

Original article

Double-blind placebo-controlled challenges for peanut allergy the efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes

Background: A firm diagnosis of double-blind placebo-controlled food challenge (DBPCFC) would facilitate the diagnosis in patients with uncertain history of reaction. Guidelines are lacking for an upper provoking dose and how to hide high concentrations of peanuts.

Aim: To develop and evaluate a double-blind recipe with minimum 10% of peanut. To compare the recipe with published recipes regarding blindness, taste, texture and immunoglobulin (Ig)E antibody binding to peanut.

Methods: A recipe (I) with 10% of peanut was developed evaluated and used in DBPCFC. The challenges were followed by development of a concentrated recipe (II) (15% peanut, 25% fat). Recipe II was compared with the only published recipe (III) (11% peanut, 7% fat) regarding taste, texture and availability of peanut. Recipe IV (12% peanut, 10% fat) was developed using the same methods. The binding of IgE in the recipes was measured using an inhibition method.

Results: During challenges, one patient reacted after 4 g, emphasizing the need for blinding recipes containing high doses of peanut. Evaluation between recipes II and III, only recipe II was regarded as blind by the taste panels. A tenfold lower availability of peanut protein in the recipe II was found at 50% of inhibition. Recipe IV had a better IgE binding that did not differ from the original peanut extract.

Conclusion: The peanut taste and texture can be hidden in a challenge medium. The fat content was important for the availability of the allergenic protein in challenges. The availability of allergens must be taken into consideration when used for DBPCFC.

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Key words: double-blind placebo-controlled food challenge; fat; immunoglobulin E; peanut hypersensitivity; symptoms.

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Background

Peanuts are reported as one of the most fatal food allergens (1) and can be common even in countries with low peanut consumption (2). Because of its anaphylactic nature, diagnosis of peanut allergy is based on patient's history and measurements of quantitative immunoglobulin (Ig)E antibodies in blood and/or in combination with skin tests (3).

The double-blind placebo-controlled food challenges (DBPCFC) have been standardized regarding patient selection, setting and challenge procedure (4, 5), but not how to optimize the blinding of the challenge without

affecting the reactivity. Thus, the information on how to prepare these recipes in practice has not been well documented, especially not for peanut. Hiding peanuts are complicated by its characteristic taste, especially after the roasting procedure. Roasted peanuts have been found to bind higher levels of IgE than raw or boiled steamed peanuts, which may affect the outcome of the clinical reaction (6).

About 5% of peanut allergic patients reacts to 1 mg of peanut or less (3), and this is the proposed starting dose in patients with suspected immediate reactions (5). Such small amount is easily hidden in a challenge vehicle, and was described by several authors with the primary aim to find the minimum provoking of for challenges of peanut and hazelnut (7–10). When challenges are performed for establishment or exclusion of diagnosis in less sensitive subjects or in subjects with no previous reaction, the reacting dose may be much higher. In a study where

Abbreviations: DBPCFC, double-blind placebo-controlled food challenge; KuA/l, kilo unit antibodies per litre; SIK, The Swedish Institute for Food and Biotechnology, Sweden.

103 patients were challenged for peanut around 40% reacted to a cumulative dose between 0.9–7 g (11).

There is also little information available how efficient the suspected ingredient was masked. This problem was recently elucidated but the recipes used were not published (12). In another study, food challenges with low doses of peanut (0.4% peanut) were evaluated but a drawback was the use of nose clips and that the availability of peanut was not assessed (13).

A recent publication has also raised the question whether the fat content of a challenge vehicle may affect the outcome of the challenge, as seen in a case report (14). It has not been clarified further how fat affects the availability of the allergen.

The aim of the current study was to:

1. Develop a recipe suitable for DBPCFC where more than 10% (or 1 g) of peanut could be disguised and to use such a recipe in DBPCFC.
2. To compare the developed recipes with already published recipes regarding blinding of peanut, taste and texture and to test the availability of the peanut in the recipes to bind IgE antibodies.

Methods

Development and evaluation of recipes

Pure cocoa powder, caster sugar, gluten free oatmeal, and milk free margarine was used to make a mouldable mixture for the test vehicle. The oatmeal sweets were provided with up to 10% of peanut and evaluated in sensorial tests as blind and used in DBPCFC (results not shown).

As a result of the comments about the rich taste of the challenge product from the test panel and from the patients, recipe I was modified to provide smaller size with higher concentrations of peanut without affecting the blindness. Increased concentration of mint oil was used together with changed amount and structure of oat exchanging cocoa powder for dark chocolate. This recipe (II) contained 15% of peanut and 25% fat.

Searching the literature, only one published recipe could be found (4). This recipe has been used in different studies with some modifications of the ingredients (3, 8, 9). To test if recipe II was comparable with regard to masked taste of peanut, and detectable peanut protein in the published recipe (III), both recipes were evaluated. In the active recipe, roasted peanuts were washed and grounded three times in a food mill, strained and air-dried. In the placebo recipe as well as in the active recipe, crushed oat was roasted dry together with salt to resemble roasted peanut (Table 1). All ingredients were measured on a digital scale (Mettler PC 4400; Mettler Toledo GmbH, Greifensee, Switzerland) and the recipes were calculated using a computer programme.

Analysis by taste panels

Sensorial evaluations using triangle tests were performed with test panels comprising of students at Göteborg University, where the objective to discover a perceivable difference between the three samples (15). The tests were performed individually and all subjects received both verbal and written information. A panel of 46 persons received two separate samples (Recipe II and III) with three samples

Table 1. Description of the developed recipes

	Active	Placebo
Recipe I		
Cocoa powder (g)	20	20
Caster sugar (g)	90	90
Oatmeal (g)	80	110
Peppermint oil (drops)	20	20
Milk free margarine 80% fat (g)	80	80
Moulded roasted peanut (g)	30 g	–
Salt (g)	1 g	2 g
Pearl sugar (g/item)	1 g	1 g
Total amount	301	302
Concentration of peanut and fat (%)	9.7% (29%)	–
Content of peanut (peanut protein)	1 g (0.24 g)	–
per 10 g serving		
Recipe II		
Dark milk free chocolate 70% (g)	50	50
Icing sugar (g)	40	40
Crushed roasted oat (g)	30	40
Oatmeal (g)	5	15
Peppermint oil (drops)	20	20
Milk free margarine 80% fat (g)	15	15
Moulded roasted peanut (g)	25	–
Salt (g)	1	2
Pearl sugar (g/item)	1	1
Total amount	166	162
Concentration of peanut and fat (%)	15% (25%)	–
Content of peanut (peanut protein)	1.5 g (0.35 g)	–
per 10 g serving		
Recipe IV		
Dark milk free chocolate 70% (g)	30	30
Cocoa powder (g)	10	10
Icing sugar (g)	40	40
Crushed roasted oats (g)	20	20
Oatmeal (g)	20	42
Peppermint oil (drops)	20 drops	20 drops
Oatbased custard cream + 'RAPS' Frutty' (g)	50	50
Moulded roasted peanut (g)	22	–
Salt (g)	1	2
Pearl sugar (g/item)	1	1
Total amount (g)	193	194
Concentration peanut and fat (%)	11% (12%)	–
Content of peanut (peanut protein)	1.1 g (0.26 g)	–
per 10 g serving		

to be consumed during 2 days. Water was allowed for rinsing the mouth before and after testing. All test vehicles were served cool in medical pots coded for each participant. Both panels were asked to assess the recipes regarding texture and edibility. The questions were scored with a scale with a 5-graded scale (unacceptable, not so good, neutral, good, very good).

Using the same protocol, recipe (IV) with lower fat content (12%) and 11% of peanut was also developed and tested separately on a panel of 23 persons. Recipe IV had a lower chocolate content altered with cocoa powder. An oat based custard cream (OATLY) mixed with rice yoghurt (RAPS), was used instead of margarine.

Binding of peanut to IgE antibodies

In vitro evaluation of peanut availability in all three recipes was carried out with inhibition of IgE antibody binding utilizing a

commercial test (Pharmacia Diagnostics AB, Uppsala, Sweden) and by inhibition on microplates coated with peanut allergens. The recipes were adjusted to contain 10% of peanut each. The inhibition was performed using a standard method (16).

Statistics

For comparison of distributions between different groups in the evaluation of recipes the chi²-test was used. The cut off values for critical numbers corresponds to the reference material of triangle tests (15).

Results

Development of recipe I

Recipe I was regarded as a good base recipe. No detectable peanut taste was reported by the first test panel and was well tolerated and used in the DBPCFC.

Blinding and evaluation of recipe II–IV

The panel could not identify which sample in the triangle test that contained peanut, despite the higher concentration of peanut in recipe II. There were significantly fewer subjects that could detect the peanut taste in recipe II compared to recipe III (Table 2). Thirty-seven per cent (17 of 46) could identify the peanut taste, compared with 74% (34 of 46) in the test of recipe III ($P < 0.001$). Only recipe II had less correct answers than expected in the triangle test according to the cut off for critical number of correct answers ($P < 0.05$) (15).

The oat recipe (II) was also preferred with regard to taste and texture compared with the rice recipe (III). Recipe II received higher scores than recipe III, since 12% (17 of 138 [each subject responded to three samples each]) scored this recipe as ‘not good’ or ‘unacceptable’ compared with recipe III where 46% (64 of 138) gave score ‘not good’ or ‘unacceptable’ ($P < 0.001$). The difference was even more clear with regard to texture where 29% (39 of 138) scored recipe II as ‘not good’ or ‘unacceptable’ compared to 62% (85 of 138) in recipe III ($P < 0.001$).

To optimize the developed recipe, a sensorial panel of 23 subjects evaluated recipe IV. There was no significant difference between the recipes measured by the same triangle test method as previously used (Table 2).

Table 2. Comparison of test of detection of peanut taste in the recipes, number of correct answers

	Recipe II Correct answers 16% peanut	Recipe III Correct answers 11% g peanut	Recipe IV Correct answers 11% peanut
Correct answer			
Panel	17/46*	34/46 ns	9/23*

*No significant difference in taste measured as maximum of correct answers for a level of significant difference ($P < 0.05$) between the products.

Inhibition of IgE antibody binding

The inhibition of recipes II and III showed both recipes had less allergen available than the standard product during the inhibition procedure. There was a 10-fold lower degree of binding in recipe II compared with recipe III at 50% of inhibition showing that fat content has a negative effect on the inhibition of the extract. Five different preparations of the three recipes showed the same inhibition results excluding the possibility that the difference seen could be due to sample preparation (data not shown). In the evaluation between recipe III and IV there was no difference in availability of peanut protein between recipe IV and the peanut extract (Fig. 1B).

Discussion

We have shown that it is possible to hide large concentrations of peanuts. The developed recipe with high content of fat, that was better for blinding and preferred with regard to taste, also was less effective with regard to accessible peanut allergens. As a consequence, we continued to develop a recipe with lower fat content, documented undetectable peanut taste and with better availability of peanut protein.

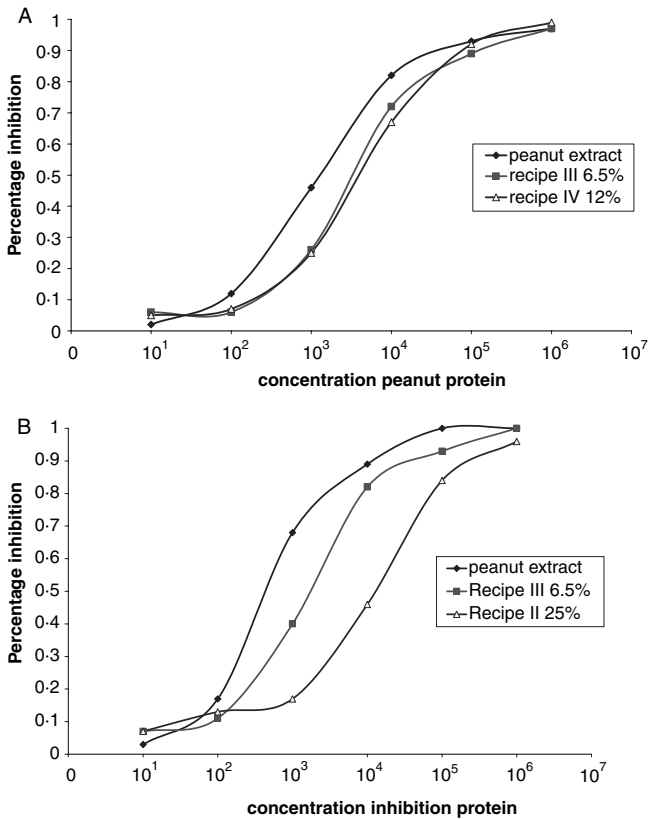


Figure 1. Inhibition of peanut extract: (A) recipe II with 25% fat, and recipe III with 6.5% fat, (B) recipe III with 6.5% fat, and recipe IV with 12% fat.

The triangle test, that was used as a test model, is the recommended method when the test objective is to determine whether a sensory difference exists between two products (15). The evaluation of the vehicles is important as there is a risk that subjective symptoms can play a role when evaluating the challenge result if the taste is not properly hidden. The test panels considered the oatmeal recipes more tasteful, which can also be of importance when increasing the challenge doses or when challenging children.

To combine a recipe with an undetectable taste of peanut with few hypoallergenic ingredients and a low fat content can be difficult. We have shown a pronounced decrease in binding of the peanut protein in recipes with high fat content, by using an *in vitro* assessment. A similar finding has been presented where four patients with repeated challenges reported longer onset of symptoms and more severe symptoms in a peanut challenge with high fat (31.5%) compared with lower fat (23%) (14). This result could be explained by our findings *in vitro*. The fat content might play an important role when evaluating a food challenge. It may prevent IgE antibodies to bind to the epitopes, with a risk of negative outcome or delayed reaction, which could have serious implications for the patient.

Conclusion

We have in several steps developed a double-blind recipe for peanut challenges. With its high content of peanut and agreeable taste and texture it is suitable for challenges for both children and adults. The oatmeal recipe was also better in hiding peanut taste despite a higher peanut content than an already published recipe. As the inhibition method showed a decreased availability of binding IgE antibodies in the high fat recipe, the amount of available peanut allergen may be lower in a recipe with high fat content. The consequence of the fat content in food challenges must be taken into consideration when creating standardization procedures for DBPCFC.

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