

A novel wheat gliadin as a cause of exercise-induced anaphylaxis

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Background: Food-dependent, exercise-induced anaphylaxis is a severe form of allergy; the reaction is caused by ingestion of a specific food before exercise. This disorder often escapes diagnosis because neither the ingested food nor the exercise alone induces the symptoms.

Objective: The aim of the study was to characterize the allergens involved in wheat-dependent, exercise-induced anaphylaxis and to describe the clinical outcome in a series of 18 adult patients.

Methods: All 18 patients had experienced recurrent episodes of generalized urticaria during exercise, 17 patients in association with collapse and 15 patients with an anaphylactic reaction. The symptoms appeared only when the patients had eaten food containing wheat before exercise. Wheat allergens were detected by immunoblotting, purified by gel filtration and reversed-phase chromatography, and subjected to N-terminal sequencing. The IgE-binding ability of the purified proteins was studied by ELISA, and their *in vivo* reactivity was studied by skin prick testing.

Results: IgE antibodies from pooled patient sera were bound to 65-kd and 40-kd wheat proteins in immunoblotting. The 65-kd allergen was a previously undescribed wheat protein, showing 61% sequence identity to γ -gliadin, whereas the 40-kd allergen had 100% identity to α -gliadin. In ELISA, all 18 patients showed elevated IgE levels to the novel γ -like gliadin, and 13 of the patients showed elevated IgE levels to the α -gliadin. None of the 54 control subjects with wheat allergy, urticaria, or coeliac disease had IgE antibodies to the γ -like gliadin. The *in vivo* reactivity of the γ -like gliadin was verified by positive skin prick test responses in all of the 15 patients who were tested. During the follow-up on a gluten-free or wheat-free diet, 3 patients experienced reactions after having unknowingly eaten wheat before exercise, but all the other patients who were adhering to the diet remained symptom-free.

Conclusion: This study shows that wheat is a frequent cause of food-dependent, exercise-induced anaphylaxis and suggests that the major allergen is a previously undescribed γ -like gliadin. For screening of this life-threatening allergy, we recommend skin prick testing with crude gliadin and we recom-

mend a gluten-free diet for treatment. (*J Allergy Clin Immunol* 1999;103:912-7.)

Key words: Wheat, food hypersensitivity, anaphylaxis, urticaria, IgE antibody, enzyme-linked immunosorbent assay

Food-dependent, exercise-induced anaphylaxis is a severe form of allergy for which ingestion of a specific food before physical exercise induces symptoms of anaphylaxis. Patients typically have IgE antibodies to the food that triggers the reactions; however, ingestion of the food causes no symptoms if not followed by exercise.¹ Food-dependent, exercise-induced anaphylaxis is regarded as a subset of classic exercise-induced anaphylaxis, a distinct form of physical allergy for which the reaction is precipitated by exercise only.^{2,3} Although various foods (including shellfish,⁴ celery,¹ hazelnut, peanut,⁵ soy, pea, and banana⁶) have been associated with food-dependent, exercise-induced anaphylaxis, the most frequently reported cause of these reactions seems to be wheat.^{5,7-9} Wheat ingestion seldom causes severe allergic reactions that are not related to exercise. Food-induced anaphylactic reactions, occurring mainly in children and adolescents, are most commonly caused by peanut, nut, seafood, or cow's milk allergy.¹⁰⁻¹³

Wheat allergens have previously been characterized in Baker's asthma, an IgE-mediated allergy to inhaled cereal flour. Water-soluble proteins belonging to the family of α -amylase inhibitors¹⁴⁻¹⁶ and a wheat flour peroxidase¹⁷ have been identified as major allergens. In addition, a few studies suggest that water-insoluble wheat prolamins (ie, gliadin and glutenin proteins) may be allergens in Baker's asthma.^{18,19} However, the allergens involved in wheat-dependent, exercise-induced anaphylaxis have not been characterized.

The aim of this study was to characterize the wheat allergens associated with wheat-dependent, exercise-induced anaphylaxis and to describe the clinical outcome in 18 patients.

METHODS

Patients

Serum samples were obtained from 18 adult patients with wheat-dependent, exercise-induced anaphylaxis, 5 of whom were described previously.⁹ Each patient had a history of anaphylactic or generalized urticarial reactions during exercise and positive skin prick test responses to wheat flour. The study was approved by the ethics committees of the respective hospitals.

All 18 patients had experienced reactions of generalized urticaria, 17 of the patients in association with collapse and 15 of the patients with an anaphylactic reaction (Table I). The age at onset

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Abbreviation used

TFA: Trifluoroacetic acid

was 18 to 59 years, and wheat allergy was diagnosed after a mean of 7.5 years (range, 0.5 to 26 years). The foods inducing the most severe reactions included wheat bread, pastries, hamburgers, pizza, pasta, and muesli. The level of exertion ranged from light indoor activities and walking to more strenuous exercise, such as bicycling, jogging, skiing, dancing, and playing ball games (Table I). The time from eating to the beginning of exercise varied from 10 minutes to 4 hours, and the duration of exercise before the onset of symptoms ranged from 10 to 60 minutes. The prodromal symptoms were pruritus and flushing, followed by whealing and generalized urticaria with angioedema in 10 patients. Five patients reported dyspnea. In more advanced reactions, the patients collapsed. Fifteen patients experienced 1 to 5 anaphylactic reactions with at least 2 of the main indicators of anaphylaxis (generalized urticaria/angioedema, hypotension, and dyspnea).^{11,13} Seventeen patients received emergency treatment with epinephrine, antihistamines, or systemic corticosteroids. All 18 patients had positive skin prick test responses to wheat flour extract (mean wheal diameter, 6.6 mm) prepared as described previously²⁰ and a positive wheat RAST (mean, 9.0 KU/L; Pharmacia, Uppsala, Sweden; Table II). The mean total IgE was 507 KU/L (Table II). Five patients had 1 to 5 positive prick test reactions to inhalant or food allergens such as pollens, hazelnut, and apple. One patient had a history of mild asthma. All patients had normal complement (C3, C4) and C1 esterase inhibitor levels. Exercise challenge tests performed as previously described⁹ showed negative responses in all patients. After diagnosis, the patients were advised to start a gluten-free diet (ie, to avoid wheat, barley, rye, and oats) because of possible cross-reactions between these cereals.⁹

Extraction and purification of wheat proteins

To extract water-insoluble proteins, wheat grains (*Triticum aestivum*, cv. Tjalve) were ground and extracted with 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) overnight at room temperature. The sample was centrifuged (30,000g for 20 minutes); the supernatant was collected, filtered, and concentrated to one third in a vacuum centrifuge, which resulted in precipitation of the acetonitrile-soluble proteins. The supernatant was collected, and the precipitate was dissolved in 10% acetonitrile-0.1% TFA.

The acetonitrile-soluble wheat proteins were purified by reversed-phase chromatography on a 3-mL column (Resource RPC; Pharmacia) with a linear gradient of acetonitrile (10% to 60% in 60 minutes) in 0.1% TFA. The supernatant, containing mainly water-soluble wheat proteins, was purified by gel filtration on a Superdex 75 HighLoad 16/60 column (Pharmacia). Fractions (500 μ L) were collected and analyzed by SDS-PAGE and Coomassie-blue staining. The fractions showing intense protein bands were further purified by reversed-phase chromatography on a ProRPC HR 5/10 column (Pharmacia) with a linear gradient of acetonitrile (10% to 50% in 60 minutes) in 0.1% TFA. Monitoring was done at 280 nm, and the fractions were collected and analyzed by SDS-PAGE and Coomassie-blue staining as described previously.²¹

Immunoblotting and N-terminal sequencing

The purified wheat proteins were analyzed by immunoblotting to detect the proteins that bind IgE in the sera of patients with wheat-dependent, exercise-induced anaphylaxis. Immunoblotting was performed as described previously with pooled sera (diluted 1:5) from 11 patients.²¹ Two purified proteins (65 kd and 40 kd) that showed

strong binding of IgE in the pooled sera were submitted to N-terminal amino acid sequencing (ABI 494 A ProciseTM Sequencer; Applied Biosystems, Foster City, Calif) and were used in ELISA, ELISA-inhibition, and skin prick tests.

ELISA and ELISA inhibition

ELISA was performed as described earlier.²² The protein concentration for coating microtiter plates (100 μ L/well) was adjusted to 2 μ g/mL. The wells were then postcoated with 1% human serum albumin in 50 mmol/L carbonate buffer, and 100 μ L of patient or control serum (diluted 1:10) was added. Biotinylated goat anti-human IgE (diluted 1:1000; Vector, Burlingame, Calif) was added, followed with streptavidin-conjugated alkaline phosphatase (diluted 1:3000; Zymed, San Francisco, Calif) and color substrate (Bio-Rad, Hercules, Calif). The color formed was read at 405 nm with an automated ELISA reader (Titertek Multiscan; Eflab, Turku, Finland). The mean OD value plus 3 standard deviations of the control sera were used as the cut-off limit for positivity.

The control sera were from 54 patients; 16 of the patients had Baker's asthma or allergic occupational rhinitis from wheat flour (mean age, 31 years; range, 20 to 53 years), and 15 of the patients had mucocutaneous or gastrointestinal symptoms from wheat (mean age, 29 years; range, 18 to 50 years). All of these 31 patients with allergy to wheat showed positive skin prick test and RAST responses to wheat. In addition, 12 patients had recurrent urticaria (mean age, 37 years; range 18 to 82 years) but negative skin prick test and RAST responses to wheat; 11 patients had coeliac disease (mean age, 48 years; range, 29 to 71 years).

In ELISA inhibition, microtiter plates were coated with an extract of wheat proteins (concentration adjusted to 15 μ g/mL), and 50 μ L of pooled patient sera (diluted 1:10) was incubated with an equal volume of inhibitor solution. Serial 10-fold dilutions (concentrations ranging from 50 μ g/mL to 0.05 ng/mL) of the 2 purified wheat proteins (65 kd and 40 kd) were used as inhibitors. Further steps were performed as described earlier for ELISA.

Skin prick testing with purified wheat proteins and gliadin

The in vivo reactivity of the 2 purified wheat proteins was examined in 15 patients and 6 control subjects, which included 3 healthy adults and 3 adults with wheat atopic flour sensitivity. Serial 10-fold dilutions (concentrations ranging from 0.05 to 500 μ g/mL in PBS) were tested until the size of the wheal was equal to that of the histamine control (10 mg/mL; ALK, Copenhagen, Denmark) or until the highest concentration gave a negative result. In addition, prick testing with a commercially available crude gliadin (1 mg/mL in 20% ethanol; Sigma Chemical Co, St Louis, Mo) was performed.

RESULTS

Purification and molecular characterization of IgE-binding wheat proteins

Immunoblotting with pooled patient sera showed strong binding of IgE to 65-kd and 40-kd wheat proteins. These proteins were purified into apparent homogeneity by HPLC gel filtration and reversed-phase chromatography (Fig 1). The N-terminal sequence of the 65-kd protein was previously undescribed (SRLLSPRGKELHTPQEQF-PQQQFPQPQQFPQ; 32 amino acids analyzed), but it showed 61% identity to wheat γ -gliadin (amino acids 65-101). The 40-kd wheat protein gave 100% identity to α -gliadin (VRVPVPLQPQNPSQQQPQEQV; 22 amino acids analyzed).

TABLE I. Triggering factors and clinical findings of 18 patients with wheat-dependent, exercise-induced anaphylaxis

Patient	Sex/ age at onset (y)	Triggering factors		Clinical findings						No. of emer- gency treat- ments
		Food*	Exercise*	Average no. of attacks/y	Generalized urticaria/ angioedema	Dyspnea	Hypo- tension	Collapse	Anaphy- lactic reaction	
1	F/30	Bread, pizza	Walking, dancing	3	+	+	+	+	+	4
2	M/22	Bread	Walking	4	+/+			+		1
3	M/28	Pasta	Walking	3	+/+	+	+	+	+	1
4	M/31	Bread	Walking	1	+			+		2
5	M/49	Pizza	Badminton	1	+/+	+	+	+	+	1
6	M/20	Bread	Indoor floorball	10	+/+		+	+	+	5
7	F/18	Muesli	Jogging	5	+/+	+	+	+	+	2
8	F/20	Pizza, hamburger	Cycling, dancing	5	+/+	+		+	+	2
9	M/39	Bread	Walking, cycling	5	+/+		+	+	+	1
10	M/20	Doughnut	Squash	4	+					0
11	F/36	Pie	Walking	3	+		+	+	+	2
12	F/59	Cookies	Walking	4	+		+	+	+	2
13	M/30	Pizza	Golf	5	+		+	+	+	1
14	M/56	Bun	Jogging	1	+		+	+	+	2
15	M/30	Bread	Jogging	3	+		+	+	+	5
16	M/32	Bread	Jogging	3	+/+		+	+	+	1
17	F/36	Pasta, pizza	Skiing, walking	5	+/+		+	+	+	5
18	F/25	Pasta	Walking	3	+/+		+	+	+	3

*Causing the most severe reaction.

TABLE II. Skin prick test and RAST results of 18 patients with wheat-dependent, exercise-induced anaphylaxis

Patient	Skin prick tests (wheal diameter in mm)					Wheat RAST (KU/L)	Total IgE (KU/L)
	Histamine (10 mg/ mL)	Wheat	γ -like gliadin/ concentration (μ g/mL)*	α -gliadin (500 μ g/mL)	Crude gliadin 1 mg/mL)		
1	3	6	5/0.5*	2	4	6.7	210
2	4	10	4/0.05	0	6	1.9	126
3	4	4	4/5	0	5	7.0	185
4	5	5	ND	ND	ND	1.5	137
5	5	8	5/0.05	3	5	8.0	1114
6	5	7	5/0.5	0	6	2.4	385
7	4	4	4/0.5	0	4	2.7	86
8	4	10	6/5	5	6	3.6	348
9	5	10	5/0.05	4	7	22.0	1669
10	5	5	5/50	0	4	0.4	74
11	5	8	ND	ND	ND	18.0	274
12	6	8	ND	ND	ND	6.1	137
13	6	6	6/5	0	5	19.0	1096
14	4	5	4/50	0	5	16.0	955
15	5	6	5/5	3	5	30.0	978
16	5	5	5/50	0	5	12.0	682
17	5	5	5/0.5	0	6	4.1	129
18	5	6	5/50	0	5	1.1	554

ND, Not done.

*Concentration causing a wheal at least the size of that caused by histamine.

TABLE III. Dietary treatment of 18 patients with wheat-dependent, exercise-induced anaphylaxis

Patient	Diet	Duration (mo)	No. of allergic reactions during follow-up
1	Gluten-free	12	1
2	Wheat-free	18	1
3	Avoids wheat before exercise	12	3
4	Wheat-free	14	0
5	Gluten-free	14	0
6	Avoids wheat before exercise	24	5
7	Gluten-free	7	0
8	Wheat-free	6	1
9	Gluten-free	10	0
10	Gluten-free	3	0
11	Gluten-free	18	0
12	Gluten-free	42	0
13	Gluten-free	8	0
14	Gluten-free	78	0
15	Gluten-free	84	0
16	Gluten-free	3	0
17	Gluten-free	3	0
18	Gluten-free	2	0

ELISA results

All 18 patients showed IgE antibodies to the γ -like gliadin, and 13 patients showed IgE antibodies to the α -gliadin (Fig 2). Patient 10 (Table I), with borderline levels of IgE to the γ -like gliadin, was the only one who had not experienced any systemic symptoms other than generalized urticaria. None of the 54 control subjects showed IgE antibodies to the γ -like gliadin. One control subject with Baker's asthma and 1 control subject with occupational allergic rhinitis had slightly elevated IgE levels to the α -gliadin (Fig 2).

In ELISA inhibition, the γ -like gliadin inhibited up to 80% of the IgE binding to solid-phase wheat proteins (Fig 3). Only a weak inhibition (<15%) of IgE binding was achieved when α -gliadin was used as the inhibitor (Fig 3).

In vivo reactivity of the purified gliadins

The prick tests performed with the γ -like gliadin showed positive reactions in all 15 patients tested (Table II). The concentration causing a wheal at least the size of that caused by histamine was 0.05 μ g/mL in 3 patients, 0.5 μ g/mL in 4 patients, 5 μ g/mL in 4 patients, and 50 μ g/mL in 4 patients. Five patients showed positive reactions to the purified α -gliadin with the highest concentration (500 μ g/mL) tested. Commercial crude gliadin (1 mg/mL) gave positive skin prick test reactions in all 15 patients (Table II). All 6 control subjects had negative results when tested with the purified or crude gliadins.

Dietary treatment

Thirteen patients adhered to a gluten-free diet; 3 patients avoided only wheat, and 2 patients did not eat wheat before

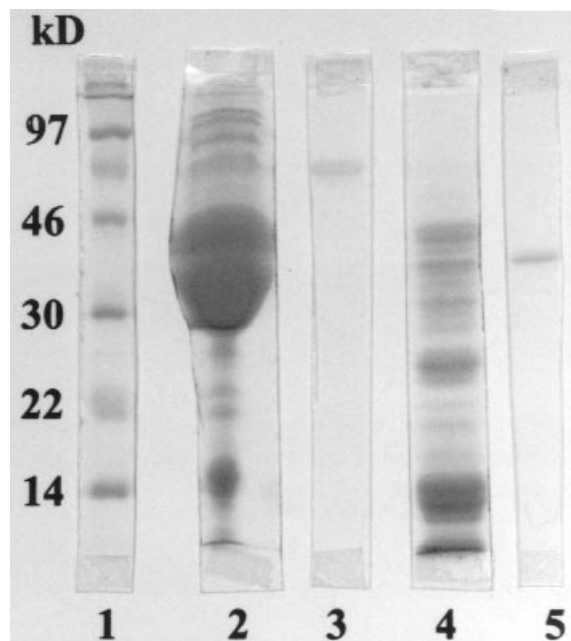


FIG 1. SDS-PAGE and Coomassie-blue staining of the purified 65-kd and 40-kd wheat proteins. Lane 1, Molecular weight marker; lane 2, wheat protein extract (acetonitrile-soluble); lane 3, γ -like gliadin (65-kd protein); lane 4, wheat protein extract (mainly water-soluble); lane 5, α -gliadin (40-kd protein).

exercise (Table III). All but 1 of the patients continued their previous exercise habits. During the follow-up (mean, 20 months), 2 patients on a wheat-free diet and 1 patient on a gluten-free diet experienced reactions after having unknowingly eaten wheat (licorice) before walking, bicycling, or jogging. All 3 patients experienced generalized urticaria; 1 patient also collapsed. The 2 patients who avoided wheat only in connection with exercise experienced annually 1 to 3 urticaria reactions (Table III).

DISCUSSION

This study describes 18 patients with wheat-dependent, exercise-induced anaphylaxis and shows that IgE antibodies to gliadins are frequently detected in such patients. In ELISA, all 18 patients (but none of the 54 control subjects) had IgE antibodies to a novel γ -like gliadin, while 13 patients and 2 control subjects had IgE antibodies to α -gliadin. The γ -like gliadin caused positive skin prick test reactions in all 15 patients tested, whereas the α -gliadin was positive in 5 patients. The γ -like gliadin inhibited 80% and the α -gliadin inhibited less than 15% of the IgE-binding to an extract of wheat proteins. These results suggest that the IgE antibodies in patients with wheat-dependent, exercise-induced anaphylaxis are directed mainly against the γ -like gliadin and, to a lesser extent, against the α -gliadin. The binding of IgE antibodies to α -gliadin could, however, be due to the presence of cross-reacting epitopes in the γ -like and α -gliadin.

The major allergens in Baker's asthma belong to the

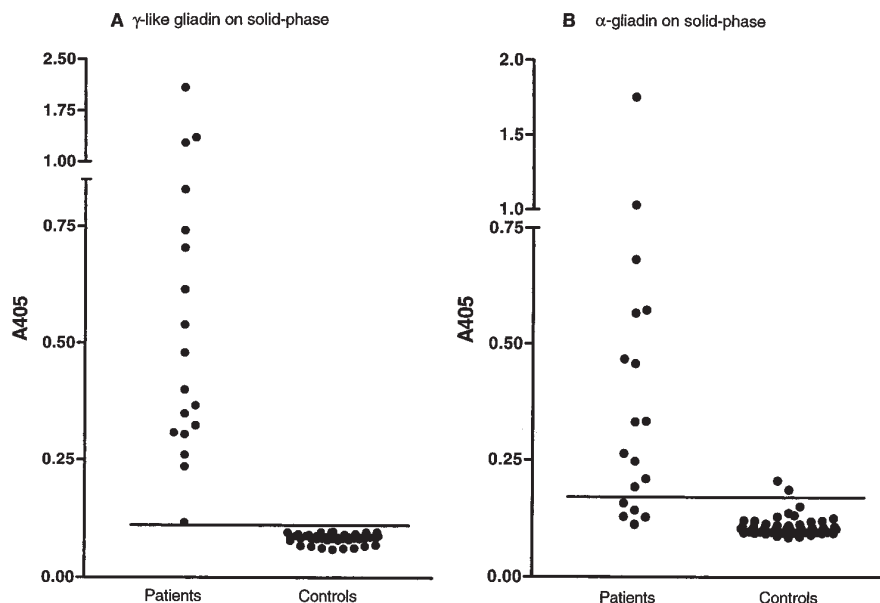


FIG 2. IgE antibody levels to the γ -like gliadin (A) and α -gliadin (B) in ELISA (absorbance units) in 18 patients with wheat-dependent, exercise-induced anaphylaxis and 54 control subjects.

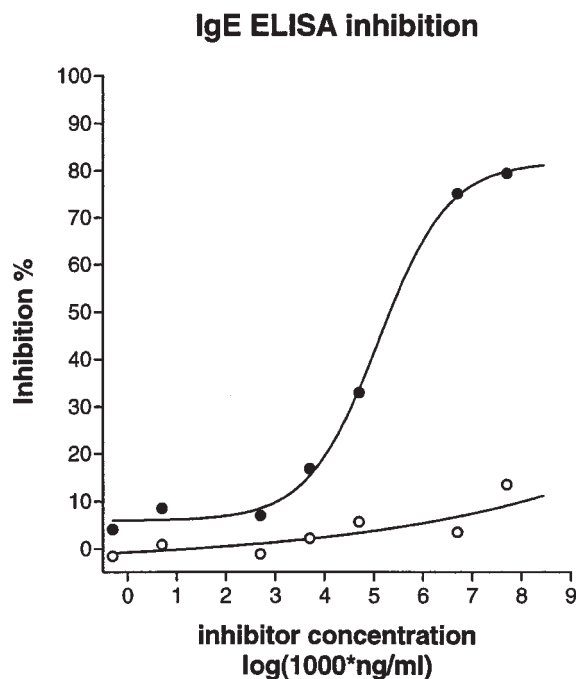


FIG 3. Inhibition of IgE binding from pooled patient sera to solid-phase wheat proteins with the γ -like gliadin (closed circles) and α -gliadin (open circles).

family of α -amylase inhibitors, a group of water-soluble proteins present in wheat, barley, and rye.^{14,15,23,24} In this study, we examined by ELISA whether the patients with wheat-dependent, exercise-induced anaphylaxis have IgE antibodies to a commercial α -amylase inhibitor. However, none of the patients showed IgE to this allergen (data not shown). In contrast to previously character-

ized wheat allergens, gliadins are mainly water-insoluble and have not until recently been regarded as important allergens.^{16,19} It is well known, however, that gliadin peptides damage the gut mucosa in coeliac disease²⁵ and that untreated patients frequently have IgA-class antigliadin antibodies.²⁶ A recent study showed that gut-derived T cells from patients with coeliac disease recognize an epitope in γ -gliadin, suggesting that γ -gliadin is an important wheat antigen also in coeliac disease.²⁷

The mechanism by which exercise provokes the reactions in food-dependent, exercise-induced anaphylaxis remains unclear. Mast-cell degranulation and elevated plasma histamine levels are important factors,^{8,28} and it has been suggested that the interaction of IgE and food antigen could lower the mast-cell threshold to another stimulus, such as exercise.⁶ On the other hand, it can be speculated that exercise may induce changes in mucosal enzyme activities or barrier functions, which could facilitate the absorption of gliadin or gliadin peptides from the gut. This, in turn, could cause IgE-mediated allergic reactions in sensitized subjects. Interestingly, Kushimoto and Aoki⁷ showed by skin testing that digestion with pepsin increased and that with trypsin decreased the activity of wheat allergens in patients with wheat-dependent, exercise-induced anaphylaxis. The allergenic activity of gliadin could thus be enhanced in the stomach and maintained in the duodenum, where the allergens could be absorbed during exercise.

Wheat-dependent, exercise-induced anaphylaxis has been previously reported in 11 patients in Japan,^{7,8,29,30} 10 patients in France,⁵ 2 patients in Italy,³¹ and 1 patient in Spain.³² We found 13 patients during a 2-year period, and 5 were from our previous study.⁹ This suggests that wheat-dependent, exercise-induced anaphylaxis is not as rare as previously thought but rather is a poorly recognized

disorder. Skin prick testing with crude gliadin is a simple screening method that should be performed for all patients with exercise-induced urticaria or anaphylactic reactions.

In the patients in this study, the diagnosis was based on a positive clinical history (ie, wheat ingestion preceded the clinical symptoms of exercise-induced anaphylaxis) and on a positive skin prick test response to wheat. The finding that bicycle ergometry did not cause systemic reactions excluded classic exercise-induced anaphylaxis. Because of the severity of the reactions, exercise challenge tests with ingestion of wheat were not performed.³³ Previously, these tests have clearly demonstrated that wheat ingestion before exercise can induce severe and life-threatening anaphylactic reactions.^{29,30} Only 5 of the present patients had a history of atopic disorders or positive skin prick test responses to common inhalant or food allergens. The total IgE levels were, however, elevated in most patients (Table II), a finding that could be explained by high levels of anti-gliadin IgE antibodies.

In conclusion, this study showed that wheat is a frequent cause of food-dependent, exercise-induced anaphylaxis and that a novel γ -like gliadin seems to be the major allergen. Further studies are needed to characterize the IgE-binding epitopes of the γ -like gliadin and its cross-reactivities to other gliadins and cereal prolamins. Studies are also needed to clarify the mechanisms by which exercise provokes food-dependent, anaphylactic reactions. Skin prick testing with crude gliadin is a screening method that facilitates the recognition and diagnosis of wheat-dependent, exercise-induced anaphylaxis. For treatment of this life-threatening allergy, we recommend a gluten-free diet.

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