce its intensity, particularly when the flavor molecule is lipophilic [20]. This is thought to be because lipophilic flavors are released more slowly from oils than from water [18]. As peanuts are such a high-fat food (46% fat), it was assumed that the unidentified flavor molecule was lipophilic; therefore,

in an effort to improve blinding, the fat content of the challenge vehicle was increased from the established recipe.

We have shown that peanut-allergic subjects consumed larger doses of allergen when the fat content of the vehicle was higher. They did not experience their oral "early warning sign" that a reaction was developing, which is generally seen in all peanut-allergic patients [21]. The lack of these early/oral symptoms is thought to be because allergens contained in a high-fat food matrix are released, and thereby absorbed, more slowly than if they were in a lower-fat matrix. Our finding suggests that food-allergic people eating high-fat foods that contain an allergen do not get the same warning symptoms as they would if the allergen was in a low-fat food or eaten in its unadulterated native form. Elimination or lack of these premonitory oral sensations results in more of the food being eaten before a reaction starts. This suggestion is substantiated by observations made during DBPCFC using capsules to disguise the food. Symptoms experienced by subjects differed from community reactions, as the allergen did not come into contact with the oral mucosa [22]. We suggest that subject 3 in our study consumed the same dose of peanut in both challenges because she had never experienced oral symptoms; masking the allergen from mast cells found in her oral mucosa did not affect her disease manifestation during challenge.

As all rechallenges occurred between 3 and 6 months after the initial challenge, we did not anticipate that any immunomodulatory effects from the first challenge would still be acting, so the reduced severity of the reactions is unlikely to be due to a hangover effect from the first challenge.

Both in vitro tests show that the presence of fat significantly inhibits allergen detection (although the effect was less marked on RAST inhibition than on ELISA). We propose that the lack of early/oral symptoms is because allergenic epitopes are concealed by the relatively high-fat food matrix and are detected only after digestion of the fat that occurs in the stomach and small intestine. Once the allergen is released from its fatty matrix and is encountered by epithelial cells, it is available to circulating allergen-specific IgE. However, as larger doses of allergen will have been eaten when this occurs, the allergic reaction will be more severe. Reactions to allergen can vary according to circumstances. Hospital-based challenges are designed to minimize confounding factors. There are few reports of repeat challenge with peanut [23]. Our data suggest that the food matrix has a critical impact on allergen availability, and we infer that it may critically affect the reactions to allergen exposure in the community.

Our study has some limitations. Firstly, the peanut concentration of each recipe was not exactly equal due to volume factors. However, the difference in peanut content was only 0.71% and would not result in the large difference seen in the detectable peanut between the two recipes, which can be explained only by a concealing effect of fat in the challenge vehicles. The results of the in vitro tests support the hypothesis that the allergen is less available in the higher-fat recipe compared with the original recipe, despite near equivalence of peanut protein. The ELISA results indicate that it is harder to extract peanut proteins from the higher-fat recipe, and this has implications in using ELISA test kits for detecting peanut in different types of foods.

Secondly, it has been reported that certain methods of food processing can enhance, reduce, or eliminate the allergenic potential of a food [6, 24], but these studies have focused

on the effect of processing on specific IgE binding in vitro and not on the bioavailability of the allergen. Our findings are very important for food allergy sufferers, who appear to experience more reactions to foods prepared by others, in restaurants, etc. It could explain the strong tendency for fatal reactions to occur to hidden rather than native allergens.

Thirdly, the numbers involved are small, and a more complete observation could be made if additional challenges were carried out. However, it would be unethical to ask peanut-allergic patients to undergo two challenges, one of which would be likely to cause a severe reaction. We obtained ethical approval to rechallenge four subjects, as they had already had a serious reaction and rechallenge would clinically clarify the unexpected nature of their previous challenge result.

Finally, reactivity may vary with time, but this variability can usually be accounted for by other circumstances such as location and dose [25]. By repeating the challenges in the same location, to the same protocol, with everything identical apart from the fat content of the vehicle, this variability in reactivity can be considered to be minimal by comparison.

Conclusion

From our observations from DBPCFC, we have been able to show that the fat content of challenge recipes has a profound effect on the reaction of the challenge subject. In vitro tests also show that fat has an effect on allergen bioavailability. This finding is significant for all people with food allergy, physicians involved in the treatment of food-allergic patients, food and catering industries, and all people working in restaurants and other eating establishments.

The knowledge that it is not just the presence of an allergen in a food that is important but how it behaves in a food matrix adds another dimension to clinical and research practice and to the approaches taken by the food and catering industry to the issue of food safety for food-allergic consumers.

Finally, when complex food matrices are being further investigated, the in vitro test used must be considered carefully, as the measured allergen content of the food preparation may differ considerably between tests because of the effects of food matrices on the efficiency of allergy extraction.

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