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ORIGINAL RESEARCH—ALLERGY

Potential non–immunoglobulin E–mediated food allergies: Comparison of open challenge and double-blind placebo-controlled food challenge

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OBJECTIVE: Comparison of open food challenge (OFC) with double-blind placebo-controlled food challenge (DBPCFC).
STUDY DESIGN: Prospective sequential randomized challenges.
METHODS: Twenty adults with chronic allergy symptoms and at least 1 positive intradermal food wheal response recorded symptoms during DBPCFC and OFC provoked using organic foods, normal portions, and normal food preparation. Acoustic Rhinometry and biochemical tests were done during DBPCFC.
RESULTS: All patients reacted to at least 1 food and to all challenges with the same food, with multiorgan symptoms in the nose, nervous system, throat, and lung. There was a correlation in the type and severity of symptoms ($P = 0.015$) for OFC and DBPCFC, and both were significantly ($P < 0.01$) more severe than placebo. Compared with DBPCFC, OFC sensitivity was 66%, and positive predictive value was 89%.
CONCLUSION: This is the first study showing both concordance of OFC and DBPCFC and also that intradermal tests can identify reactive foods that can be verified by DBPCFC. Because most tests for IgE-mediated food allergy were negative, observed reactions were probably non–IgE mediated.

Food allergy, intolerance, or hypersensitivity, however defined, remains one of the most controversial subjects in modern medicine. This is because of the complexity of the mechanisms underlying the disease and the lack of universally accepted definitions or diagnostic tools for identifying the various types of food allergy.

Allergic food reactions may occur in any organ, regardless of the immunopathogenic mechanism.[1] Skin-prick testing (SPT) or in vitro serum-specific IgE (sIgE) testing are accurate diagnostic methods for IgE-mediated food sensitivity;[1,2] negative SPT or sIgE confirm the absence of IgE-mediated reactivity (negative predictive accuracy >95%).[2,3] Some food allergy studies have shown negative SPT or sIgE in patients with positive oral food challenges.[4-8] Except for challenges, there has been no universally accepted diagnostic test for food reactions that do not appear to involve IgE. However, various forms of intradermal tests have been used to attempt to identify non-IgE food allergies. These tests have been controversial,[9] and, although 1 study compared intradermal progressive dilution food testing (IPDFT) with open challenges,[10] no intradermal food test has been compared with blinded challenges.

Double-blind placebo-controlled food challenges (DBPCFC) are accepted as the gold standard but are not standardized.[11] Details that need standardization include (1) method(s) to identify what foods are tested; (2) methods to disguise foods for DBPCFC; (3) food purity (lack of chemical contaminants); (4) food preparation methods to retain the same allergenicity as in normally consumed food; (5) determining a starting dose quantity; (6) when safe, challenging with large, normal-sized portions of food; (7) length of prechallenge washout period; (8) length of time to consume all of the test food, (9) length of observation period between completing ingestion and symptom development; (10) minimum time between two challenges that allows symptom clearing; (11) number of repetitions required to confirm a negative or equivocal challenge; and (12) a complete list of symptoms to be evaluated.

Open food challenges are much easier to perform than DBPCFC and are widely used for office diagnosis,[12,13] but there is no standard protocol or data validating the value of open challenge as compared with DBPCFC. This study was performed to compare results of open food challenges with DBPCFC, with careful examination of the 12 variables listed previously. We used organic foods; standard portion sizes; preparation and masking methods similar to normal meals; and adequate, experimentally determined, prechallenge washout, postchallenge observation, and intervals between challenges.

METHODS

The National University Hospital Institutional Review Board approved all procedures, and all patients gave in-

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formed consent. Twenty patients, 6 men and 14 women, aged 25 to 61 years (mean age, 46), 18 Asians and 2 Caucasians, all Singapore residents, were recruited from the National University Hospital ENT allergy clinic or a Camden Medical Center, Singapore, private clinic. All of them had food allergy compatible chronic symptoms, no food anaphylaxis history, and at least 1 positive intradermal skin wheal response to a commonly eaten food. They were not allowed any antiallergic drugs including H1 antihistamines, chromoglycate, leukotriene antagonists, nonsteroidal anti-inflammatory agents, and oral or topical steroids for 1 month before and during the entire study.

ALLERGY TESTS

There is no agreement on the value of pre-DBPCFC screening tests including in vitro serum specific IgE (sIgE) for inhalant or food antigens and in vivo skin-prick tests or intradermal tests.[11] Therefore, in this study, multiple techniques have been used and compared with open food challenge (OFC) and DBPCFC results.

Intradermal Progressive Dilution Food Test

Common positive foods in Singapore were tested, including the following hidden ingredient foods: baker's yeast, black pepper, corn, egg, garlic, soy, malt, cow's milk, and wheat. Also tested were all foods a specific patient ate twice or more per week, including apple, banana, barley, beef, brewer's yeast, carrot, cabbage, chicken, chili, chocolate, coconut, coffee, grape, ginger, oats, onion, orange, oyster, papaya, peanut, pork, potato, rice, rye, sesame, tea, and tomato. Food extracts, 1:20 weight/volume (W/V) in 50% glycerin (Antigen Laboratories Inc, Liberty, MO), were diluted with 0.9% saline containing 0.4% phenol. Negative concentration-matched glycerin,[14] and positive histamine controls were used. All patients denied food anaphylaxis and were eating all foods to be tested. IPDFT used 0.05-mL intradermal wheals of 1:100 W/V diluted food extracts.[10] A positive wheal size was at least 2 mm larger in average diameter than the concentration-matched glycerin control after 10 minutes.

SPT

Single-prick technique (Solofix; B. Braun Petzold GmbH, Melsungen, Germany) was done for foods selected for DBPCFC. The same 1:20 W/V food extracts used for IPDFT were used for SPT. Negative (0.4% phenol in 50% glycerin) and positive (histamine in 50% glycerin) controls were used. A positive result was antigen wheal size after 15 minutes at least 3 mm larger than the negative control.

In Vitro sIgE

Serum sIgE to the following inhalants was determined by ImmunoCAP (Phadia, Uppsala, Sweden): Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis, Asperillus fumigatus, Blattella germanica, Cynodon dactylon, and Ambrosia elatior. Positive lower cutoff was sIgE $\geq$0.35 IU/mL. The sIgE for each food selected for DBPCFC was also determined.

SYMPTOM ASSESSMENTS

The severity of each distinct symptom was separately recorded by using an 11-point written response Likert rating scale. Symptom scoring sheets contained printed severity definitions: 0 = symptom free, 1-3 = mild symptoms, 4-6 = moderate symptoms, and 7-10 = severe symptoms. Also recorded at each time point were symptom description, time of first appearance, and duration. An SRE 2100 acoustic rhinometer (Rhinometrics A/S, Denmark) measured nasal geometry[15] responses to DBPCFC. At each time, triplicate measurements were made on each nasal cavity. Minimum cross-sectional areas (MCA) and mean of left and right sides were calculated.

BIOCHEMICAL ASSESSMENTS

An enzyme immunoassay was used to measure serum tryptase and eosinophil cationic protein (ImmunoCAP) and serum histamine (Cayman Chemical, Ann Arbor, MI). The analytic range was 2 to 50 pg/mL for tryptase, 2 to 200 pg/mL for eosinophil cationic protein, and 40 to 5,500 pg/mL for histamine.

Serum Th1/Th2 cytokines interleukin (IL)-2, IL-4, IL-5, IL-10, tumor necrosis factor α, and interferon γ and complement C3α, C4α, and C5α were measured by fluorescent flow cytometry (BD Cytometric Bead Array; BD Biosciences, San Diego, CA). The lower detection limit was 20 pg/mL for cytokines and 10 pg/mL for complement.

PROCEDURES FOR OFCS

Food Diary

During an initial 14-day period, patients recorded the intake of all foods and symptoms experienced by using the 0 to 10 scale (see earlier). Every patient was instructed to eat all nonanaphylactic foods that he/she used to avoid. Diaries were read by both the dietitian and physician. All foods identified as positive by pretests or as causing symptoms and also those eaten more frequently than twice weekly were evaluated for allergic potential.

Open Oral Food Challenge

Patients were then instructed to eliminate all suspicious foods for 1 to 2 weeks. This withdrawal period was found to be adequate (see Results section). Once patients were symptom free, open challenges of suspected allergic foods were performed under dietitian supervision, testing single foods,

given as 2 normal servings in 1 day, and evaluated by diary sheet records for the following 3 days. The dietitian selected 1 reactive food for each patient for further DBPCFC. Each selected food was available in organic form, could be masked, and provoked clearly recognizable symptoms in OFC. After OFC, patients resumed eating their positive foods until a second avoidance period 5 days before their DBPCFC.

Based on pretests, we anticipated that the open challenges would be of non–IgE-mediated reactions. Eighteen of the foods selected for challenge had no positive sIgE or prick test for that food. One food had a positive sIgE, and 2 others had a positive prick test to that food. It is likely that challenge reactions from all 20 of these foods were primarily non–IgE-mediated because, in addition to the almost completely negative IgE tests, there was no anaphylaxis, large doses were needed to trigger symptoms, and before pretests were done, patients were not aware that these foods were causing symptoms.

Double-blind, placebo-controlled food challenge

DBPCFC was conducted on 2 days, separated by an interval of 5 to 7 days, with 2 identical meals (at 9 AM and 12 AM) in each day containing allergic food (verum challenge) or placebo food in a double-blind presentation (phases A and B). A randomization list, generated by SPSS Version 11.5.1 statistical software selected the order of A and B for each patient. Only the dietitian knew randomization details. The food quantity for each meal was determined from normal portion sizes and sufficient symptom production in OFC. Placebo and masking ingredients were selected to eliminate potentially allergic foods identified by either pretests or dietary history.

On DBPCFC days, each patient was provided a single room with comfortable temperature in the clinical trials unit. Tests began at 8 to 9 AM after an overnight fast for food diary review of the past 5 days, and medical examination of blood pressure, pulse, temperature, respiratory rate, weight, and girth. Each patient was given a diary sheet with completion instructions. Symptom assessment and acoustic rhinometry were performed before the first meal and then every hour until 3 hours after the second meal. In all patients, 3 mL of peripheral blood were taken before and 30 minutes and 3 hours after the first and second meals (5 samples per person) for biochemical measurements. Patients were discharged 4 hours after the second meal and continued with diet control and recording symptoms for 3 days after each challenge.

STATISTICAL ANALYSIS

An independent statistician analyzed data using SPSS Version 13.0 (SPSS Inc, Chicago, IL). Results were presented at the departmental research meeting before opening the randomization code. Wilcoxon signed rank test compared the severity score, onset, and duration of symptoms between OFC and DBPCFC phases A and B because the distributions of these variables did not satisfy normality assumptions, as determined by the Kolomogorov-Smirnov goodness of fit test. The sensitivity and positive predictive value were determined for the OFC as compared with the DBPCFC. Biochemical measurements were graphically compared by plotting time point means and standard errors.

RESULTS

All patients completed the study. Eleven were positive by sIgE tests to at least 1 house dust mite. All were positive to more than 1 food by intradermal tests, with a mean of 6.5 (range, 2-12) reactive foods. Only 1 of 20 patients showed a positive sIgE to a test food, and only 2 patients had a positive SPT to a test food (not done in 3 patients). Appendix 1 (see online issue http://journal.entnet.org) shows the amounts and ingredients for each allergic food or placebo tested. Large amounts of protein, ranging from 5 to 31 g, corresponding to usual portion sizes, were required to produce significant symptoms. Appendix 2 (http://journal.entnet.org) shows for each patient historic symptoms, intradermal food test positives, sIgE inhalant test results, and SPT and sIgE results for the 1 food for each patient that was selected for DBPCFC.

SYMPTOM COMPARISON AMONG CHALLENGES

Table 1 shows symptoms appearing during OFC and both DBPCFC phases. All 20 patients reported 1 or more identical symptoms after both open and double-blind challenge with allergic food. After a meal that produced a positive challenge, the average time to symptom resolution was observed to be 20.4 ± 25 hours, although in 1 patient nasal obstruction persisted for 96 hours. Seven patients (35%) reacted also to placebo with similar but statistically significantly less severe symptoms. In these 7 patients, placebo challenges, open challenges, and verum challenges produced a total of 12 symptoms that were identical across the 3 challenges in each patient (Table 2). Because these were common allergy symptoms, these patients might have had underlying allergies unrelated to their food challenges. By excluding these 12 sets of correlated symptoms and comparing correlations of patient symptoms that appeared in only verum DBPCFC with those in OFC and not in placebo challenge (Table 3), the sensitivity of open challenge was calculated as 66% (25/38), and the OFC positive predictive value (PPV) was 89% (25/28). Without excluding positive placebo results, OFC performance is slightly better, with sensitivity of 74% and PPV of 92.5%.

Table 1
Complaints of distinct symptoms produced during open challenge (20 patients), DBPCFC with allergic food (20 patients), and DBPCFC placebo (7 patients)*

	Complaints of distinct symptoms		
		DBPCFC	
Type of symptom	Open challenge	Allergic food	Placebo
Nasal obstruction	7	9	3
Headache	6	7	1
Sneezing	4	6	1
Phlegm	5	5	4
Runny nose	5	5	3
Cough	2	3	0
Itchy nose	2	2	0
Post nasal drip	1	2	1
Itchy throat	2	2	0
Bloating	1	1	0
Eye swelling	1	1	0
Hives	0	1	0
Itchy ears	0	1	0
Itchy eyes	1	1	0
Itchy palate	0	1	0
Sore throat	1	1	0
Tearing	1	1	0
Wheezing	0	1	0
Sensitive lip	1	0	0
Tongue sensation	0	0	1
Total	**40**	**50**	**14**

*Thirteen patients had no symptoms with placebo.

ORGAN INVOLVEMENT, SYMPTOM SEVERITY, ONSET, AND DURATION

We observed strong responses to oral challenges but found no single pattern of clinical symptoms, rather, there was multiorgan involvement. Nasal obstruction, headache, sneezing, phlegm, and runny nose were most common.

Table 2
Complaints of identical symptoms reported after placebo DBPCFC and also with both open challenge and serum DBPCFC

Type of symptom	Frequency (no.)	Patient number
Nasal obstruction	3	7,8,16
Phlegm	3	7,14,18
Runny nose	3	8,18,19
Sneezing	1	19
Headache	1	20
Post nasal drip	1	20
Total number	12	

Table 3
Complaints of distinct symptoms reported after DBPCFC with allergic food and in open challenge but not during placebo challenge

	Complaints of distinct symptoms		
		Open challenge	
Type of symptom	DPBCFC	Also in DPBCFC	Not seen in DPBCFC
Headache	6	5	0
Nasal obstruction	6	3	1
Sneezing	5	3	0
Cough	3	2	0
Itchy nose	2	2	0
Runny nose	2	1	1
Phlegm	2	2	0
Itchy throat	2	2	0
Sore throat	1	1	0
Bloating	1	1	0
Eye swelling	1	1	0
Itchy eye	1	1	0
Hives	1	0	0
Wheezing	1	0	0
Itchy palate	1	0	0
Post nasal drip	1	0	0
Tearing	1	1	0
Itchy ear	1	0	0
Sensitive lip	0	0	1
Total	**38**	**25**	**3**

Data exclude the 12 placebo-associated complaints listed in Table 2.

Ordinal data from Likert scales are additive; therefore, all symptom observations were combined (Table 4), producing mean severity scores for each challenge condition (OFC, DBPCFC with allergic food, and DBPCFC with placebo). Mean symptom onset time after ingestion, and mean symptom duration were also calculated for the 3 challenge conditions. The symptom severity, onset time, and duration, calculated for each of the 3 challenge conditions, were then compared by using the pair-wise Wilcoxon signed rank test, resulting in 18 combinations. Symptoms that appeared both in open challenge (mean severity score 3.8 $\pm$ 1.3) and DBPCFC with allergic foods (3.8 $\pm$ 1.8) were significantly ($P < 0.01$) more severe than those appearing after placebo (1.9 $\pm$ 0.8).

The mean onset of symptoms induced by DBPCFC with allergic food and with open food challenge was 2.6 $\pm$ 1.6 hours and 4.3 $\pm$ 5.8 hours, respectively. These were not significantly different, indicating that, after oral challenge, a long period of surveillance is necessary to ensure that symptoms can be observed that might otherwise have been lost by a patient leaving the laboratory too early or obscured by symptoms from subsequent ingestion of another meal. Once symptoms began, they lasted from 1 hour to 4 days. For reactive foods, the mean symptom duration was 20.4 to 24.5

Table 4
Comparisons of severity score, onset time, and duration of symptoms for three pairs of challenge conditions: open challenge (OFC) versus DBPCFC with allergic food (AF), OFC versus DBPCFC with placebo (PL), and DBPCFC with AF versus PL

	OFC versus AF (n = 37 symptoms)			OFC versus PL (n = 12 symptoms)			AF versus PL (n = 12 symptoms)		
	OFC	AF	*P* value*	OFC	PL	*P* value*	AF	PL	*P* value*
Score (severity)			0.015			0.005			0.009
Mean (SD)	3.6 ± 1.4	4.5 ± 2.1		3.8 ± 1.3	1.9 ± 0.8		3.8 ± 1.8	1.9 ± 0.8	
Median	3.0	4.0		3.5	2.0		3.0	2.0	
Range	1-7	2-8		2-6	1-4		2-8	1-4	
Onset (h)			NS			NS			NS
Mean (SD)	4.3 ± 5.8	2.6 ± 1.6		4.6 ± 7.2	2.4 ± 1.5		3.3 ± 2.2	2.4 ± 1.5	
Median	2.0	2.0		1.0	2.0		3.5	2.0	
Range	0.5-24	1-8		0.5-24	1-5		1.0-8	1-5	
Duration (h)			NS			NS			0.036
Mean (SD)	24.5 ± 26.5	20.4 ± 25		17.4 ± 24.1	8.8 ± 20		23.3 ± 31.9	8.8 ± 20	
Median	12.0	7.0		4.0	3.0		5.0	3.0	
Range	1-96	1-96		1-72	1-72		1-96	1-72	

Summary of all pooled symptom data from all patient challenges (see Results).
NS, not significant.

*Wilcoxon signed rank test.

hours versus 8.8 hours for placebo responses, but these were statistically identical. Because symptoms can continue for up to 4 days, subsequent challenges should be performed only after this interval.

OBJECTIVE MEASUREMENTS

We found significant increases in nasal obstruction symptom severity at 1 ($P = 0.016$) and 3 ($P = 0.042$) hours after DBPCFC with allergic food as compared with placebo but did not find significant differences for acoustic measurements between the 2 DBPCFC phases. However, in subgroup analysis, there were 9 patients complaining of nasal obstruction after verum food challenge. Eight of these had a measurable decrease in MCA after DBPCFC: mean 0.46 ± 0.13 cm^2 compared with prechallenge 0.52 ± 0.16 cm^2 ($P = 0.068$, Wilcoxon signed rank test). The ninth patient had a minimum obstruction severity score (1/10) and no MCA change. Three of these patients also had obstruction complaints after placebo but had no MCA change. Biochemical markers did not show any significant changes ($P < 0.05$) before and after DBPCFC challenges whether with allergic food or placebo (Appendix 3; http://journal.entnet.org).

DISCUSSION

Sicherer[12] suggested that food allergy is not a single disease, and it is not caused by a solitary pathophysiologic disturbance. The myriad manifestations of food allergy can be influenced by many determinants such as food-protein chemistry, absorption and processing of ingested allergen, immune responses, and target organ hyperreactivity. This complexity, the existence of both IgE-mediated and non–IgE-mediated mechanisms, both producing identical symptoms after food ingestion,[16] coupled with the lack of any validated diagnostic test for non–IgE-mediated food sensitivities, are major reasons why progress in this field has been so difficult.[17]

Methods for performing DBPCFC tests have not been standardized.[1,11] Many DBPCFC studies have used dehydrated food capsules,[7] and, although convenient, this differs from the natural process of eating and whether or not it influences the outcomes of food challenge is unclear. Our study was performed by using standard portion sizes of naturally prepared food. Under these conditions, all patients were able to reproduce, during challenge, symptoms typical of those they had historically experienced.

This is the first study that validates the clinical value of open challenge for the diagnosis of food allergy. Our data show that the sensitivity of OFC is 66%, with 89% of the possible positive predictive value of DBPCFC. Also, positive OFC responses are as robust as those after DBPCFC: the mean (±SD) symptom score was 4.5 ± 2.1 after active DBPCFC and 3.6 ± 1.4 after open challenge. The onset and duration of symptoms are also statistically identical between these techniques. We therefore recommend open food challenge as a simple, valuable clinical tool for office diagnosis. In this study, 7 (35%) patients developed similar symptoms but markedly less intense after placebo challenges. Whether this represented a pla-

cebo effect, insufficiently controlled allergen exposure, or the potential allergic action of masking ingredients is not known. Because of this finding, repeated challenges with a single food may be required for maximum certainty when designing therapeutic allergy diets.

Whether food hypersensitivity can induce isolated nasal symptoms has been contentious. Although it is known that ingested antigen can reach the nasal mucosa and provoke allergic rhinitis,[18] this study shows clearly that 3 of the top 5 symptoms induced by DBPCFC are nasal, most frequently nasal obstruction, as objectively verified both by symptom scores and by acoustic rhinometry. Also, the onset, duration, and severity of food-induced nasal symptoms differ from those after the inhalant nasal challenge.[19] After DBPCFC, symptoms begin in from 1 to 8 hours compared with 5 minutes after the inhalant challenge and last as long as 96 hours compared with 24 to 48 hours after the inhalant challenge. Our timing data agree with a prior DBPCFC study[7] and show that both prolonged observation and long periods between individual tests are critical for accurate outcome assessment in oral challenges.

This is also the first DBPCFC study that supports the potential usefulness of intradermal food tests. Non–IgE-mediated immunologic mechanisms remain controversial because most studies have shown mainly IgE-mediated food allergy. But, although good diagnostic tests for IgE-mediated allergy have been available for decades, there have been no reliable tests for identifying non–IgE-mediated cases. Some studies have shown positive open challenges or DBPCFC in from 10% to 100% of patients despite negative SPT and in vitro tests.[4-8,20] Our study also found a high number of possible non-IgE cases; in our patients with positive DBPCFC responses, 88% had negative SPT and 95% had negative sIgE. We used IPDFT as a screening test to identify patients who might have non-IgE food reactions. However, this study was not designed to determine sensitivity and specificity of IPDFT; the protocol was designed only to evaluate the methods and results of open and double-blind challenge.

We could not show significant changes in serum type I inflammatory mediators, T-cell cytokines, or complement components. This could be explained by cellular reactions occurring only within target tissues so that soluble markers were undetectable in serum.

In conclusion, this is the first study to use DBPCFC to confirm the existence of food allergy in patients who had positive skin responses to high-dose intradermal challenges by IPDFT. These patients showed evidence of non-IgE food reactions because most were negative to food-specific SPT and in vitro testing for sIgE, and all cases had no serologic markers of type I reactions. Most patients showed simultaneous, multiple-organ involvement of the nervous system, throat, lung, and, especially, the nose. Finally, this is also the first study to prove that open oral food challenge is a useful method for identifying or confirming food allergy and is nearly as efficient as DBPCFC.

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FINANCIAL DISCLOSURE

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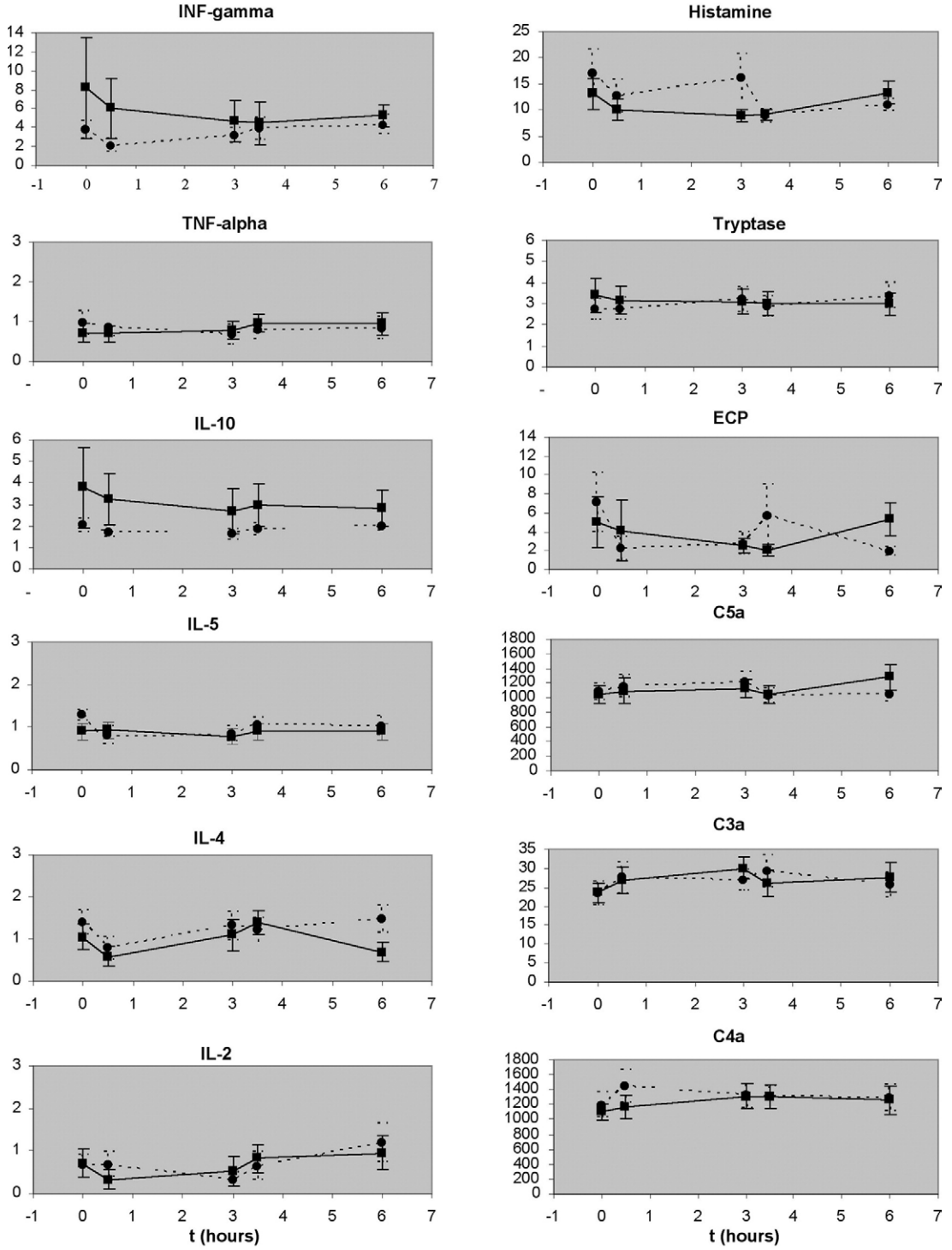
Patient no.	Challenge food	Food Challenge Doses	
		OFC	DBPCFC
1	Cow's milk	300 mL cow's milk (≈3.84 g milk protein)	400 mL cow's milk (≈5.12 g milk protein)
2	Wheat	11 yeast free wheat biscuits (200 g wheat flour, ≈22.98 g wheat protein)	300 g uncooked wheat pasta (≈22.4 g wheat protein)
3	Soy	300 g soy bean curd (≈33.9 g soy protein)	250 g soy bean curd (≈28 g soy protein)
4	Wheat	442 g fried noodles plus 80 g uncooked pasta (262.8 g wheat, ≈25.05 g wheat protein)	300g uncooked wheat pasta (≈22.4 g wheat protein)
5	Baker's yeast	30 mL vinegar (yeast)	15 g dried instant yeast powder (=6 g protein)
6	Malt	150 g Milo*	90 g Milo*
7	Soy	500 mL soymilk (≈20.8 g soy protein)	300 mL soymilk plus 200 g soy bean curd (≈35.3 g soy protein)
8	Peanut	50 g peanuts (≈8.23 g peanut protein)	50 g peanuts (≈8.23 g peanut protein)
9	Baker's Yeast	15 g dried instant yeast powder	15 g dried instant yeast powder (=6 g protein)
10	Baker's Yeast	15 g dried instant yeast powder	15 g dried instant yeast powder (=6 g protein)
11	Cow's milk	500 mL cow's milk (≈6.4 g milk protein)	400 mL cow's milk (≈5.12 g milk protein)
12	Corn	260 g corn (≈8.06 g corn protein)	600 g corn flour (≈16.7 g corn protein)
13	Baker's Yeast	12 g dried instant yeast powder	15 g dried instant yeast powder (=6 g protein)
14	Malt	90 g Milo*	90 g Milo*
15	Corn	400 g corn (≈8.06 g corn protein)	50 g finely mashed corn kernels plus 400 g corn flour (450 g corn, ≈12.12 g corn protein)
16	Soy	450 mL soy milk (≈18.8 g soy protein)	200 mL soy milk plus 200 g soy bean curd (≈31 g soy protein)
17	Soy	250 mL soy milk plus 147 g soy bean curd (≈26.4 g soy protein)	200 mL soy milk plus 200 g soy bean curd (≈31 g soy protein)
18	Corn	1 corn on the cob (130 g corn, ≈4.03 g corn protein)	50 g finely mashed corn kernels plus 300 g corn flour (350 g corn, ≈9.34 g corn protein)
19	Soy	200 mL soy milk plus 200 g soy bean curb (≈31.12 g soy protein)	200 mL soy milk 200 g soy bean curd (≈31 g soy protein)
20	Baker's yeast	15 g dried instant yeast powder	15 g dried instant yeast powder (=6 g protein)

* Ingredients of Milo in descending order: Sugar, barley malt extract, milk solids (butter fats), cocoa powder, vegetable oil (palm oil), lecithin, minerals, vanillin, and vitamins.

Appendix 1

Patient serial no.	Medical history of symptoms*	Positive intradermal for foods (food underlined was used for DBPCFC)	SPT to DBPCFC food	sIgE to DBPCFC food	sIgE to inhalan allergen
1	Tearing, nasal obstruction, headache, joint pain	Cow's milk, ginger, malt, garlic, carrot, chocolate, oyster	+	-	-
2	Post nasal drip, cough, reflux sneezing, headache	Wheat, baker's yeast, garlic, chicken, oyster, ginger	-	-	-
3	Eczema, hives, itchy skin, runny nose, bloating, sneezing, diarrhea	Soy, sesame, corn, wheat, chicken, oyster, chocolate, pepper, ginger, barley, grape, watermelon	ND	-	+
4	Nasal obstruction, runny nose, tearing, headache, bloating	Wheat, peanut, tea, barley, sunflower, orange, chili, garlic	ND	+	+
5	Nasal obstruction, sneezing, itchy nose, runny nose, muscle aches, bloating, itchy skin and eyes, chest tightness, headache	Baker's yeast, soy, oyster	-	-	+
6	Nasal obstruction, sneezing, runny nose, eye redness, cough, joint pain, muscle aches, bloating, diarrhea	Malt, garlic, cabbage, coconut, broccoli	-	-	+
7	Nasal obstruction, sneezing, runny nose, bloating	Soy, cow's milk, malt, soy, garlic, chili, coconut	ND	-	+
8	Nasal obstruction, sneezing, join pain, muscle aches	Peanut, banana, tea	-	-	-
9	Itchy nose, runny nose	Baker's yeast, ginger	-	-	+
10	Bloating, nasal obstruction	Baker's yeast, egg, tea, tomato, ginger, olive	-	-	-
11	Nasal obstruction, sneezing, itchy nose, runny nose, itchy eyes, tearing, joint pain, bloating	Cow's milk, garlic, sugar, banana, chili	-	-	-
12	Nasal obstruction, sneezing, itchy nose, runny nose, itchy eyes, chest tightness	Corn, malt, soy, pepper, tomato, tea, carrot	-	-	+
13	Headache, muscle aches, bloating, hives, nasal obstruction	Baker's yeast, soy, pepper, ginger, watermelon, oyster, chili	-	-	-
14	Cough, phlegm, headache, runny nose	Malt, wheat, pepper, chicken, oyster	-	-	-
15	Eczema, eye swelling, tearing, itchy eyes, nasal obstruction, sneezing, runny nose, cough, headache, muscle aches	Corn, garlic, carrot, broccoli, tomato, ginger, onion, oyster	-	-	-
16	Eczema, nasal obstruction, chest tightness, cough, headache	Soy, corn, malt, egg, chocolate, tea, oyster	-	-	-
17	Eczema, cough	Soy, corn, wheat, malt, garlic, rice, apple, oyster	-	-	+
18	Eczema, itchy skin	Corn, milk, malt, egg, pepper, pork, coffee, ginger, chocolate	-	-	+
19	Hives, itchy eyes, tearing, eye redness, nasal obstruction, sneezing, itchy nose, runny nose, cough, joint pain, muscle aches, bloating, diarrhea	Soy, wheat, malt, coffee, pepper, coconut, ginger, onion, chili, oyster	-	-	+
20	Hives, tearing, headache	Baker's yeast, soy, onion, tea, peanut	-	-	+

Appendix 2



Sample means (n=20) with standard error bars. There is no significant change ($P<0.05$) before and after DBPCFC challenges, whether with allergic food or placebo.
Time 1: Baseline serum sample.
Time 2: 1/2 hour after ingestion of 1st food serving.
Time 3: 3 hours after ingestion of 1st food serving.
Time 4: 1/2 hour after ingestion of 2nd food serving.
Time 5: 3 hours after ingestion of 2nd food serving.

Appendix 3