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An Evaluation of the Sensitivity of Subjects with Peanut Allergy to Very Low Doses of Peanut Protein: A Randomized, Double-Blind, Placebo-Controlled Food Challenge Study

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Background
The minimum dose of food protein to which subjects with food allergy have reacted in double-blind, placebo-controlled food challenges is between 50 and 100 mg. However, subjects with peanut allergy often report severe reactions after minimal contact with peanuts, even through intact skin. *Objective:* We sought to determine whether adults previously proven by challenge to be allergic to peanut react to very low doses of peanut protein. *Methods:* We used a randomized, double-blind, placebo-controlled food challenge of 14 subjects allergic to peanuts with doses of peanut ranging from 10 μg to 50 mg, administered in the form of a commercially available peanut flour. *Results:* One subject had a systemic reaction to 5 mg of peanut protein, and two subjects had mild objective reactions to 2 mg and 50 mg of peanut protein, respectively. Five subjects had mild subjective reactions (1 to 5 mg and 4 to 50 mg). All subjects with convincing objective reactions had short-lived subjective reactions to

preceding doses, as low as 100 μg in two cases. Five subjects did not react to any dose up to 50 mg. *Conclusion:* Even in a group of well-characterized, highly sensitive subjects with peanut allergy, the threshold dose of peanut protein varies. As little as 100 μg of peanut protein provokes symptoms in some subjects with peanut allergy.

Keywords: peanut allergy, food challenge, low dose

Abbreviations
DBPCFC: Double-blind, placebo-controlled food challenge
OAS: Oral allergy syndrome

Peanut allergy is an increasingly common manifestation of atopy.[1,2] It is clinically characterized by the usually rapid onset of symptoms after exposure to small amounts of peanut protein. Symptoms are often severe,[3–5] and peanut allergy is considered to persist indefinitely.[6]

Peanut protein is consumed in several forms. Infants and young children eat peanut protein almost exclusively in the form of peanut butter, whereas older children and adults also eat the kernels as a snack food. Roasting of peanut kernels does not appear to decrease their in vitro allergenicity.[7] Peanut protein can also be eaten in the form of flour, having been defatted and roasted before grinding. Peanut flours have been shown to bind peanut-specific IgE.[8]

The threshold dose of peanut protein is likely to vary among subjects. There have been anecdotal reports (not supported by challenge studies) of subjects reacting strongly to the smell of peanuts or to being in the vicinity of an open jar of peanut butter.[9,10] The dose of presumably airborne peanut protein involved in these reactions must be very low. The more common scenario is an allergic reaction after a minimal contact with peanuts,[5] through intact skin (e.g., being touched by someone who has handled peanuts, accidental ingestion of small amounts of peanut protein, or eating bread buttered with a knife previously used to make a peanut butter sandwich for someone else).

Crude peanut oil can contain allergenic peanut protein. In contrast, refined oil does not contain allergenic protein.[11] In a study of the in vivo allergenicity of peanut oils, only six of 60 subjects with peanut allergy reacted to crude peanut oil, but no subject reacted to refined peanut oil.[12]

The gold standard for diagnosis of a food allergy is a double-blind, placebo-controlled food challenge (DBPCFC).[13] The minimum doses of protein that have elicited definite reactions in DBPCFCs have been between 50 and 100 mg, administered in capsules.[14,16] Despite the apparent sensitivity of subjects with peanut allergy to very low doses of peanut protein, the precise sensitivity of these subjects (or conversely the minimum provoking dose of peanut protein) has not been established. The identification of such a threshold dose would have considerable implications for such subjects, the food and catering industries, and the appropriate regulatory authorities.

Oral allergy syndrome (OAS) is often elicited by minimal contact with food proteins.[17] Subjects with peanut allergy often report OAS.[5] The minimum dose of protein that elicits OAS has, to our knowledge, not been documented because, until recently, most food

challenges have used capsules that bypass the oral mucosa. More recently, real-life exposures have been mimicked in challenges by using test doses in milk shakes, drinks, and solid meals.[13]

Using a commercially available peanut flour in very small doses (equivalent to a dose of peanut protein as low as 10 μg), we investigated whether subjects with peanut allergy react to lower doses of protein than have been demonstrated to cause reactions in studies of other types of food allergy.

Methods

Subjects

Fourteen adult subjects (2 men and 12 women), from a group of 60 subjects who participated in a previous study of the in vivo allergenicity of peanut oils, were enrolled in the study.[12] Six subjects (nos. 2, 3, 4, 8, 10, and 13) were selected for this study on the basis of having reacted to the crude peanut oil (implying high sensitivity). Two of the six subjects who reacted to crude oil also had positive reactions to peanut challenge, and four were not challenged with peanut protein after reacting to the crude peanut oil. Eight other subjects were enrolled. They had not reacted to peanut oils but had reacted to the minimum dose in the open peanut challenge (peanut protein rubbed 10 times on the left side of the lower lip). All subjects were in good general health at the time of the DBPCFC. Nine subjects had successfully avoided peanuts since their last visit for the oil challenge. Five had taken inhaled β-agonists (including one who had also used inhaled fluticasone) on the day of the challenge. Intravenous access was established, and the challenges were conducted in a dedicated research ward that was fully equipped for physiologic monitoring and resuscitation.[18]

Ethical approval and consent

Ethical approval for this study was obtained from the regional ethics subcommittee. All subjects gave written informed consent.

Peanut flour

A food-grade peanut flour was obtained from Pert Labs/Seabrook Enterprises. The product information supplied stated that the peanuts were 85% defatted, roasted, and ground to the consistency of wheat flour. The protein content of the flour was found by Kjeldahl's analysis to be 46.23% of the dry weight.[19]

Doses

The challenge started with a dose of 10 μg of peanut protein (21.63 μg of flour). Doses increased stepwise thereafter: 20 μg, 50 μg, 100 μg, 250 μg, 500 μg, 1 mg, 2 mg, 5 mg, 10 mg, 20 mg, up to a maximum dose of 50 mg (108.15 mg of flour).

Dose manufacture

The 24 test doses (12 peanut protein and 12 placebo) were manufactured by the pharmacy department of Southampton University Hospital National Health Service Trust. To disguise the color of the flour, each dose and placebo was combined with commercial

wholegrain wheat flour bought in a local supermarket up to a total weight of 200 mg. The dose schedule of peanut flour or placebo was randomly allocated by the pharmacy and supplied in a sealed envelope with the containers.

Blinding and challenge process

The 12 peanut protein doses were randomly interspersed with an equal number of placebo doses.[13,20] Rice pudding was used as the vehicle, and peppermint or cocoa was used as flavoring.[12] No subject was able to detect any taste of peanut in any dose. The doses (peanut and placebo) were administered by a research nurse who was blinded to the sequence. The supervising investigator was blinded to the order either until a reaction severe enough to terminate the challenge was provoked or until the twenty-fourth dose had been consumed without any observable reaction. Subjects were not aware of the total or relative number of doses or placebos to be ingested. To minimize anticipation of increasing doses and the increasing likelihood of reaction during the series, the subjects were told the doses were in random, not increasing, order.

Assessment of reactions

If no reaction to an administered dose was reported or observed, a 10- to 15-minute interval was allowed to elapse before the next dose.

Subjective reactions consisted of symptoms that were not supported by objectively measurable signs. Subjective reactions did not usually require cessation of the challenge and were allowed to resolve completely before the subject was asked to continue with the next dose.

Objective reactions were those in which symptoms were supported by observable signs such as (ranging from mildest to most severe) urticaria, rhinitis, lip swelling, angioedema, wheeze, or hypotension.

Results

Results for each subject are shown in Table I. No subject reacted to any dose of peanut protein from 10 to 50 μg. Five subjects did not react to any dose of peanut flour. Four challenges were stopped before the twenty-fourth dose was taken. In one subject (number 1) the dose that caused the challenge to be terminated was shown, when "unblinded," to have been a placebo dose. Challenge of patient 2 was stopped after the seventeenth dose (5 mg of peanut protein) caused her to become anxious and she reported lip tingling and oral itching (see Table II). She had reported similar, less severe symptoms in response to each dose from 250 μg upward and no reaction to any placebo dose. The chances of making a 50/50 guess correctly 17 times in a row is 0.5^{17} or 1 in 131,072. Two other terminated challenges had objectively positive results and are described later. One other subject had an objectively positive reaction to the 50 mg dose. Four subjects had subjective reactions to the highest dose (50 mg) not supported by measurable signs of reaction.

Table I. DBPCFC with peanut flour

Patient No.	Age (yr)	SPT wheal (mm)	Duration of challenge (min)	Total no. of doses (peanut and placebo)	Reactions to preceding doses	Provoking dose
1	26	7	115	11		Placebo
2	17	10	240	18	250 μg, 500 μg, 1 mg, 2 mg	5 mg
3	37	10	330	24	5 mg, 10 mg, 20 mg	50 mg
4	18	12	300	23	10 mg, 20 mg	50 mg
5	32	5	290	24		None
6	37	20	315	24		None
7	34	6	280	24		None
8	17	10	320	24	10 mg, 20 mg	50 mg
9	26	10	345	24	5 mg, 20 mg	50 mg
10	24	15	195	17	100 μg, 250 μg, 1 mg	5 mg
11	20	18	205	17	100 μg, 250 μg, 500 μg, 1 mg	2 mg
12	26	9	315	24		None
13	16	15	390	24	1 mg, 2 mg, 5 mg, 10 mg, 20 mg	50 mg
14	29	9	295	24		None

Table II. Double-blind peanut flour challenge of patient 2 (consistent subjective reactions)

Dose No.	Peanut protein dose or placebo	Reaction	Dose No.	Peanut protein dose or placebo	Reaction
1	10 μg	Nil	13	P	Nil
2	20 μg	Nil	14	P	Nil
3	P	Nil	15	l mg	Lip tingling (resolved)
4	P	Nil	16	P	Nil
5	P	Nil	17	2 mg	Mild lip tingling (resolved)
6	50 μg	Nil	18	5 mg	Anxiety, both lips tingling, throat sore
7	P	Nil	19	P	NA
8	100 μg	Nil	20	10 mg	NA
9	P	Nil	21	20 mg	NA
10	P	Nil	22	P	NA
11	250 μg	Warm feeling in throat (resolved)	23	P	NA
12	500 μg	Scratching in throat (resolved)	24	50 mg	NA

P, Placebo; NA, not administered

Minor subjective reactions to placebo were seen in seven cases, including both of the challenges with objectively positive results that were stopped prematurely. Most of the other minor subjective reactions were to early doses and may have been related to anxiety caused by the challenge procedure or possibly to the short dose interval.

In two cases (subjects 3 and 13), a sequence of early subjective reactions prompted the unblinding of a doctor not involved in the challenge process and maintaining the blinding of the doctor supervising the challenge. In both cases the challenge was continued.

Subject 3 had reacted to three of the first four doses (in order: placebo, placebo, 20 μg) but of the next 20 reacted only subjectively to the peanut doses from 5 mg upward. Her series was completed with no reaction to two placebo doses after the top dose of 50 mg was consumed as the twenty-second dose. The chances of getting 20 consecutive 50/50 guesses correct is 1 in 1,048,576.

Subject 13 reported symptoms after five of the first 11 doses. Eventual unblinding revealed that four of these early subjective reactions were to placebo. For the 13 subsequent doses, she reported subjective symptoms in response to only real doses from 1 mg upward and not to any placebo dose (a chance of 1 in 8192).

Two subjects (subjects 10 and 11) reacted objectively to doses of peanut flour, and the challenge was stopped before the last dose. One other subject (subject 4) reacted mildly to the top challenge dose (50 mg). All had premonitory, short-lived subjective symptoms to doses lower than the dose that caused the challenge to be stopped (see Table III). The three objectively convincing reactions are described briefly below.

Table III. Double-blind peanut flour challenge of patient 10 (systemic reaction to 5 mg dose)

Dose No.	Peanut protein dose or placebo	Reaction	Dose No.	Peanut protein dose or placebo	Reaction
1	10 μg	Nil	13	P	Nil
2	20 μg	Nil	14	P	Nil
3	P	Nil	15	l mg	Subjective feeling of lip swelling
4	P	Nil	16	2 mg	Nil
5	P	Nil	17	5 mg	Lip and tongue itching, nausea, vomiting, urticaria, shivering, wheeze
6	50 μg	Nil	18	P	NA
7	P	Nil	19	10 mg	NA
8	P	Nil	20	20 mg	NA
9	100 μg	Lip tingling (resolved)	21	P	NA
10	250 μg	Lip tingling (resolved)	22	P	NA
11	500 μg	Nil	23	50 mg	NA
12	P	Nil	24	P	NA

P, Placebo; NA, not administered

Subject 4 reacted to the top dose of 50 mg with flushing and oral itching. She had reported throat itching and tingling in response to 10 mg and 20 mg doses but also had throat tingling in response to one earlier placebo dose.

Subject 10 had a systemic reaction to the 5 mg dose after minor subjective reactions to doses of 100 μg, 250 μg, and 1 mg. No reaction to the 2 mg dose or any placebo dose was observed. The reaction started with itching and lip swelling but progressed over 1 hour to

nausea, shivering, and subjective wheeze. Blood pressure and peak expiratory flow rate remained normal throughout. She vomited at 1 hour, and her symptoms decreased considerably thereafter. She was treated with inhaled epinephrine and intravenous antihistamines.

Subject 11 reported oropharyngeal itching and mild lip swelling after the 2 mg dose. Preceding subjective oral symptoms had been reported in response to doses of 100 μg, 250 μg, 500 μg, and 1 mg. She reacted subjectively to the placebo dose immediately preceding the 2 mg dose to which the significant reaction was observed.

Discussion

Peanut allergy is the most common cause of fatal food-related allergic reactions.[3,4] Many subjects react to foods in which the initial source of peanut is not obvious, and they may occasionally have been reassured that peanuts were absent from the offending food.[21] The implication is that the food has become adulterated with peanut during the preparation of the meal. The dose of peanut that has adulterated the meal must be low, or persons without peanut allergy would be able to detect a peanut flavor. Anecdotally, many subjects with peanut allergy report being able to detect peanuts in foods that do not taste of peanut to other persons.

The reaction witnessed in each patient on this occasion was similar to that observed by the same supervising clinician in the previous study of peanut oils.[12] The threshold dose of peanut appears to vary with time. Some of the subjects who had reacted to the crude peanut oil did not react to any dose in this study. Conversely, others who had not reacted to the crude peanut oil had reactions to very low doses of peanut protein in this DBPCFC study.

DBPCFCs have shown objective reactivity to doses as low as 50 to 100 mg of protein.[14–16] This project has shown that convincing reactions are inducible with doses as low as 2 mg of peanut protein. Each of the subjects who had a convincing reaction to peanuts also had reported short-lived symptoms to doses as low as 100 μg.

These symptoms were not supported by objective, measurable signs but were convincing both to the patient and to the investigator. This study therefore shows evidence of an in vivo dose effect; reactions to the lowest doses (100 μg) were subjective, short-lived, and well tolerated. Increasing doses caused more generalized and long-lasting reactions.

In real life it is clear that a dose-response relationship exists and that sensitivity may vary with time or circumstances. The provoking dose may vary according to the target organ.

Cumulative dose of peanut protein	Reaction	Clinical impression
0.43 mg	Oral itching	Reaction to placebo
8.93 mg	Lip tingling, throat itching, anxiety	Subjective reaction to 5 mg dose
88.93 mg	Itchy throat	Subjective reaction to 50 mg dose
88.93 mg	Oral itching, facial flushing, felt cold	Mild reaction to 50 mg dose
88.93 mg		No reaction
88.93 mg		No reaction
88.93 mg		No reaction
88.93 mg	Throat itching	Subjective reaction to 50 mg dose
88.93 mg	Throat itching and tingling	Subjective reaction to 50 mg dose
8.93 mg	Lip swelling, urticaria, vomiting, wheeze	Systemic reaction to 5 mg dose
2.93 mg	Lip swelling, throat itching	Mild reaction to 2 mg dose
88.93 mg		No reaction
88.93 mg	Throat itching, lip itching	Subjective reaction to 50 mg dose
88.93 mg		No reaction

The protocol for this study differed from those of other studies, with regard to provoking doses, in several ways. Placebo and real doses were administered randomly in succession. This abolishes an order effect, which can be evident when a challenge is undertaken with placebo or a real test substance in a separate series. The use of doses in real foods clearly mimics a real-life situation more closely than does the use of capsules, which bypass contact with the oral mucosa. Oral allergy reactions are very common with peanuts,[5] and the elicitation of such reactions at low doses in this study clearly validates our approach. Because of the differences outlined, particularly delivery of the test dose in food rather than in capsules, the results cannot be compared with those of other challenge studies. Such studies of other foods would need to be repeated with a protocol similar to ours for such comparisons to be made.

There were frequent subjective reactions to placebo doses, probably caused by the prolonged nature of the challenge (24 doses). Only one challenge had to be stopped because of a reaction to a placebo, and another because of a sufficiently marked subjective reaction (patient 2, Table II). In the course of this DBPCFC study, we found it useful for an uninvolved physician to review the dose schedule of two subjects who were reporting unexpectedly frequent subjective symptoms. In both cases the challenge was continued, the reactions became consistent, and they were reported only after real doses of peanut protein rather than placebo after initial anxiety had settled. The decision to continue the challenge may have influenced the investigator, who remained blinded, but it was believed that the decision could only have increased his necessary clinical scepticism and improved the objective evaluation of the challenges. The usefulness of unblinding an experienced physician, who is uninvolved in the challenge itself, is an important additional feature of DBPCFCs, which increases their objectivity.

The implications of this study (that individuals with peanut allergy react, albeit mildly, to doses of peanut protein as low as 100 μg) are considerable for the food and catering industries and regulatory authorities. This study and previous clinical studies of peanut

oils[6,12,22] and clinical reports of reactions to foods cross-contaminated with peanut[23] show that extreme care must be taken in the preparation of foods that contain commonly allergenic ingredients such as peanuts and, by extrapolation, tree nuts, fish, and shellfish. More care needs to be taken in the catering industries to minimize the risk of cross-contamination of foods, both in the manufacture of prepared, prepackaged foods and in the preparation of restaurant and cafe meals. Similarly designed studies of subjects allergic to other foods are required to evaluate possible threshold doses of each allergenic food.

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