

Serum Gliadin Monitoring Extracts Patients with False Negative Results in Challenge Tests for the Diagnosis of Wheat-Dependent Exercise-Induced Anaphylaxis

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ABSTRACT

Background: Challenge testing with wheat plus exercise and/or aspirin is a gold standard for the diagnosis of wheat-dependent exercise-induced anaphylaxis (WDEIA); however, the test may often yield false-negative results. Our previous study suggested that an increase in serum wheat gliadin levels is required to induce allergic symptoms in patients with WDEIA. Based on this knowledge, we sought to extract the patients with false negative results in the challenge tests of WDEIA.

Methods: Thirty-six patients with suspected WDEIA were enrolled. First, group categorizations—Group I, challenge tests were positive; Group II, challenge tests were negative and serum gliadin were undetectable; Group III, challenge tests were negative and serum gliadin were detectable—were given according to the results of wheat plus exercise and/or aspirin challenge testing and serum gliadin levels. Second, diagnoses were made using retests and/or dietary management in Group II and III.

Results: Positive results for wheat plus exercise and/or aspirin challenge tests gave a diagnosis of definite WDEIA in 17 of 36 patients (Group I). Of the remaining 19 challenge negative patients, serum gliadin was undetectable in ten patients (Group II). Of the ten patients (Group II), three of them were diagnosed as definite WDEIA by retesting and six of them were diagnosed as probable WDEIA using a wheat elimination diet, whereas one patient was non-WDEIA. In the rest of the nine challenge negative patients, serum gliadin was detectable (Group III). No allergic episodes with a normal diet provided a diagnosis of non-WDEIA in seven of the nine patients, whereas the remaining two patients were probable WDEIA or had another food allergy because of repeated episodes.

Conclusions: Our study revealed that serum gliadin monitoring during challenge testing is useful.

KEY WORDS

challenge test, serum gliadin monitoring, wheat-dependent exercise-induced anaphylaxis, ω -5 gliadin

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the paper. All authors discussed the results and commented on the manuscript.

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ABBREVIATIONS

FDEIA, food-dependent exercise-induced anaphylaxis; HMW-glutenin, high-molecular-weight glutenin subunit; NSAIDs, non-steroidal anti-inflammatory drugs; WDEIA, wheat-dependent exercise-induced anaphylaxis.

INTRODUCTION

Food-dependent exercise-induced anaphylaxis (FDEIA) is a severe form of food allergy, in which symptoms are elicited by exercise in combination with ingestion of the culprit food.^{1,3} Other factors that elicit allergic symptoms in addition to exercise include non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, patient's general conditions and atmospheric conditions.^{3,5} In Japanese patients, the most frequent cause of FDEIA is wheat, which is found in almost 60% of culprit foods.^{3,6} In contrast, vegetables are the most frequent culprit food in European cases of FDEIA.⁷

Diagnosis of FDEIA is made based on detailed interviews, allergy skin testing, detection of serum allergen-specific IgE, and challenge testing. In the case of wheat-dependent exercise-induced anaphylaxis (WDEIA), skin testing with commercial wheat extract is not always reliable because the wheat extract consists largely of water-soluble proteins and only a trace amount of water-insoluble proteins (gluten) that are the major cause of WDEIA.⁸ A specific IgE test with recombinant ω -5 gliadin is highly reliable and now widely used to identify the patients with WDEIA. However, the test did not positively identify approximately 20% of cases.⁹⁻¹³ The exercise challenge test combined with wheat ingestion is the most definitive test to diagnose WDEIA, if the test elicits allergic symptoms. However, WDEIA, cannot be excluded when the challenge test is negative, because the challenge tests are not always successful. The success of a test is highly dependent on the amount of wheat, intensity of exercise and the condition of the patients.^{3,14} Therefore, a negative test may turn out to be a false-negative result.

In a previous study, we found that gliadin concentrations markedly increased in the sera of patients with WDEIA in parallel with allergic symptoms during positive challenge testing.¹⁵ We also demonstrated that aspirin, a well-documented trigger of WDEIA even in patients without a history of exacerbation due to NSAIDs,^{5,6} had an ability to increase the serum concentration of gliadin when taken before wheat ingestion.¹⁶ These observations indicated that both exercise and aspirin intake cause an absorption of non-digested gliadin from the intestine into the blood circulation. In addition, we found that the increase in serum gliadin also occurred in healthy subjects.^{15,16} Taken together, we consider that the amount of allergen-absorbed via the intestine is critical for eliciting the allergic symptoms of WDEIA seen after wheat ingestion.

Herein, we propose a new index to determine whether there was a sufficient challenge to cause gliadin absorption, by combining wheat plus exercise and/or aspirin challenge testing with serum gliadin monitoring.

METHODS

SUBJECTS

Thirty-six patients (17 female and 19 male; mean age, 34.9 years; range, 9-71 years), who experienced allergic symptoms after ingesting wheat-containing foods in combination with exercise and/or NSAIDs were enrolled in this study. Table 1 lists the patient characteristics, clinical histories, total IgE and specific IgE levels for wheat, gluten, ω -5 gliadin and high-molecular-weight glutenin subunit (HMW-glutenin). Twenty-nine of the 36 patients experienced more than three allergic episodes (patients 1-18, 20-25, 27-29, 33 and 35), two patients had two episodes (patient 26 and 34) and five had one episode (patients 19, 30-32, and 36). Clinical histories included urticaria, generalized erythema, palpebral edema, sneezing, laryngeal edema, dyspnea, nausea and anaphylaxis. All patients provided informed consent prior to participation in the study. Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine at Shimane University (No. 121).

MEASUREMENT OF SERUM ALLERGEN-SPECIFIC IgE

IgE antibodies specific for wheat, gluten, ω -5 gliadin and HMW-glutenin were detected using the CAP-fluorescent-enzyme immunoassay (ImmunoCAP, Phadia, Uppsala, Sweden) as previously described.^{10,11} Values >0.35 kUA/L were judged to be positive in this study.

CHALLENGE TESTS

Challenge tests for WDEIA were performed as previously described.¹⁵ The tests involved wheat ingestion, exercise, pretreatment with aspirin or a combination of these challenges on a separated day (Fig. 1). Briefly, the wheat products included noodles, bread or pasta containing 60-135 g of wheat flour. Thirty minutes after wheat ingestion, exercise was performed using a treadmill for 15-20 min according to the standard Bruce protocol. Aspirin (500 mg) was administered 30 min prior to wheat ingestion. A positive test was determined if allergic symptoms such as wheals and/or anaphylaxis were observed. During the challenge tests, 9 mL of blood was drawn at each time point via an indwelling needle. Patients with

Table 1 Patient characteristics and clinical and laboratory data

Group	Patient No.	Age (years)	Gender	Episodes	Symptoms during episodes	Total IgE (kIU/L)	Specific IgE (kUA/L)			
							Wheat	Gluten	ω -5 gliadin	HMW-glutenin
Group I (n = 17)	1	32	M	>3	Anaphylaxis, Urticaria	146	<0.35	<0.35	5.35	<0.35
	2	61	M	>3	Anaphylaxis, Systemic urticaria	45	1.76	1.55	9.08	<0.35
	3	36	F	>3	Anaphylaxis, Urticaria	201	0.42	0.65	12.3	<0.35
	4	16	M	>3	Anaphylaxis, Systemic urticaria	80	<0.35	<0.35	3.72	<0.35
	5	44	M	>3	Anaphylaxis, Systemic urticaria	150	<0.35	<0.35	5.45	<0.35
	6	52	F	>3	Anaphylaxis, Urticaria, Dyspnea	260	0.37	0.66	5.15	<0.35
	7	15	M	>3	Dyspnea, Urticaria	590	<0.35	2.10	<0.35	<0.35
	8	17	M	>3	Anaphylaxis, Urticaria	602	1.34	0.52	13.1	<0.35
	9	43	F	>3	Anaphylaxis, Systemic urticaria	105	<0.35	<0.35	2.14	<0.35
	10	25	F	>3	Anaphylaxis, Systemic urticaria	1007	1.32	2.84	16.3	<0.35
	11	19	M	>3	Anaphylaxis, Wheezing, urticaria	109	<0.35	<0.35	<0.35	3.53
	12	38	F	>3	Anaphylaxis, Systemic urticaria	660	0.66	1.52	4.22	<0.35
	13	23	M	>3	Dyspnea, Generalized erythema	212	15.3	17.3	<0.35	<0.35
	14	47	F	>3	Anaphylaxis, Urticaria	107	<0.35	<0.35	2.15	-
	15	57	F	>3	Anaphylaxis, Systemic urticaria	292	<0.35	0.51	5.96	<0.35
	16	58	M	>3	Anaphylaxis, Systematic urticaria	22	<0.35	0.51	2.20	<0.35
	17	17	M	>3	Anaphylaxis, Systematic urticaria	2220	13.1	<0.35	<0.35	<0.35
Group II (n = 10)	18	34	F	>3	Anaphylaxis, Generalized erythema	770	0.76	0.90	14.1	<0.35
	19	65	F	1	Systemic urticaria, Dyspnea	1190	0.78	0.90	15.5	<0.35
	20	12	M	>3	Anaphylaxis, Systemic urticaria	126	1.21	<0.35	<0.35	1.36
	21	34	M	>3	Anaphylaxis, Systemic urticaria	5420	4.26	13.5	74.9	<0.35
	22	14	M	>3	Palpebral edema, Systemic urticaria	36	<0.35	<0.35	<0.35	0.42
	23	48	M	>3	Anaphylaxis, Urticaria	2028	<0.35	<0.35	1.63	<0.35
	24	9	M	>3	Anaphylaxis, Systemic urticaria	343	<0.35	<0.35	<0.35	1.72
	25	18	F	>3	Systemic urticaria	20022	11.7	1.06	0.49	0.39
	26	24	F	2	Urticaria	434	<0.35	0.83	5.72	<0.35
	27	24	F	>3	Impetiginous eczema	10001	0.95	<0.35	<0.35	<0.35
Group III (n = 9)	28	40	F	>3	Anaphylaxis, Urticaria, Nausea	111	<0.35	<0.35	2.44	<0.35
	29	28	F	>3	Palpebral and laryngeal edema, Sneezing, Dyspnea	360	0.94	0.44	<0.35	<0.35
	30	28	F	1	Systemic urticaria	278	1.19	1.23	<0.35	<0.35
	31	18	M	1	Dyspnea, Palpebral edema	749	0.50	<0.35	<0.35	<0.35
	32	71	F	1	Anaphylaxis, Systemic urticaria	412	2.17	0.55	<0.35	<0.35
	33	64	M	>3	Anaphylaxis	541	<0.35	<0.35	0.36	<0.35
	34	18	M	2	Anaphylaxis, Systemic urticaria	1699	<0.35	<0.35	<0.35	<0.35
	35	60	M	>3	Anaphylaxis, Systemic urticaria	186	<0.35	<0.35	<0.35	<0.35
	36	48	F	1	Anaphylaxis, Systemic urticaria	160	<0.35	<0.35	<0.35	<0.35

Gender: M; male, F; female.

Anaphylaxis: a condition showing symptoms caused by insufficient blood circulation.

-, not done.

negative challenge test results were divided into either an undetectable (<15 pg/ml) (Group II) or a detectable ($\geq$ 15 pg/m) (Group III) according to their serum gliadin concentration.

SERUM GLIADIN MONITORING

Serum gliadin concentrations were determined using an ELISA kit for wheat gliadin (Morinaga Institute of

Biological Science, Yokohama, Japan) as previously described.¹⁵ Briefly, 0.25 ml of serum was mixed with 0.05 ml deionized water and 0.7 ml ethanol and the mixture was vortexed for 1 min. The mixture was then centrifuged at 20,000 g for 20 min at room temperature. The gliadin-containing supernatant was decanted into a new tube, and evaporated in a vacuum centrifuge evaporator (VEC-260, Iwaki Glass Co.,

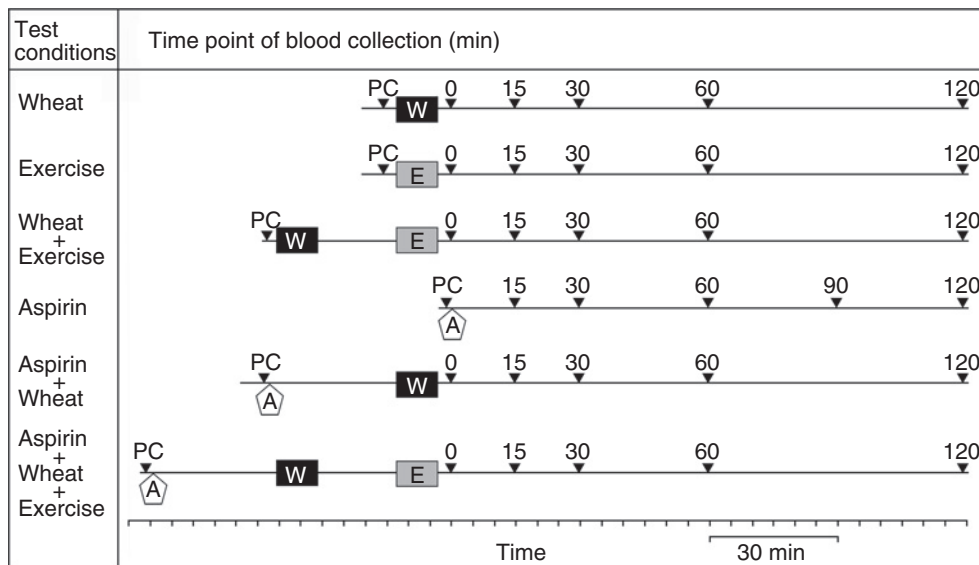


Fig. 1 Challenge test conditions and time-course of blood sampling. Test conditions are shown in the left-hand column and time points for blood collection in the right-hand column. PC, pre-challenge; W in a black square indicates wheat ingestion; E in a gray square indicates exercise; A in a white pentagon indicates aspirin ingestion; the black inverted triangle indicates point of blood sampling.

Ltd., Chiba, Japan). The dried residue was dissolved in 0.05 ml of the sample dilution buffer included in the kit and the gliadin concentration measured according to the manufacturer's instructions. The gliadin concentration in each sample was calculated using a gliadin standard (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan). All measurements were performed in duplicate. The detection limit of serum gliadin level was 15 pg/mL.

DIAGNOSTIC PROCEDURE

The patients were divided into three groups after challenge testing combined with serum gliadin monitoring as illustrated in Figure 2. Patients who were evaluated as having a positive result in the challenge tests were included in the group of definite WDEIA (Group I). Patients with negative challenge test results were divided into either an undetectable (Group II) or a detectable (Group III) according to their serum gliadin concentration. The Group II patients were considered insufficiently challenged and were required to undergo retesting. Patient with positive retests were considered as definite WDEIA. Patients with a negative retest, with undetectable serum gliadin, were considered as a failure of retesting and followed with a diet management. Those who declined retests also followed with a diet management. The diagnostic endpoint of the diet management was the clinical outcomes during a 1-year follow-up of wheat elimination diet or exercise-restriction after their meals. If the patient did not experience symptoms during the 1-year follow-up, a diagnosis of probable

WDEIA was given. If a patient in this group had recurrent symptoms without wheat plus exercise and/or NSAIDs, a diagnosis of non-WDEIA was given. The Group III patients maintained a normal diet for a year, and during this time their allergic symptoms were carefully monitored. If a patient in this group had no symptoms during the year, a diagnosis of non-WDEIA was given. If a patient in this group had recurrent symptoms with wheat plus exercise and/or NSAIDs, a diagnosis of probable WDEIA or other food allergy was considered.

RESULTS

CHALLENGE TESTS AND SERUM GLIADIN MONITORING

All patients participated in the challenge testing and serum gliadin was monitored throughout the challenge test. The results of the challenge testing and the maximal serum gliadin concentrations are summarized in Table 2. All 36 patients had a negative reaction to a single challenge with wheat, exercise or aspirin. Of the 36 patients, 17 had a positive reaction with either wheat plus exercise, aspirin plus wheat or aspirin plus wheat plus exercise. However, 19 patients had a negative reaction even in the combination challenge of wheat and exercised and/or aspirin.

Representative patterns of serum gliadin kinetics are shown in Figure 3. Figure 3A shows the kinetics of patient 1 who had a positive challenge test with wheat and exercise. A similar pattern was seen for another seven patients (patients 2-7 and 16). Figure 3B shows the kinetics of patient 11, who had a positive

Serum Gliadin Monitoring in WDEIA

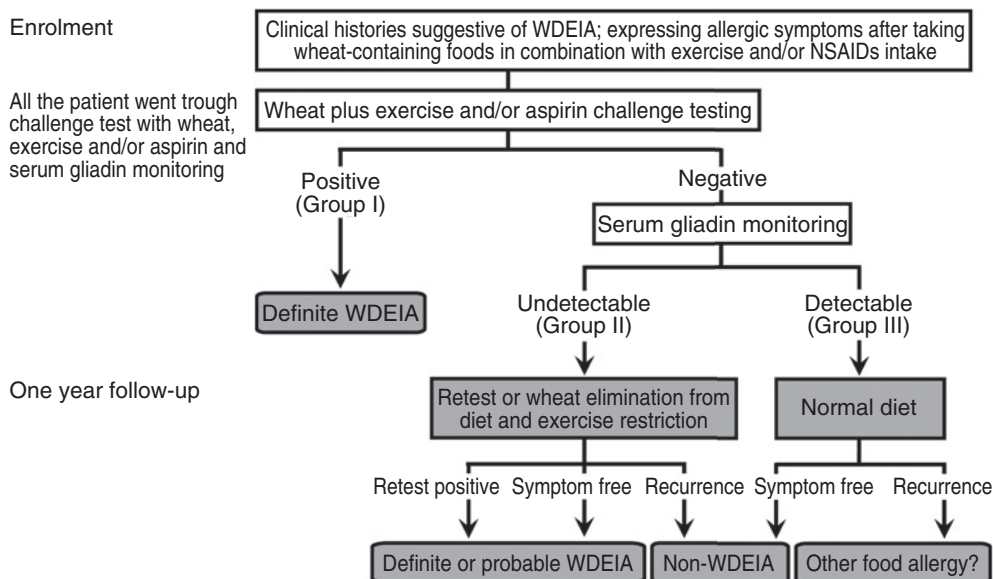


Fig. 2 Diagnostic procedure for WDEIA. Black-framed rectangles indicate criteria for the group categorization; black-framed gray rectangles indicate the follow-up for the diagnosis; rounded gray squares indicate the diagnosis.

challenge test with aspirin and wheat. A similar pattern was seen for another seven patients (patients 6-10, 12 and 16). Figure 3C shows the kinetics of patient 13, who developed allergic symptoms after a triple challenge. This pattern was also observed for patient 14-17. Neither symptoms nor an increase in serum gliadin concentrations were seen under any of the test conditions in ten patients (patients 18-27, Fig. 3D). Serum gliadin was detected in patient 31 even after a negative challenge test with aspirin and wheat (Fig. 3E). A similar pattern was observed for patients 28 and 30-35. In patients 29 and 36, serum gliadin was detected without allergic symptoms after a triple challenge. In the 17 patients with negative challenge testing (patient 18-34), serum gliadin was undetectable in 10 patients (patient 18-27) but was detectable in seven patients (patient 28-36).

CATEGORIZATION OF PATIENTS BY DIAGNOSTIC PROCEDURE

According to the diagnostic procedure, patients were categorized into three groups (Fig. 2). A diagnosis of definite WDEIA was made in 17 patients and they were categorized into Group I (patients 1-17). Those who were negative to wheat plus exercise and/or aspirin challenge with undetectable serum gliadin were categorized into Group II (patients 18-27). Those who had negative challenge testing with detectable serum gliadin concentration were categorized into Group III (patients 28-36).

ELIMINATION DIET TREATMENT AND FINAL DIAGNOSES IN THE GROUP II

Patients in Group II were proceeded to either retest and/or follow a wheat elimination diet. Table 3 summarizes the results. Three patients (patient 18-20) were found to be positive by retesting, whereas patient 21 had negative test. Patient 21 then followed a further elimination diet and experienced recurrence of urticaria with accidental ingestion of wheat product and exercise. Patient 22 visited the emergency room with anaphylactic shock after accidental ingestion of curry, and had a serum gliadin level of 137 pg/mL. The other four patients (patient 23-26) had no allergic symptoms after following a wheat-free diet for more than 1 year, resulting in a diagnosis of probable WDEIA. Patient 27 experienced recurrent symptoms despite wheat elimination and was diagnosed as non-WDEIA. In this procedure, definite WDEIA was diagnosed in three patients, probable WDEIA was diagnosed in six patients, and non-WDEIA was diagnosed in one patient.

NORMAL DIET TREATMENT AND FINAL DIAGNOSIS IN THE GROUP III

A final diagnosis of non-WDEIA was given for 7/9 patients (patients 30-36) in Group III who experienced no symptoms after a normal diet for more than 1 year. Patient 28, who experienced repetitive episodes after a combination of wheat and exercise, was given a final diagnosis of probable WDEIA. In patient 28, challenge testing and serum gliadin monitoring excluded WDEIA. Because she has ω -5 gliadin specific IgE (2.44 kUA/L), which is higher than the cutoff

Table 2 Challenge test and serum gliadin results during testing

Group	Patient No.	Wheat		Exercise		Wheat + Exercise		Aspirin		Aspirin + Wheat		Aspirin + Wheat + Exercise		Retest (based on the episode)	
		Result	Gliadin	Result	Gliadin	Result	Gliadin	Result	Gliadin	Result	Gliadin	Result	Gliadin	Result	Gliadin
Group I (n = 17)	1	N	ud	N	ud	P	127	-	-	-	-	-	-	-	-
	2	N	ud	N	ud	P	216	-	-	-	-	-	-	-	-
	3	N	ud	N	ud	P	15	N	-	N	-	-	-	-	-
	4	N	-	N	-	P	27	-	-	-	-	-	-	-	-
	5	N	-	N	-	P	65	N	-	N	-	P	-	-	-
	6	N	ud	N	ud	P	17	N	-	P	43	-	-	-	-
	7	N	16	N	ud	P	96	N	-	P	15	-	-	-	-
	8	N	ud	N	ud	N	18	N	ud	P	18	-	-	-	-
	9	N	30	-	-	-	-	N	-	P	132	-	-	-	-
	10	N	25	-	-	-	-	N	ud	P	76	-	-	-	-
	11	N	18	-	-	-	-	N	ud	P	107	-	-	-	-
	12	N	ud	-	-	-	-	-	-	P	200	-	-	-	-
	13	N	ud	N	ud	N	ud	N	ud	N	ud	P	18	-	-
	14	N	ud	-	-	N	ud	N	-	N	ud	P	19	-	-
	15	N	-	-	-	N	ud	-	-	-	-	P	91	-	-
	16	N	-	N	ud	P	53	N	-	P	52	P	122	-	-
	17	N	-	N	-	N	-	-	-	-	-	P	333	-	-
Group II (n = 10)	18	N	ud	-	-	-	-	-	-	N	ud	-	-	P	18
	19	N	ud	-	-	N	ud	N	-	N	-	N	ud	P	ud
	20	N	ud	-	-	N	ud	-	-	-	-	N	ud	P	62
	21	N	ud	-	-	-	-	-	-	N	ud	-	-	N	ud
	22	N	ud	-	-	-	-	N	ud	N	ud	-	-	-	-
	23	N	ud	-	-	N	ud	-	-	N	ud	-	-	-	-
	24	N	ud	N	ud	N	ud	-	-	-	-	-	-	-	-
	25	N	ud	-	-	-	-	-	-	N	ud	-	-	-	-
	26	N	ud	N	ud	N	ud	-	-	-	-	-	-	-	-
	27	N	-	-	-	-	-	-	-	N	ud	-	-	-	-
Group III (n = 9)	28	N	ud	N	ud	N	ud	-	-	N	20	-	-	-	-
	29	N	ud	N	ud	N	ud	-	-	-	-	N	29	-	-
	30	N	ud	-	-	N	ud	-	-	N	133	-	-	-	-
	31	N	ud	-	-	-	-	N	ud	N	61	-	-	-	-
	32	N	35	-	-	-	-	-	-	N	70	-	-	-	-
	33	N	ud	-	-	-	-	N	-	N	31	-	-	-	-
	34	N	-	-	-	-	-	N	-	N	181	-	-	-	-
	35	N	-	-	-	-	-	-	-	N	234	-	-	-	-
	36	N	-	-	-	-	-	-	-	-	-	N	255	-	-

Gliadin, serum gliadin concentration (pg/mL); P, positive; N, negative; ud, under the detection limit; -, not done.

Patient 18 showed urticaria when exercising after ingesting a sweet bun. Patient 19 showed urticaria after a combination of bread, aspirin, rebamipide, itopride, and exercise (in accordance with her medical history), although neither rebamipide nor itopride induced symptoms individually. Patient 20 showed urticaria after ingesting bread and exercise. Patient 21 was retested with additional condition; sweet bun with cream was ingested instead of noodles, neither serum gliadin nor symptom were induced.

value we have previously reported, she was considered as probable WDEIA. Although patient 29 must be retested within additional conditions, we could not get the patients cooperation to do so. Patient 29 did not have specific IgEs to ω -5 gliadin nor HMW-

glutenin. Thus there is still the possibility that patient has another food allergy.

DISCUSSION

In the present study, we provided evidence that se-

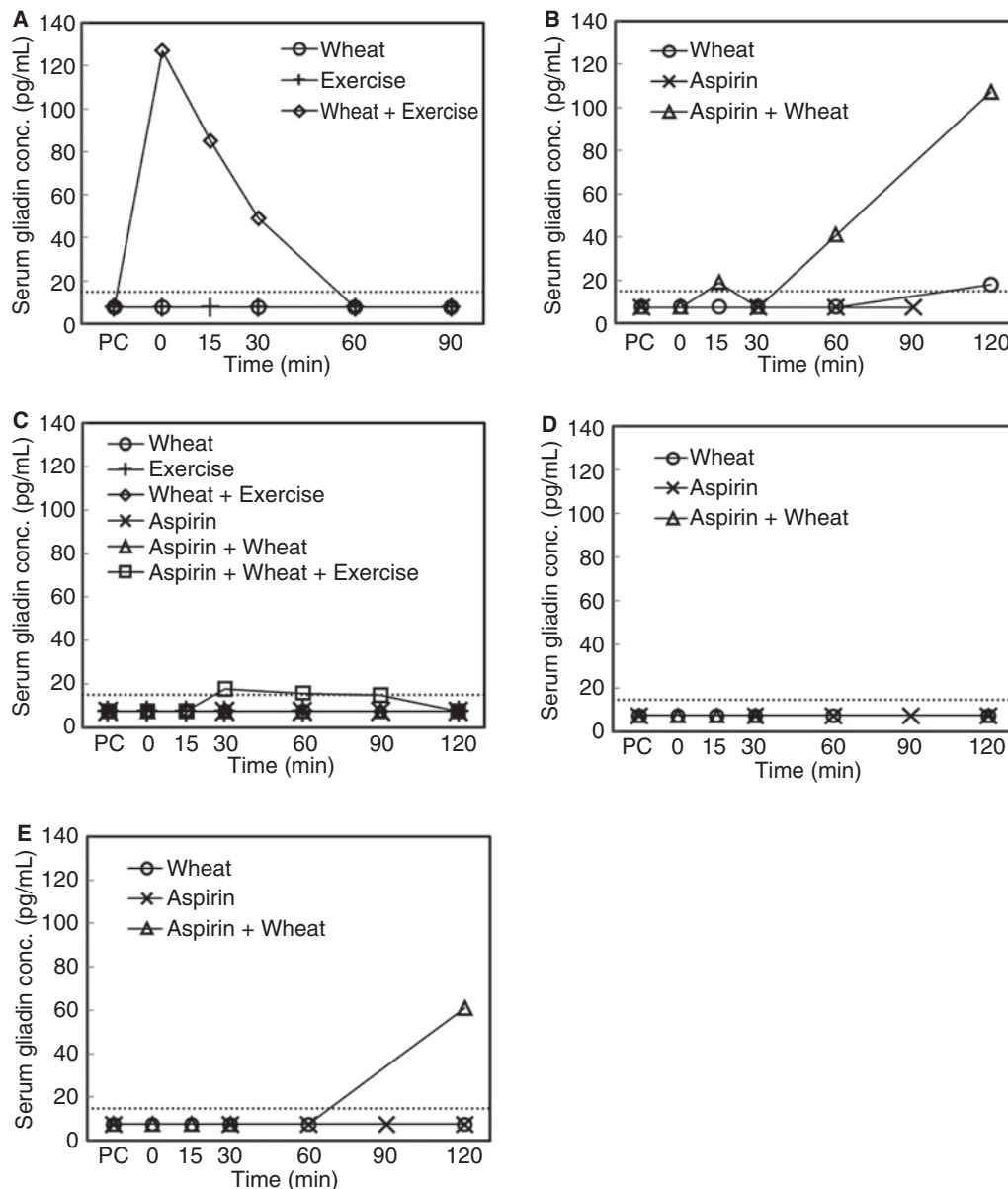


Fig. 3 Time course of serum gliadin kinetics. **A, B, C, D** and **E** show patterns in patients 1, 11, 13, 21 and 31, respectively. Circle, cross, diamond, x, triangle and square indicate challenge with wheat, exercise, wheat + exercise, aspirin, aspirin + wheat and aspirin + wheat + exercise, respectively. The dotted line indicates the detection limit (<15 pg/mL). PC, pre-challenge; conc., concentration.

rum gliadin monitoring is a good tool to use in checking the efficacy of challenge tests for the diagnosis of WDEIA, since a lack of increased serum gliadin can prove an insufficient challenge test. We focused on an extraction of WDEIA-suspected patients who had negative results of the challenge test, because it was believed that an elevation of serum gliadin is required to develop allergic symptoms in WDEIA. As with our previous study, increased serum gliadin levels were observed in the patients with positive challenge testing, supporting research that gliadin levels in the sera

correlate with allergic symptoms in WDEIA.¹⁵ The patients who had shown negative challenge testing were divided into two groups based on their serum gliadin levels; patients who had been tested by an efficient challenge with increased serum gliadin level (Group III) and patients who had not been tested efficiently without serum gliadin-increase (Group II). The false-negative challenge performed in Group II was confirmed by the retesting or wheat-elimination diet and the result showed that 9 out of 10 Group II patients were definite/probable WDEIA, whereas the

Table 3 Results of retests or wheat elimination diet of Group II

Patient No.	Retests	Wheat elimination diet	Final diagnosis
18	Positive	Symptom-free	Definite WDEIA
19	Positive	Symptom-free	Definite WDEIA
20	Positive	Symptom-free	Definite WDEIA
21	Negative	Recurrent allergic symptoms were observed during exercise after ingestion of wheat containing meal and exercise.	Probable WDEIA
22	ND	Anaphylaxis was observed after accidental exposure of wheat and exercise.	Probable WDEIA
23	ND	Symptom-free	Probable WDEIA
24	ND	Symptom-free	Probable WDEIA
25	ND	Symptom-free	Probable WDEIA
26	ND	Symptom-free	Probable WDEIA
27	ND	Symptoms were induced in spite of wheat eliminations.	Non-WDEIA

result of normal diet treatment showed that 7 out of 9 Group III patients were non-WDEIA. Thus, the detection of serum gliadin and classification of patients by its level during the challenge testing is of importance for interpreting the negative challenge test result.

The cutoff line between Group II and III were divided according to their serum gliadin level as being undetectable or detectable (15 pg/mL of cutoff value). Although the cutoff line might be debatable, we consider that this is the limitation of our current methodology.

Previously we observed that the absorption of wheat gliadin from the intestine varies according to the individual.¹⁶ This might be because of individual differences in intestinal sensitivity to exercise or aspirin. In the present study, we detected gliadins using anti-gliadin antibodies that bound to several types of gliadin such as α/β -gliadin, γ -gliadin, and ω -gliadin. Tanaka *et al.* recently reported that ω -5 gliadin is absorbed from the gastrointestinal tract more effectively than other wheat components in a mouse model.¹⁷ The same observation was obtained using an *in vitro* caco-2 cell line model.¹⁸ Thus, the specific detection of ω -5 gliadin seemed to provide effective details for the diagnosis of WDEIA. From a practical application standpoint, further study is required to decide the optimum time to measure the serum gliadin and to improve the complicated measurement method.

Retesting with a wheat product other than noodles, was succeeded in three of four patients (patients 18-20). Patients 18 and 20 induced urticaria following a retest that used bread instead of noodles. The difference of challenge test between noodles and bread may be due to amount of gluten contained in the same weight of wheat flour. Usually, wheat flour for making bread contains more gluten than that for making noodles.¹⁴

A high serum gliadin level (137 pg/mL) was detected during an allergic reaction induced by accidental ingestion of wheat products in patient 22. Serum

gliadin was not detected during the negative-challenge tests (Table 2), indicating that this patient was challenged with an insufficient amount of wheat product or needed secondary factors. The lack of allergen absorption in the gastrointestinal tract may explain the failure of challenge tests.

In this study, the diagnosis of WDEIA was made in 27 patients, including seven with probable WDEIA. Of these 27 patients, 19 patients were challenged with wheat plus exercise, and eight had a positive test (Table 2). This shows a positive-induction rate of 42.1% for the wheat plus exercise test. The induction rate of the aspirin plus wheat test was the same as that for the wheat plus exercise test (8/19; 42.1%). However, of the eight patients challenged with aspirin plus wheat plus exercise, six had a positive test (75.0% positive induction rate), indicating an additive effect of aspirin plus exercise. Exercise is known to increase intestinal permeability.¹⁹ Aspirin also increases gastrointestinal permeability²⁰ and increases serum gliadin levels in a dose-dependent fashion when taken in combination with wheat.¹⁶ The combined effects of NSAIDs plus exercise promote greater gastrointestinal permeability to high-molecular-weight substances.^{21,22} The mechanism of NSAID-enhanced gastrointestinal permeability is thought to involve decreased prostaglandin synthesis mediated via the inhibition of cyclooxygenase. This is supported by studies showing that co-administration of prostaglandin E₁ suppresses both allergic symptoms and serum gliadin levels during challenge tests involving aspirin and wheat.^{23,24}

Recently, two cases of WDEIA were reportedly induced by aspirin, but not exercise.²⁵ In our challenge tests, patients 7 and 8 did not show symptoms, despite their serum gliadin concentrations increased after ingesting wheat alone or in combination with exercise. However, symptoms were induced when challenged with aspirin plus wheat, with similar levels of gliadin. These results suggest that co-administration with aspirin may mediate mast cell activation in addi-

tion to increasing gastrointestinal tract permeability.

As we reported previously, HMW-glutenin showed additional diagnostic effectiveness when combined with ω -5 gliadin, because patients who have specific IgE to HMW-glutenin do not have specific IgE to ω -5 gliadin. From the 36 patients in the study, 24/27 (88.8%) of those with WDEIA, and 1/8 (12.5%) of those with non-WDEIA, were positive against ω -5 gliadin- and/or HMW-glutenin-specific IgE tests, respectively. The limitation of the ω -5 gliadin-specific IgE test has been demonstrated by another group.¹³ In fact, three patients (patients 7, 13 and 17) with definite WDEIA in the present study did not have IgE against ω -5 gliadin/HMW-glutenin. Patient 33 had specific IgE against ω -5 gliadin (0.36 kUA/L); however, he was diagnosed as non-WDEIA. We have recently shown that the specific IgE test to ω -5 gliadin can produce false positive results in 1.4% of Japanese adults, and that could be the case with patient 33.²⁶

In conclusion, serum gliadin monitoring during challenge testing for WDEIA is helpful to extract the patients with false negative results. However further study is needed for the practical application of this method.

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