Wheat allergy: A double-blind, placebo-controlled study in adults

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Background: Wheat is believed to be an uncommon cause of food allergy in adults; the number of studies that address IgE mediated wheat allergy in adults is all too few.
Objective: Determine how many subjects with a history of wheat allergy have real allergy by double-blind, placebo-controlled food challenge; identify the symptoms manifested during the challenge; determine the lowest provocation dose; determine the performance characteristics of wheat skin prick test and specific IgE; identify subjects with real wheat allergy for potential immunoblotting studies.
Methods: Patients underwent skin test with commercial wheat extract; specific wheat IgE was determined. Subjects were challenged with 25 g wheat. Subjects who were positive to raw wheat challenge underwent cooked wheat challenge.
Results: Thirty-seven double-blind placebo-controlled wheat challenges were performed on 27 patients. A total of 13 of 27 (48%) patients had a positive result. Eleven subjects with positive raw wheat challenge underwent cooked wheat challenge: 10 were positive. The provocation dose range was 0.1 to 25 g. Twenty-seven percent of the subjects allergic to wheat had a provocation dose that was ≤1.6 g.
Conclusion: Wheat causes real food allergy in adults. More than a quarter of the patients allergic to wheat reacted to less than 1.6 g wheat. Specific IgE was more sensitive than skin test for wheat; however, specificity and predictive values were low for both tests. Thus, these tests should not be used to validate diagnosis of wheat allergy. (J Allergy Clin Immunol 2006;117:433-9.)

Key words: *Food allergy, wheat allergy, hypersensitivity, anaphylaxis, symptoms, double-blind, placebo-controlled food challenge, provocation dose, performance characteristics, exercise-induced*

Abbreviations used
DBPCFC: Double-blind, placebo-controlled food challenge
NPV: Negative predictive value
OAS: Oral allergy syndrome
PD: Provocation dose
PPV: Positive predictive value
SPT: Skin prick test
WDEI: Wheat-dependent, exercise-induced

Wheat is widely consumed all over the world; thus, its potential to cause disease is a matter of concern. IgE-mediated reactions to wheat have been demonstrated as early as the beginning of the 20th century for ''baker's asthma.''[1] Food allergic reactions to wheat can give way to a array of clinical manifestations that can be immediate and/or delayed, and their severity can vary from mild to life-threatening. Typical immediate symptoms include oropharyngeal symptoms, urticaria, angioedema, atopic dermatitis flare, rhinitis, asthma, gastrointestinal symptoms, and anaphylaxis.[2-5] Delayed wheat hypersensitivity has been described in children by Scandinavian authors; in particular, they reported erythema, pruritus, eczema, and gastrointestinal reactions several hours after oral provocation with wheat[6,7]; in the study by Majamaa et al,[7] oral wheat provocations were positive in children with negative skin test results and wheat specific IgE.

Wheat food allergy is frequent in children and infants. In fact, along with milk, soy, peanuts, eggs, and fish, wheat is listed as 1 of the 6 most commonly implicated allergens in children with skin allergies.[5,8,9] Wheat allergy in children has been confirmed by the large number of double-blind, placebo-controlled food challenges (DBPCFCs) performed in subjects with atopic dermatitis.[5,10-12] Although there have been increasing reports of food allergy to a wide range of foods, Ellman et al[13] recently found that children with atopic dermatitis who were followed for a span of 10 years did not manifest reactions to an increasing variety of foods and that wheat, milk, egg, soy, peanut, tree nuts, and seafood continued to account for nearly 90% of the food-allergic reactions.

Despite the large consumption of wheat all over the world, there are few reports that address the clinical aspects of wheat food allergy in controlled adults allergic to wheat. Two reports are found in the literature. One regards severe exercise-induced wheat anaphylaxis in 18 adult patients[14]; the other evaluated the allergenic

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reactivity of ingested and inhaled cereal allergens in different age groups and found that the most important cereal allergen in adults was barley, followed by rye.[15] The only other study that considers wheat allergy in adults conducted open oral wheat challenges in 20 patients with gastrointestinal symptoms.[16] In the study by Armentia et al,[15] positive wheat DBPCFCs were performed in 5 young adults; however, the authors provided little detail about the challenge protocol, the test meal used, the incremental doses, the PDs, and the symptoms observed. When investigating the clinical aspects of food allergy, the characteristics of the test meal used are important from a food processing point of view. Allergens can be created or eliminated with cooking. Thus, it is important that the characteristics of the food items used are specified when conducting DBPCFCs. Also, knowledge of the amount of food necessary to provoke a reaction is important inasmuch as the provocation dose (PD) provides feedback for the patient who will need to modify dietary habits accordingly—for example, less vigilance will be required in case of mild reactions for high PDs. The DBPCFC procedure provides the control conditions necessary for determining evidence-based PDs for the offending foods.

Last, the high frequency of serological cross-reactivity between cereal allergens and grass pollen contributes to poor predictive values for wheat specific IgE end skin tests.[17,18] Although skin testing and specific IgE are routinely used to identify sensitization to a food, the true performance characteristics of these tests need to be calculated with respect to the DBPCFC. Currently, the answer to this problem still depends on carefully obtained clinical histories and the application of DBPCFCs.[19,20]

The aim of this study was to identify the range and severity of symptoms manifested during wheat DBPCFC in adults from southern and northern Europe; evaluate the clinical reactivity after oral provocation with raw and cooked wheat; determine the lowest PD during DBPCFC; determine the performance characteristics of wheat skin prick test (SPT) and wheat specific IgE compared with DBPCFC; identify subjects with real wheat allergy; and obtain sera for potential immunoblotting studies.

METHODS

Patients

Subjects with presumed wheat allergy were admitted to 1 of 3 Allergy Units in Europe, Niguarda Cà Granda Hospital (Milan, Italy), Milan University Hospital (Milan, Italy), or Odense University Hospital (Odense, Denmark), during the period March 2002 to April 2004. Approval for the study was obtained by the Ethics Committee of all 3 hospitals.

Patients were selected on the basis of suspected wheat allergy and not on the basis of allergy to grass pollen or positive wheat skin test or specific IgE. All of the patients selected reported a clinical history of symptoms shortly after the ingestion of wheat products. Patients with a history of severe symptoms after wheat ingestion were not recruited.

Antitransglutaminase antibody titers were used to screen for gluten sensitivity before enrollment in the study.

Treatment with antihistamines and glucocorticoids was suspended at least 10 days before the challenge. The patient was asymptomatic on the day of the challenge. In case of atopic dermatitis, the patient obtained the best possible skin condition before undergoing challenge.

Skin testing

All of the subjects underwent SPT with commercial wheat extract (Stallergenes, Antony Cedex, France) as well as extracts for grass, birch, mugwort, parietaria and ragweed (Stallergenes). Skin testing was performed on the volar aspect of the forearm with a monodentate lancet; histamine (1%) was used as positive control and saline as negative control. Tests were considered positive if the wheal produced had a mean diameter of at least 3 mm.[21]

Specific IgE

All patients were tested for wheat specific IgE antibodies (CAP-FEIA; Pharmacia-Upjohn, Uppsala, Sweden).

Challenge testing

Wheat was eliminated from the diet at least 1 week before the challenge. The patients were given the test meals as inpatients on separate days after an overnight fast. The physician and patient were both blind to the protocol.

The test meal was developed by the dieticians of the Niguarda Cà Granda Hospital.[22] The ingredients used for the preparation of the raw wheat test meals were wheat flour (50% durum and 50% tender), water, cocoa, sugar, and lemon aroma syrup. The same ingredients were used for the placebo meal; potato starch was used instead of wheat. The cooked wheat test meal was made with wheat flour (50% durum and 50% tender), water, cocoa, sugar, and lemon aroma syrup; the placebo meal contained potato starch, water, cocoa, partially cooked minced rice, and lemon aroma syrup. Allergies to the ingredients used for the test meal were excluded before submitting the patient to the challenge.

The triangle test was in used in a group of wheat-tolerant subjects to evaluate for sensory difference between the active and placebo tests.[23]

Double-blind placebo-controlled food challenge with 25 g raw wheat flour was performed on all of the patients. In the case of a positive raw wheat DBPCFC, the patient also underwent cooked wheat DBPCFC.

Challenge procedure

Informed consent was obtained from all of the patients; consent was obtained by the parents of minors (ie, younger than 18 years). Precautions were taken for possible systemic reactions. Intravenous access was obtained for all of the patients.

Before administering the test meal, the oral cavity and skin were carefully inspected for pre-existing lesions; blood pressure and FEV_1 were measured (spirometry was performed before each subsequent dose if there was a history of asthma and whenever respiratory symptoms were reported during the challenge). A positive reaction for asthma was defined as a decrease in FEV_1 of at least 15% compared with baseline.

Only immediate skin reactions were recorded as positive in patients with a history of atopic dermatitis.

The Master 2-step test was used after each challenge dose when testing for exercise-induced wheat allergy.

DBPCFC

Doses were administered at 20-minute intervals. A minimum starting dose of 100 mg raw wheat flour was administered; the next dose was 500 mg, then 1 g, and 1.5 g; the last dose was then doubled

(3 g, 6 g, 12 g) until symptoms were reported/observed or until the entire test meal was eaten. Hence, the cumulative dose schedule was as follows: 100 mg, 600 mg, 1.6 g, 3.1 g, 6.1 g, 12.1 g, and 25 g. The same dose schedule was used for cooked wheat DBPCFC in patients who had a positive result to raw wheat.

Open challenge

Open challenge with 25 g cooked wheat pasta was performed on subjects with a negative wheat DBPCFC. Provided that no symptoms occurred, each subject ate 1 g pasta; this amount was doubled every 20 minutes until completion of the test meal.

Data analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for wheat skin test and specific IgE were calculated according to the method of Goldman.[24]

We defined these statistics as follows:

P (prevalence) = positive oral provocations/total patients tested
TP (true positive): patients with positive oral provocation and positive SPT and/or specific IgE
FP (false positive): patients with negative oral provocation and positive SPT and/or specific IgE
TN (true negative): patients with negative oral provocation and negative SPT and/or specific IgE
FN (false negative): patients with positive oral provocation and negative SPT and/or specific IgE
Sensitivity = TP/(TP + FN)
Specificity = TN/(TN + FP)
PPV = (TP × P)/[(TP × P) + FP(1 − P)]
NPV = TN(1 − P)/[TN(1 − P) + FN × P]

RESULTS

Patients

A total of 27 patients (24 Italian and 3 Danish) with suspected wheat allergy were included in the study. Patient characteristics were distributed as follows for sex and age: 18 female, 15 male; age, 14 to 60 years (mean, 33.7 years). Table I lists demographic data, SPT and specific IgE, and clinical history to wheat and sensitizing pollens. Only 14 of 27 (52%) patients had a positive result for grass pollen.

Clinical histories for wheat were in keeping with IgE mediated symptoms. Most of the subjects recruited also reported food allergy to other fruits and vegetables: tomato (patients 1, 4, 7, 8), carrot (patient 7), fennel (patient 1), soy (patient 7), peach (patients 1, 2, 5), cherry (patients 4, 5), grape (patients 1, 2), melon (patient 7), apple (patients 1, 4, 5), hazelnut (patients 5, 7), chestnut (patients 2, 7), barley (patient 1), rye (patient 1), corn (patients 2, 4), and rice (patient 2). Food-allergic symptoms to wheat appeared in adulthood in all but 2 patients (patients 5, 27). Patient 2 complained of immediate symptoms as well as migraine headache 12 to 15 hours after the ingestion of wheat. Patients 6 and 11 reported wheat-dependent, exercise-induced (WDEI) allergy.

DBPCFC

A total of 37 DBPCFCs were performed on 27 patients. All of the meals tested were well tolerated by all of the patients, and no severe reaction was manifested during all 37 challenges. Table II shows the results of the positive oral provocations, the symptoms provoked, and the lowest PD.

Thirteen of the 27 (48%) patients had a positive DBPCFC with raw wheat. Two of the positive patient had WDEI allergy. DBPCFC using raw wheat was performed on 27 subjects (23 Italian, 4 Danish).

Double-blind placebo-controlled food challenge using cooked wheat was performed on 11 patients who had a positive result to raw wheat DBPCFC: 10 were positive. Patient 3 tolerated 25 g cooked wheat; this patient underwent a second DBPCFC with raw wheat 18 months after the initial challenges (not included in the calculation of total number of challenges performed) and once again had a positive result.

Patients 14 to 27 were negative to DBPCFC; 5 of 14 patients were placebo responders and 9 of 14 were

TABLE I. Demographic data, clinical history for wheat, pollen sensitization, wheat skin test, and wheat specific IgE values

N	Sex	Age (y)	History	Wheat SPT	Wheat CAP (kU/L)	Pollen
1	M	44	GI, A	++	38.7	B,P
2	F	50	P, OA S, A, R, HA	+	6.3	G,B,P,M, R
3	F	54	AE, GI	−	1.4	−
4	F	31	OAS, AE, A	−	<0.35	B
5	F	16	AD, U, GI, R	++++	>100	B,R
6	M	42	EIU, AE	++	17.2	P
7	F	60	OAS, U	−	3.6	G,B
8	M	40	A	−	0.54	G,B,P,M
9	F	28	U	−	0.89	G,B,P,R, M
10	F	37	EIU	−	1.97	−
11*	M	45	LE	−	<0.35	−
12*	F	39	U	+	2.0	−
13	F	32	GI, AE	+	6.6	G
14	F	47	GI	+	12.2	G,B,R,M
15	F	20	AD	−	1.8	−
16	F	28	U, OAS	+	0.38	G,B,P,M
17	F	25	P	+	12.2	G,B,M
18	F	21	OAS	−	10.4	G,B,P,M,R
19	F	47	P	−	2.1	−
20	M	23	U	−	<0.35	−
21	M	22	AE	−	1.63	−
22	M	43	E, P	−	<0.35	−
23	F	25	OAS	−	20.8	G,B,P
24	F	31	GI	+	<0.35	G,R,M
25	M	26	U, A	+	3.0	G,R
26	M	19	P	+	5.0	G
27*	F	14	P	++	36	G

A, Asthma; *AD*, atopic dermatitis; *AE*, angioedema; *B*, birch; *E*, erythema; *EIU*, exercise food urticaria; *G*, grass; *GI*, abdominal colic; *HA*, headache; *LE*, larynx edema; *M*, mugwort; *P*, parietaria; *P*, pruritus; *R*, ragweed; *R*, rhinitis; *U*, urticaria; *V*, nausea/vomiting.
*Danish patients.

TABLE II. Detailed results of the positive raw and cooked wheat DBPCFC

N	Symptoms during raw wheat DBPCFC		Symptoms during cooked wheat DBPCFC		PD raw (g)	PD cooked (g)
	Active	Placebo	Active	Placebo		
1	E, P, C	-ve	U, E, P	-ve	0.6	3.1
2	E, P, HA	-ve	E, P, HA	-ve	25	25
3	V, GI	-ve	-ve	-ve	25	ND
4	OAS, AE	-ve	OAS	-ve	0.1	0.1
5	U, GI	-ve	U	-ve	3.1	6.1
6*	EI-E+GI	-ve	EI-E+GI	-ve	25	25
7	OAS, U	-ve	OAS	-ve	25	12.1
8	E, A	-ve	C	-ve	25	0.1
9	U, A	-ve	E, P	-ve	1.6	3.1
10*	EI-U	-ve	EI-U+E+AE	-ve	12.1	25
11	OAS, R, V	-ve	np	np	0.15	ND
12	E, U	-ve	np	np	3.9	ND
13	AE, GI	-ve	GI	-ve	3.1	6

A, Asthma; *AD,* atopic dermatitis; *AE,* angioedema; *C,* cough; *E,* erythema; *EI,* exercise food induced; *GI,* abdominal colic; *LE,* larynx edema; *ND,* not determined; *NP,* not performed; *OAS,* oral allergy syndrome; *P,* pruritus; *PD,* provocation dose; *R,* rhinitis; *U,* urticaria; *V,* nausea and vomit.
*Symptoms after wheat challenge and exercise.

nonresponders. The 9 nonresponders reintroduced wheat openly; 6 showed no symptoms and 3 had nonspecific symptoms such as bloating, drowsiness, and lightheadedness.

Symptoms observed during positive DBPCFC

Nearly all of the patients with positive challenge developed symptoms either during the challenge or immediately after test completion. The symptoms documented during the 24 positive wheat DBPCFCs (14 raw wheat, 10 cooked wheat) were as follows: persistent erythema, 8; generalized pruritus, 4; oral allergy syndrome (OAS), 5; urticaria, 8 (1 exercise induced); angioedema, 3; abdominal pain, 5 (1 exercise induced); nausea/vomiting, 3; asthma, 2; persistent cough, 1; rhinitis, 1; and migraine headache, 3.

The 2 patients who presented with asthma showed a significant decrease in FEV_1 compared with baseline (patients 8, 9) and responded promptly to inhaled albuterol. One patient with a history of wheat-induced asthma (patient 1) presented abrupt and insistent cough during the challenge; however, FEV_1 had decreased only 8% compared with baseline. The cough discontinued after the inhalation of albuterol.

Patient 2 complained of generalized erythema and pruritus minutes after completion of the active meal; 15 hours after the active meal, the patient also developed a severe migraine headache. The headache was reproduced also when cooked wheat DBPCFC was performed. To confirm the food-induced nature of the migraine headache, a third wheat DBPCFC was repeated in patient 2 twelve months later. The patient once again had acute migraine headache 12 hours after having been challenged with the active meal.

A review of Table II shows that 8 of 13 (62%) patients with a positive result (patients 1, 5-9, 11, 13) manifested symptoms that involved at least 2 organ systems.

PD

The range of PD for positive wheat challenges was 0.1 to 25 g. Table II shows the lowest PD for all of the positive challenges. Three patients had a PD for raw wheat that was <1 g (patient 1, 4, 9); 5 patients had a PD between 1.6 and 12.1 g (patients 5, 9, 10, 12, 13); 5 patients reacted after having ingested 25 g raw wheat. When cooked wheat was used, 2 patients had a PD of 100 mg (patient 4, 8); 5 patients had a PD between 1.6 and 12.1 g (patients 1, 5, 7, 9, 13); 3 reacted after 25 g cooked wheat. Patients 1 and 9 developed symptoms with low doses of cooked wheat but were able to complete the entire test meal before manifesting objective symptoms.

When we considered the PD limit $\leq$1.6 g, we found that 31% and 20% reacted to raw and cooked wheat, respectively. When considering raw and cooked wheat together, 26% (6/23) of the challenges showed a PD that was $\leq$1.6 g. Both patients with WDEI food allergy reacted to higher PDs (Table II, patients 6 and 10).

Data analysis

Table III shows the results of SPT with commercial wheat extract and CAP for wheat in patients with positive and negative wheat DBPCFC. *Part A* of Table IV shows the performance characteristics of SPT with commercial wheat extract and CAP for wheat specific IgE in patients with positive and negative wheat DBPCFC; *part B* shows the performance characteristics of SPT with commercial wheat extract and wheat specific IgE in patients with positive and negative wheat DBPCFC who were not sensitized to grass pollen.

DISCUSSION

This study demonstrates that real wheat allergy takes place in adults in Italy and Denmark. This is the first report

TABLE III. Results of SPT with commercial wheat extract, and specific IgE for wheat in patients with positive and negative wheat DBPCFC

	DBPCFC	
Tests	**+ve***	**−ve†**
Commercial SPT +ve	6	7
Commercial SPT −ve	7	7
IgE +ve (>0.35 kU/L)	11	11
IgE −ve (<0.35 kU/L)	2	3

*Active +ve, placebo −ve.
†Negative DBPCFC; 2 cases active +ve and placebo +ve (patients 18 and 21) were not considered.

TABLE IV. A, sensitivity, specificity, PPV, and NPV values for corresponding results of SPT with commercial wheat extract and CAP for wheat specific IgE in patients with positive and negative wheat DBPCFC; **B,** values calculated after excluding grass pollen–positive patients (2, 7-9, 13, 14, 16, 17, 23-27)

Part A	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Commercial SPT	0.462	0.417	0.482	0.397
CAP-FEIA	0.846	0.273	0.570	0.581
Part B	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Commercial SPT	0.500	0.100	0.100	0.333
CAP-FEIA	0.750	0.500	0.857	0.333

of suspected wheat allergy confirmed by DBPCFC in a large group of adult patients: 37 DBPCFCs were performed on 27 patients.

Nearly half of the patients tested (13/27) were positive to DBPCFC. The fact that 48% of the adults tested were positive to wheat DBPCFC confirms that wheat is an important food allergen in adults and should not be overlooked, especially when the clinical history implicates this food as well as other food items.

In this study, only 38% of challenge-positive patients were grass pollen–positive, compared with 64% in the challenge negative group. Thus, our study shows that in about 62% (8/13) of the cases, wheat food allergy was not associated with grass pollen cross-reactivity, indicating that in these patients, wheat sensitization occurred via the gastrointestinal route and did not require grass pollen presensitization.

With the exception of the studies on atopic dermatitis by Sampson et al,[5,10] literature reports of wheat food allergy do not thoroughly describe the array of symptoms observed during wheat DBPCFC. In our study, the multiplicity and diversity of the symptoms were in keeping with the symptoms of typical IgE mediated food allergy; all of the symptoms were manifested either during the challenge or immediately after test completion. The fact that the symptoms reported in our study were observed during oral provocation and not simply those reported by the clinical history makes these wheat-induced symptoms evidence-based.

There were no differences in the types of symptom observed during the challenge between Italian and Danish patients. The most severe manifestations observed were 2 cases of asthma (Italian patients) and 1 case of larynx edema (Danish patient).

It is noteworthy that in this study, more than half (62%) of the challenge-positive patients reported symptoms that involved 2 or more organ systems, thus fulfilling the criteria for wheat-induced anaphylaxis.

It is interesting to note that those patients who reported only a history of wheat-induced pruritus ended up having a negative challenge with wheat (patients 17, 19, 26, 27). This consideration is important when choosing patients for a diagnostic wheat challenge, because in our study, the presence of pruritus alone was associated with negative challenge outcome.

Although the mechanism of WDEI allergy is different from that of typical IgE mediated food allergy, we challenged 2 patients with exercise-induced symptoms to evaluate clinical history, PD, and symptom severity. We found that the symptoms observed during the challenge were in keeping with the clinical history, both patients were not grass pollen–allergic, both reacted after 25 g raw and cooked wheat, and no severe reaction was documented after DBPCFC and exercise. Only patient 6 fulfilled the criteria of WDEI anaphylaxis (gastrointestinal and cutaneous symptoms); patient 11 showed only WDEI urticaria. The fact that in our study only 2 of the 13 challenge-positive patients (15%) had WDEI allergy brings us to conclude that the exercise-induced nature of wheat allergy was a minor feature in this group of adults allergic to wheat.

One patient (5) with a history of wheat induced atopic dermatitis did not complain of exacerbation of the eczema during wheat challenge but rather urticaria and gastrointestinal symptoms. An explanation for the difference between reported and observed clinical reactivity could be that the patient had eliminated dietary wheat for quite some time; thus, the lack of a desensitizing effect of a constant exposure to wheat could have resulted in the appearance of more immediate-type symptoms such as urticaria and abdominal pain.

Patient 2 manifested generalized erythema and pruritus during raw and cooked wheat challenges. This patient also reported a clinical history of severe migraine headache after the ingestion of wheat products. We did not consider the headache as a symptom for determining challenge outcome; however, the fact that this patient complained of severe migraine headache 12 hours and 14 hours after the respective raw and cooked wheat DBPCFCs made us reconsider the nonspecific nature of this atypical symptom. Thus, we placed the patient on a wheat-free diet, and the migraine headache had a decrease in frequency and intensity; after 6 months, we performed a third raw wheat DBPCFC with a 48-hour interval between the active and

placebo tests, and the patient manifested immediate hypersensitivity (erythema and pruritus) during the active meal as well as migraine headache (latency, 12 hours). This is the first report of wheat-associated headache confirmed by 3 DBPCFCs in an adult patient.

In this study, we also show that the clinical reactivity did not differ significantly when patients underwent DBPCFC with raw wheat or cooked wheat. Nearly all of the patients manifested the same symptoms when we compared the symptoms provoked with raw and cooked wheat. An interesting finding was that patient 3 was positive to challenge with raw wheat but was able to tolerate 25 g cooked wheat, thus confirming the clinical history of symptoms only after raw wheat (eg, on flour on the crust of pizza or freshly baked bread). Eighteen months after the first 2 challenges, the patient was asked to repeat the raw wheat challenge, and a positive result was confirmed (PD, 25 g); the patient also reported to have tolerated cooked wheat products at home (bread, crackers, pasta). An explanation for the clinical reactivity to raw wheat but not cooked wheat in this patient could be that cooking changes the structure of the specific allergenic protein or epitope presentation.

The issue of PD has become very important because some patients with exquisite food allergy can react to very small doses of food. Perry et al[25] reviewed the reaction characteristics of positive food challenges to wheat, egg, soy, peanut, and milk and found no positive correlation between reaction severity and dose; indeed, more severe reactions were seen in subjects who reacted at lower doses. The lowest PD has been reported for some of the more commonly allergenic foods[26] (peanut, egg, fish, milk); however, there are no literature reports that verify the lowest PD for wheat under DBPCFC conditions. Our study shows that in 4 DBPCFCs with raw and cooked wheat, the lowest PD was 100 mg in 3 challenges and 150 mg in 1 challenge; all of these patients developed objective symptoms for very low doses of wheat. It is important to underline that these PDs were observed in patients who did not present a history of very severe reactions. Current guidelines discourage the use of food challenges in patients with severe food allergy; thus, one can never directly identify the threshold dose for an incriminated food in these patients with exquisite allergy.

Forty-six percent and 36% of the positive patients reacted to $\leq$3.1 g for raw and cooked wheat, respectively. When we considered the reactive dose limit $\leq$1.6 g, we found that 31% and 18% reacted to raw and cooked wheat, respectively. Thus, one can see that there was some difference in the percent of reactants when raw and cooked wheat challenges were considered separately; however, given the small population size, this difference cannot be considered statistically significant.

When we considered all 23 positive DBPCFCs (raw and cooked), we found that 26% (6/23) had a PD that was $\leq$1.6 g. Therefore, our study shows that more than a quarter of patients allergic to wheat reacted to a dose of wheat that was $\leq$1.6 g.

We were not able to identify a subset of patients who were likely to have a clinical reaction by a quantitative assessment of wheat-specific IgE. In fact, with the exception of 1 challenge-positive patient (5), the distribution of quantitative values for wheat specific IgE were comparable between the challenge-positive (patients 1-13) and challenge-negative (patients 14-27) groups (Table I).

The fact that specific IgE for wheat cannot provide a PPV greater that 95% has been attributed to the extensive cross-reactivity between wheat and grass pollen. We found that the performance characteristics of wheat specific IgE (CAP System, Pharmacia-Upjohn) were superior to skin test for sensitivity (0.85 vs 0.46); the specificity, PPV, and NPV were extensively disappointing for both tests (Table IV, *A*). The limitation of current performance characteristics is clearly seen in patients 4 and 12. These patients had negative results to wheat skin test and specific IgE; however their clinical histories strongly indicated an allergic reaction after wheat ingestion. The fact that these patients were negative to routine tests but were positive to wheat challenge and SDS-PAGE immunoblotting for wheat (E.A. Pastorello, unpublished data, July 2005) draws attention to the unsatisfactory sensitivity of these tests compared with DBPCFC. In fact, when we considered all of the challenge-positive patients, we found that wheat skin test was positive in 46% of the patients allergic to wheat, and specific IgE was positive in 85%. Thus, although specific IgE was more sensitive than the skin test, it was negative in 2 of 13 positive challenges.

To correct for grass pollen cross-reactivity, we recalculated the performance characteristics after having excluded grass-sensitized patients. We found a significant improvement in the specificity and PPV of skin test and the PPV of CAP System; however, remaining performance characteristics were still disappointing (Table IV, *B*). The poor PPV of wheat skin test and CAP System is most likely a result of grass cross-reactivity; however, the inadequate sensitivity and NPV of skin test and specific IgE is probably a result of yet unrecognized panallergens or the absence of clinically important allergens in commercial wheat extracts. Future developments may see serological tests that use epitopes for wheat IgE, thus improving the performance characteristics of these tests.

In conclusion, our study is the first controlled study performed on a large group of subjects allergic to wheat from Italy and Denmark. We have shown that wheat food allergy does occur in adult patients; in 62% of the patients allergic to wheat, there was no grass pollen sensitization. Also, we have shown that wheat ingestion was able to provoke immediate symptoms in all of the adult patients tested. In fact, we observed that 62% (8/13) of the adult patients with positive DBPCFC had symptoms that involved 2 or more organ systems; thus, a more appropriate classification of symptoms in these patients is wheat anaphylaxis. Moreover, we have shown that in 4 patients, a clinical history of only pruritus was associated with a negative challenge outcome. This is an important

consideration when choosing patients for a diagnostic challenge with wheat.

Despite the fact that wheat has been considered a trigger for migraine headache in children, in this study, we presented the first case of wheat-induced headache in an adult allergic to wheat, confirmed by 3 wheat DBPCFCs.

Last, all of the patients included in this study were adult patients who were found to be allergic to wheat after the clinical histories for wheat-induced symptoms were purposely investigated. Therefore, it is important to always consider wheat allergy in all adults with food allergy who report symptoms with this food item.

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