

Exercise-induced allergic reactions on desensitization to wheat after rush oral immunotherapy

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Abstract

Background: The effect of oral immunotherapy (OIT) on wheat allergy is promising in terms of the potential to obtain desensitization; however, the frequency of exercise-induced allergic reactions on desensitization (EIARDs) and the associated risk factors remain to be determined.

Methods: Twenty-five patients underwent rush OIT for wheat allergy, and 21 achieved the full-dose intake of wheat products (5 g of wheat protein). Exercise-provocation tests were repeatedly performed after the ingestion of a full-dose wheat product. The time-course of the levels of the specific IgEs (sIgE) to wheat extract, total gliadin, deamidated gliadin, recombinant gliadin components (α/β -, γ - and ω -5-), and glutenin (high and low molecular weight) components was analyzed using ImmunoCAP[®], ELISA, or IgE immunoblotting.

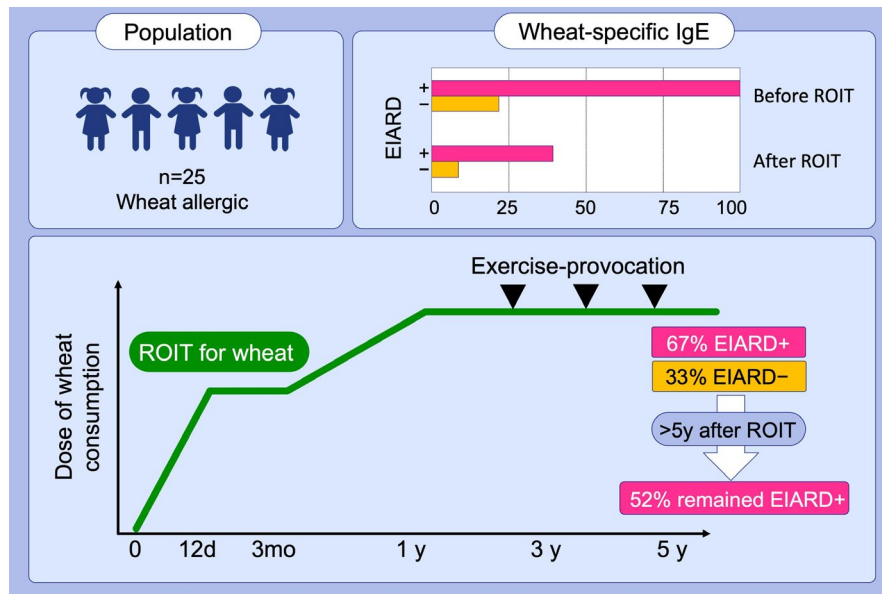
Results: Fourteen patients (66.7%) were diagnosed as EIARD+, which remained 5 years after rush OIT in 11 patients (52.4%). There were no differences in the clinical backgrounds of the EIARD+ and EIARD- patients. However, EIARD+ patients showed significantly higher sIgE levels to all gliadin and glutenin components than EIARD- patients before OIT. The sIgE levels to each component decreased equally after 1 and 2 years of OIT. On IgE immunoblotting, sera from all patients reacted to the multiple gluten bands, and some reacted to the water-soluble bands. The intensity of all IgE-reactive bands also became equally lighter after OIT.

Conclusions: EIARDs were frequently observed and remained for a long period after successful OIT for wheat allergy. None of the specific wheat components were found to contribute to EIARDs.

KEYWORDS

allergen component, exercise-induced allergic reaction, exercise-provocation test, oral immunotherapy, wheat allergy

Abbreviations: ASCA, Anaphylaxis Scoring Aichi; CBB, Coomassie Brilliant Blue; EIARD, exercise-induced allergic reaction on desensitization; ELISA, enzyme-linked immunosorbent assay; Ex-P, exercise-provocation test; FA, food allergy; HMW, high molecular weight; HWP, hydrolyzed wheat protein; LMW, low molecular weight; LTP, lipid transfer protein; ME, mercaptoethanol; OFC, oral food challenge; OIT, oral immunotherapy; PAGE, polyacrylamide gel electrophoresis; Pro, cumulative protein dose; PVDF, polyvinylidene difluoride; ROIT, rush oral immunotherapy; SDS, sodium dodecyl sulfate; sIgE, specific immunoglobulin E; TS, total score of ASCA; tTG, tissue transglutaminase; WDEIA, wheat-dependent exercise-induced anaphylaxis.



GRAPHICAL ABSTRACT

Exercise-induced allergic reaction on desensitization (EIARD).

1 | INTRODUCTION

Despite the increasing number of studies that have investigated oral immunotherapy (OIT) for food allergy (FA), OIT is not recommended as a clinical strategy for FA in Japan.¹ The European Academy of Allergy and Clinical Immunology issued the guidelines that recommended OIT for hen's egg, cow's milk, and peanuts to be performed in research centers or in clinical centers with extensive experience in OIT.² Furthermore, peanut OIT products are expected to be approved in the USA soon.³ Although OIT can induce transient desensitization or sustained unresponsiveness,⁴ these conditions are not equivalent to permanent tolerance,^{5,6} because patients may experience allergic symptoms when they eat the target food during sickness or in association with exercise after consumption.^{7,8}

Immediate-type wheat allergy is the third most common food allergy in Japan, and wheat is the third most frequent target food of OIT.⁹ Patients who received OIT for wheat allergy previously experienced repeated episodes of anaphylaxis after consuming a small amount of wheat product, and resolution is not expected in cases involving patients of 5 years of age or older. The patient is sensitized to multiple wheat allergens, including gluten (gliadin¹⁰ and glutenin^{11,12}) fractions, as well as water-soluble proteins.¹³

There are some case series on the occurrence of allergic reactions during physical exercise after eating milk¹⁴ and wheat^{15,16} after the acquisition of desensitization by OIT. Because of the absence of an appropriate term to indicate this phenomenon, we designate the term exercise-induced allergic reactions on desensitization (EIARDs) in this manuscript. EIARDs critically affect patients' quality of life. However, the risk factors associated with the persistence of EIARDs after OIT have not been revealed.

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is another type of wheat allergy.¹⁷ The onset of WDEIA is generally in adolescence or adulthood, and typically, patients can tolerate wheat without exercise. The patients are primarily sensitized to ω -5 gliadin or high-molecular-weight (HMW) glutenins.¹⁸

Transcutaneous sensitization to hydrolyzed wheat protein (HWP) triggers another type of WDEIA.¹⁹ The causative allergen component in HWP-WDEIA was reported to be γ -gliadin.²⁰ Acid hydrolyzation of wheat protein also causes deamidation of gliadin molecules, which improves the water solubility of gluten. Tissue transglutaminase (tTG) in the duodenal mucosa also catalyzes this reaction, which enhances the immune response of T cells in celiac disease.²¹ Patients exclusively allergic to deamidated gluten were reported,²² and deamidated gluten fractions showed stronger IgE-binding capacity in some patients with WDEIA.²³

Considering the difference in the clinical manifestations and contributing allergen components, the EIARDs after OIT for wheat allergy and WDEIA likely represent different clinical entities. To the best of our knowledge, no specific wheat components that contribute to EIARD after OIT for wheat allergy have been reported.

In this study, we aimed to investigate the clinical features of patients with EIARDs remaining after OIT for wheat allergy as well as the contributing allergen components.

2 | METHODS

2.1 | Study population

Twenty-five children with wheat allergy underwent rush oral immunotherapy (ROIT) for wheat allergy in Aichi Children's Health

and Medical Center between July 2010 and December 2012. ROIT was indicated for patients who showed positive results in an oral food challenge (OFC) with a cumulative dose of 8.7 g or less of udon noodles (containing 2.6% wheat protein) at 5 years of age or older.

2.2 | Oral food challenge to wheat

At the beginning of ROIT, an OFC was performed to evaluate the severity of wheat allergy. An increasing dose (0.2-0.5-1-2-5 g) of boiled udon noodles was given every 30 minutes until the objective allergic symptoms were observed. The severity of symptoms was scored using the Anaphylaxis Scoring Aichi (ASCA), a scoring system developed in our department (Figure S1).²⁴⁻²⁷ In the ASCA system, the total score (TS; maximum 240 points) is defined as the sum of the 5 organ scores (1 to 60 points) observed throughout a course of OFCs. To express the overall result of the OFC, TS/Pro (points/g) was defined by dividing the TS by the cumulative protein dose of the allergen (Pro).

2.3 | Rush oral immunotherapy

The ROIT protocol consisted of the rush phase for 12 days in the hospital, followed by the slow-increase phase and maintenance phase at home (Figure 1). The starting dose was determined from the final dose of the OFC, and the patients ingested udon noodles 4 times a day, typically with a 1.3-fold increase in each dose (Table S1). When an allergic symptom was provoked, the next intake was waved until the symptoms disappeared, and the dose was repeated or even reduced based on the severity of the symptoms. The final dose tolerated at discharge was set as the first dose for the slow-increase phase.

In the slow-increase phase, the patients ingested udon noodles every day and gradually increased the dose at home to the full dose (200 g of udon noodles, equivalent to 5 g of wheat protein). The dose escalation plan was varied among patients according to the frequency and severity of allergic symptoms, typically with a 5%-10% increase after 10 to 20 asymptomatic intakes. When the patients reached the full dose, they maintained the dose to ensure the absence of symptoms (maintenance phase). In these periods, we instructed the patients to avoid exercise and bathing for 1 hour before and 2 hours after the programmed intake.

2.4 | Exercise-provocation test

An exercise-provocation test (Ex-P) was performed in the hospital after a maintenance phase of at least 3 months, without symptoms. The patients ingested the full dose of the wheat product, and after 30 minutes, they performed free running for approximately

10 minutes to achieve a target heart rate of > 180 beats/min. The provoked symptoms were scored using the ASCA system.

2.5 | Specific IgE to wheat components

Blood samples were obtained before and one and two years after ROIT. Wheat and ω -5 gliadin-specific immunoglobulin E (sIgEs) were clinically detected using ImmunoCAP[®] (0.35-100 kU_A/L, Thermo Fisher Scientific AB, Uppsala, Sweden). The sIgEs to total gliadin, deamidated gliadin, and recombinant HMW glutenin were also analyzed using experimental ImmunoCAP[®] (0.1 - 100 kU_A/L). Specific IgE levels of ≥ 0.35 kU_A/L were defined as positive.

Recombinant gliadins (α/β , γ , ω -1,2) and low-molecular-weight (LMW) glutenin were produced in *Escherichia coli*, and the sIgEs to these components were detected by an enzyme-linked immunosorbent assay (ELISA).²⁸ Briefly, recombinant gliadins and LMW glutenin were dissolved in 1% acetic acid and 1-PrOH, respectively, and used to coat the microplate wells. ImmunoCAP[®] Specific IgE Conjugate 400 (Thermo Fisher Scientific AB) served as the secondary antibody. The cutoff value was set as the mean plus two standard deviations of the control wells.

2.6 | SDS-PAGE and IgE immunoblotting

Wheat protein was extracted from wheat flour (Nisshin Foods Inc, Tokyo, Japan) using 1 M Tris-HCl (pH 6.8) containing sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (ME). The extracted proteins were dissolved in a sample buffer containing 5% 2-ME and boiled for 5 minutes. SDS-polyacrylamide gel electrophoresis (PAGE) was performed using 15% polyacrylamide gel with 9.5 μ g of protein loaded to each lane. The protein bands were stained with Coomassie Brilliant Blue (CBB).

The separated proteins were electrically transferred to polyvinylidene difluoride (PVDF) membranes and blocked with Tris-buffered saline and 0.1% Tween-20 containing 0.3% bovine serum albumin. The membranes were incubated with patient sera (1:12.5 dilution), and alkaline phosphatase-labeled goat anti-human IgE antibody (Bethyl, Montgomery, TX, USA) served as the secondary antibody. The specific bands were visualized using a BCIP[®]/NBT (Sigma-Aldrich, Tokyo, Japan) and then photographed and analyzed using a printed graph and CS Analyzer (ver. 3.0 for Windows; Atto, Tokyo, Japan).

2.7 | Statistical analysis

All statistical analyses were performed using the Statcel 3 software program (OMS Ltd., Tokyo, Japan). In the analysis, sIgE levels of < 0.35 kU_A/L and ≥ 100 were considered as 0.34 and 100, respectively. A nonparametric analysis was performed using the Mann-Whitney U

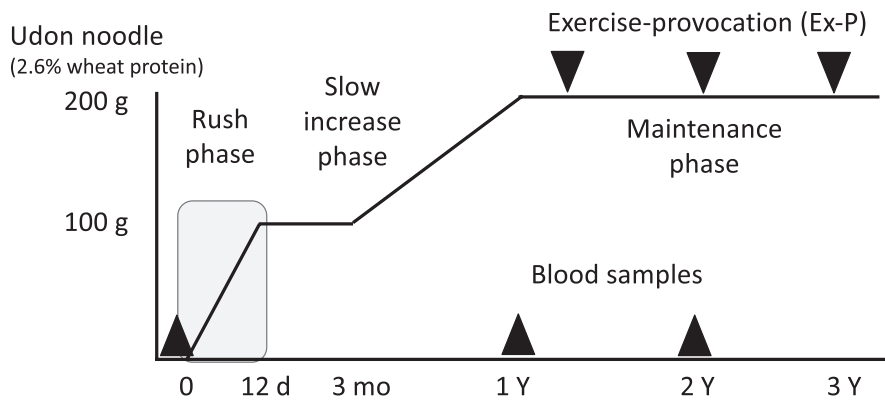


FIGURE 1 Schematic illustration of rush oral immunotherapy for wheat allergy. After reaching the full-dose intake (200 g of udon noodles), patients performed an exercise-provocation test (Ex-P) after eating 200-g udon noodles

	EIARD+	EIARD-	Total	P
Number (M:F)	14 (10:4)	7 (4:3)	21 (14:7)	
Age	7.6 (5.4-12.7)	7.0 (5.7-11.7)	7.6 (5.4-12.7)	NS
Threshold dose ^a (g)	2.1 (0.7-38.0)	3.8 (0.3-18.7)	3.0 (0.3-38.0)	NS
ASCA total score	25 (15-130)	15 (10-90)	25 (10-130)	NS
TS/Pro	454.1 (25.3-2272.7)	202.4 (22.9-1282.1)	384.6 (22.9-2272.7)	NS
Dose at discharge (g)	100 (4-100)	100 (4-100)	100 (4-100)	NS
Months to achieve the maintenance phase	13.0 (5.5-36.8)	11.6 (5.5-35.5)	11.6 (5.5-36.8)	NS
Months to the 1st Ex-P	20.7 (10.0-65.0)	15.8 (13.1-44.3)	18.3 (10.0-65.0)	NS

TABLE 1 Baseline characteristics of the subjects

Abbreviations: EIARD: exercise-induced allergic reactions on desensitization, M: male, F: female, NS: not significant by Mann-Whitney U test; ASCA: Anaphylaxis Scoring Aichi, TS: total score, Pro: cumulative protein amount, Ex-P: exercise-provocation test.

Number (parenthesis): median (range).

^aWeight of udon noodles (2.6% protein).

test and the Wilcoxon rank sum test. *P* values < .05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | The outcome of ROIT

Among the 25 patients who underwent ROIT for wheat, four could not continue the ROIT procedure because of moving (*n* = 2), mental intolerance, and acute pancreatitis; the remaining 21 patients (female, *n* = 7) were enrolled in this study (Table 1). The median age of the participants at ROIT was 7.6 (range: 5.4-12.7) years.

After achieving full-dose intake at a median 11.6 (range: 5.5-36.8) months, 20 patients underwent the 1st Ex-P at a median 17.1 (range: 10.0-65.0) months after ROIT (Figure 2). The remaining 1 patient reached full-dose intake at 23 months but did not perform

an Ex-P because of 3 convincing episodes of EIARDS. The first one was an anaphylactic episode during exercise after consuming 25-g udon noodles at 25 months that required adrenaline injection and hospitalization. The second episode that required adrenaline occurred at 41 months, when he performed hard exercise in the daytime, consumed 200-g udon noodles and took a bath after consumption. The third one was generalized urticaria, a swelling of the eyelids and coughing at 46 months when he rode a bicycle after eating a donut.

3.2 | Exercise-provocation test

As a result of the first Ex-P (*n* = 20), allergic reactions were provoked in 10 patients, while 10 patients passed the test (Figure 2). In the former 10 patients, EIARDS disappeared in 1 patient (Case 7 in Table 2 who provoked only transient coughing) at 22 months in his daily consumption and exercise. One patient (Case 16) who performed her

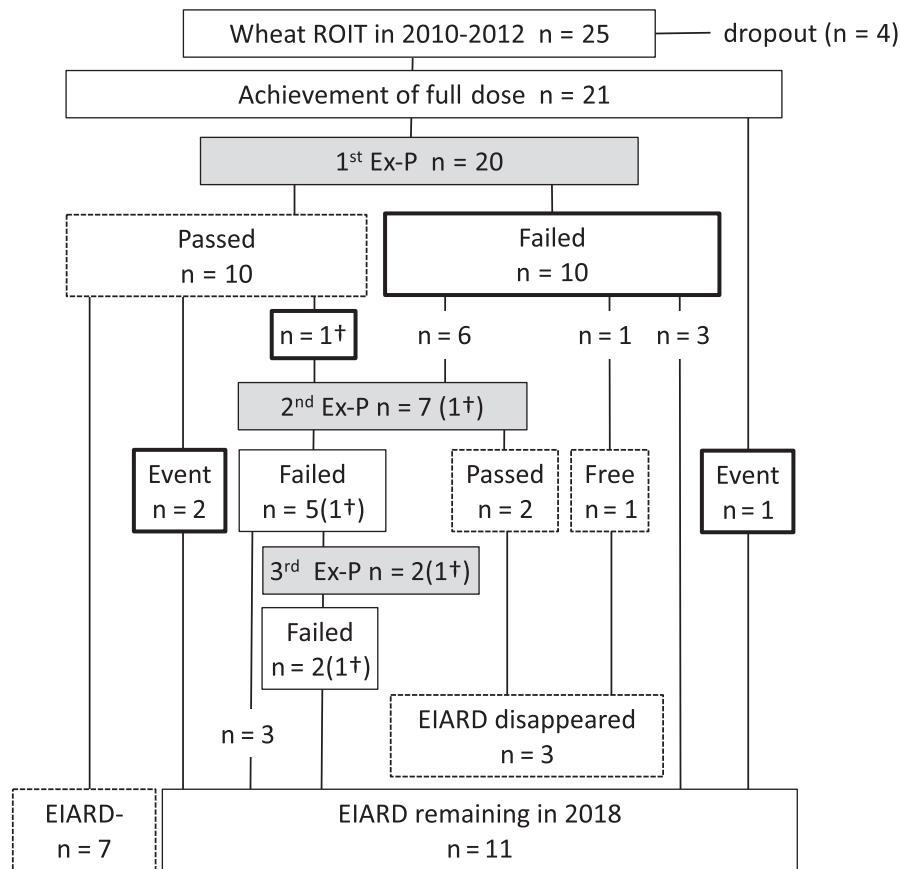


FIGURE 2 Clinical flow of the participants. Rush oral immunotherapy to wheat was performed in 25 patients, and 21 patients achieved full-dose intake. One patient repeated exercise-induced allergic reactions on desensitization (EIARDs), designated as an “event.” The remaining 20 patients performed the 1st exercise-provocation tests (Ex-Ps) and 10 patients failed. In the 10 patients who passed the 1st Ex-P, 2 patients had convincing EIARD events, and 1 patient (†) performed the 2nd Ex-P because of a suspicious EIARD event, resulted in positive. Overall, we defined the EIARD+ group ($n = 14$, shown in the thick frames) as those who experienced at least one positive Ex-P or convincing event and the EIARD- group ($n = 7$) as those who never had an event. One patient who failed the 1st Ex-P disappeared EIARDs in his daily life, designated as “free,” and 2 patients who passed the 2nd Ex-P never had an EIARD event afterward. At the final evaluation in 2018, 3 patients were free from EIARDs, and 11 patients continued to have EIARDs

1st Ex-P at 65 months experienced severe anaphylaxis ($TS = 140$) and never agreed to repeat an Ex-P. Two other patients (Cases 5, 20) experienced a repeated EIARD episode at 46 and 66 months, respectively. The remaining 6 patients and one from those who passed the 1st Ex-P but experienced a suspicious EIARD event (Case 2) underwent a 2nd Ex-P at a median of 38.6 (31.9-78.9) months after ROIT; two patients (cases 3 and 6) passed, and five failed. In the 5 failed patients, three experienced a convincing EIARD event, and two underwent a 3rd Ex-P at 59.3 and 72.2 months after ROIT; both of them failed. In addition, in the 10 patients who passed the 1st Ex-P, two patients repeatedly experienced convincing EIARD events, including coughing, wheezing, or urticaria during ball games or swimming after eating a piece of bread or steamed barley.

In summary, 14 patients were classified as the EIARD+ group (66.7%, Figure 2, thick frames), who had at least one EIARD occurrence, and 7 patients were in the EIARD- group, who had no episodes of EIARD after ROIT. We made the final evaluation at a median of 80.9 (64.9-93.9) months after ROIT in May 2018 and

found 3 patients in the EIARD + group who disappeared their EIARDs, whereas the other 11 patients (52.4%) still restricted exercise after the consumption of wheat products in their daily life.

3.3 | Allergic symptoms provoked in the exercise-provocation tests

The allergic symptoms provoked in the 17 Ex-Ps in 11 patients are listed in Table 2. Respiratory symptoms, including cough (score 5-10), wheeze (score 20), and dyspnea (score 40), were provoked in 94% (16/17) of the tests. Skin symptoms were also frequently provoked in 84% (14/17) of the tests; most of these cases involved multiple (but local) urticaria (score 10). Cardiovascular symptoms were provoked in three tests. Two severe symptoms were provoked in patients (cases 16 and 18) at 5 and 3 years after ROIT, respectively. These patients were treated with an intramuscular injection of adrenaline.

TABLE 2 Symptoms provoked in the exercise-provocation tests

Case No	Ex-P No	Months after ROIT	Anaphylaxis Scoring Aichi						
			Total score	Respiratory	Skin	Gastrointestinal	Neurological	Cardiovascular	Adrenaline
1	1st	15.5	80	20	20		40		
	2nd	31.9	10		10				
2 ^a	2nd	36.1	10	10					
	3rd	72.2	20	10	10				
3	1st	25.1	15	10	5				
4	1st	23.9	50	20	10		20		
	2nd	35.4	10	10					
	3rd	59.3	50	20	10		10	10	
5	1st	20.7	60	40	10		10		
6	1st	15.7	20	10	10				
7	1st	13.5	5	5					
16	1st	65.0	140	40	10	20	10	60	+
18	1st	27.8	20	10	10				
	2nd	40.6	110	40	20		10	40	+
19	1st	52.5	20	10	10				
	2nd	78.9	30	10	5	5	10		
20	1st	30.6	30	20	10				

Abbreviations: Ex-P: exercise-provocation test, ROIT: rush oral immunotherapy

^aThe 2nd Ex-P was performed because of an appearance of an EIARD event after passing the 1st Ex-p.

3.4 | Baseline characteristics of the EIARD+ and EIARD- groups

There was no significant difference between the EIARD+ and EIARD- groups in terms of sex, age, threshold dose, TS, or TS/Pro at the initiation of the ROIT. Furthermore, the duration from the ROIT to the first Ex-P did not differ to a statistically significant extent (Table 1).

3.5 | Specific IgE levels to wheat components

Before ROIT, the EIARD+ patients showed significantly higher sIgE levels to wheat, gliadin, deamidated gliadin, and every component in comparison with the EIARD- patients. These sIgE levels thereafter decreased in all patients; however, the statistically significant difference between the groups remained after 1 and 2 years (Table 3, Table S2).

Individual data are shown in Figure S2 (ImmunoCAP®) and Figure S3 (ELISA). As all components showed a similar decreasing trend, there were no special wheat components that showed characteristic movement.

3.6 | IgE immunoblotting before and after ROIT

IgE immunoblotting was performed using 7 serum samples from EIARD+ and 6 serum samples from EIARD- patients (Figure S4). All

sera obtained before ROIT reacted to the protein bands between 30 and 45 kDa, which were considered to be gliadin and glutenin fractions.²⁹ Some serum samples also reacted to the bands between 14 and 20 kDa, which were considered to be water-soluble α -amylase/trypsin inhibitors.¹³ However, no specific bands were exclusively detected in the EIARD+ group.

The serum samples obtained 2 years after ROIT were simultaneously subjected to IgE immunoblotting in the same conditions. The overall density of all bands, as measured by the CS Analyzer, became significantly lighter in both the EIARD+ ($P = .018$) and EIARD- groups ($P = .046$, Figure 3). The band patterns detected in each patient before and after ROIT were identical, and no specific bands exclusively remained in the serum samples from the EIARD+ group (Figure S4).

4 | DISCUSSION

In the present study, we reported, for the first time, the high prevalence and long persistence of exercise-induced allergic reactions after the achievement of desensitization to wheat by OIT (EIARDs).

Exercise after the intake of the offending food generally causes the deterioration of allergic reactions in patients with immediate-type food allergies. The definition of this phenomenon is not described in the guidelines in the USA³⁰ or Europe.² However, it is considered to be different from typical WDEIA¹⁸ in terms of the onset of illness, contributing allergen components and prognosis.

WDEIA generally develops after the daily consumption of wheat products without allergic symptoms.

Several case series on OIT for wheat allergy^{31,32} have reported favorable outcomes regarding the acquisition of desensitization. Sato et al reported that 89% of patients who underwent wheat OIT achieved desensitization, and 61% showed sustained unresponsiveness after the cessation of intake for 2 weeks.³³ A recent multicenter randomized control study reported that two years of low-dose (1445 mg) wheat protein OIT resulted in 30% desensitization and 13% sustained unresponsiveness to 7443 mg wheat protein.³⁴

In the present study, 21 patients finally achieved desensitization after OIT for wheat allergy, and an EIARD was confirmed in 14 patients (66.7%). However, it remains to be elucidated whether sustained unresponsiveness and the absence of EIARDs are associated with each other because we did not examine sustained unresponsiveness. We usually instruct the patients without EIARDs to introduce wheat products into their daily meal freely, which should be the true goal of OIT. Once free intake has been established, it is a burden for the patient to discontinue the diet for some weeks. On the other hand, the EIARD+ patients were aware that their desensitized status was not stable, and sustained unresponsiveness was not expected. Regardless of the EIARD status, patients occasionally feel mild discomfort after eating wheat products when they are sick or fatigued or during pollen seasons.

We further investigated the allergen components specifically involved in EIARDs. As a result, every component in the EIARD+ group showed significantly higher sIgE levels before ROIT. Furthermore, the sIgE levels to every component decreased equally after 1 and 2 years. IgE immunoblotting also revealed the absence of the specific component involved in the residual EIARDs. Thus, in the present study, we could not find any specific wheat components contributing to the residual EIARDs.

Adult patients with conventional WDEIA are sensitized predominantly to ω -5 gliadin and HMW glutenin.¹⁸ Patients with

HWP-WDEIA were sensitized to γ -gliadin, and deamidated epitope peptides showed stronger IgE-binding capacity in comparison with natural epitope peptides.²⁰ Three Italian cases with peach allergy caused WDEIA by wheat lipid transfer protein (LTP), which was cross-reactive to peach LTP.³⁵ However, in the present study, deamidated gliadin showed lower sIgE levels than native gliadin, and none of the patients had peach allergies. Taken together, EIARDs after OIT for wheat allergy had different characteristics of the allergen components contributing to conventional WDEIA or HWP-WDEIA.

We repeated the Ex-P for the patients with EIARDs ($n = 14$) for as long as 5 years after ROIT; however, we could find only three patients whose EIARDs disappeared, even though the patients maintained a regular full-dose intake at least 3 times a week. This long-term persistence of EIARDs suggested that they were not due to the "rush" induction procedure of the OIT. An EIARD is suggested to be a status of incomplete acquisition of tolerance, but it might also raise the hypothesis that a primary exercise-sensitive predisposition is present in some patients with refractory wheat allergy. However, we are still expecting the disappearance of EIARD in these patients because their sIgE levels continued to decrease.

EIARDs are a considerable burden with respect to the patients' quality of life.³⁶ Patients need to avoid almost every wheat product in their daily meals because we cannot estimate the safe intake dose or the safe level of exercise intensity. However, it is important to encourage patients to maintain their intake because if they stop their intake, the unstable desensitization status might be abrogated.

Although we could reveal the contribution of sIgE levels as a risk factor for the persistence of EIARDs after OIT for wheat allergy, the present study was associated with some limitations. First, the time points of blood sampling and exercise-provocation tests were not identical among the patients. Second, we did not examine other immunological factors that could reduce the occurrence

TABLE 3 Specific IgE levels (ImmunoCAP®) to wheat components during the course of oral immunotherapy

Group	EIARD+			EIARD-			EIARD+ vs. EIARD-		
Sample	Before OIT	1 y	2 y	Before OIT	1 y	2 y	Before OIT	1 y	2 y
Total IgE (IU/mL)	910 (264-3586)	1015 (250-4463)	949 (152-4246)	456 (80-1762)	427 (201-2208)	362 (101-1673)	NS	<0.05	NS
Wheat (kUA/L)	100 (11.8-100)	85 (6.85-100)	39.2 (3.6-100)	21.7 (1.0-100)	18.6 (0.46-66.2)	8.61 (2.98-21.8)	<0.05	<0.01	<0.01
Gliadin (kUA/L)	74.5 (17.8-100)	32.2 (6.8-70.4)	12.1 (4.3-40.8)	10.2 (0.7-47.6)	3.8 (0.1-15.2)	1.4 (1.3-1.5)	<0.01	<0.01	<0.05
Deamidated gliadin (kUA/L)	5.4 (0.5-26.9)	1.1 (0.1-5.2)	0.7 (0.1-2.6)	0.4 (0.1-2.0)	0.1 (0.1-0.3)	0.1 (0.1-0.1)	<0.01	<0.05	<0.05
ω -5 Gliadin (kUA/L)	8.6 (1.5-40.2)	1.73 (0.34-14)	1.2 (0.3-3.2)	0.7 (0.3-7.7)	0.38 (0.34-5.28)	0.34 (0.34-0.4)	<0.01	<0.05	<0.05
HMW glutenin (kUA/L)	12.7 (4.0-39.4)	3.8 (1.3-8.0)	2.4 (1.1-4.3)	2.2 (0.3-7.4)	0.8 (0.2-1.7)	0.2 (0.2-0.2)	<0.01	<0.01	<0.05

Note: Data are expressed as median (range). Mann-Whitney U test.

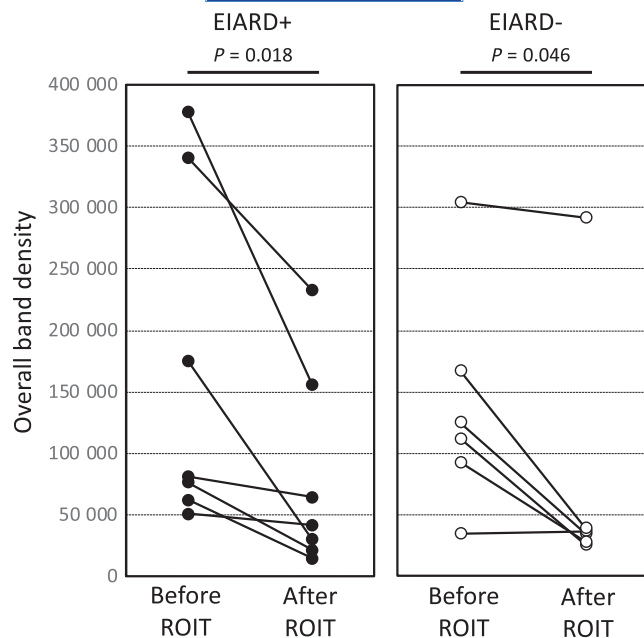


FIGURE 3 Decrease in the overall band density after oral immunotherapy. The sera from representative 7 EIARD + and 6 EIARD- patients were subjected to IgE immunoblotting (Figure S4) under the same conditions, and the overall band density was measured using the CS Analyzer. A significant decrease in the band density was detected between the individual sera obtained before and 2 y after ROIT by the Wilcoxon rank sum test. However, no difference was detected between the EIARD + and EIARD- groups by the Mann-Whitney U test

of EIARDs, such as specific IgG4 antibodies and regulatory T cells. Third, we could not perform a serum analysis at more than three years after ROIT because of the unstable frequency of blood sampling, the loss of the experimental ImmunoCAP® reagents, and the fact that in-house ELISA requires all samples to be detected at the same time.

In conclusion, many patients were able to reach “desensitization” after OIT for wheat allergy. However, 67% of them experienced EIARDs and 52% remained the status even after 5 years or longer, which strongly affect their quality of life. Unlike conventional WDEIA, EIARDs were not associated with a specific allergen component, and their nature remains to be investigated.

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CONFLICT OF INTEREST

TA is an employee of Phadia KK, a part of Thermo Fisher Scientific, and SS is an employee of Thermo Fisher Scientific AB. The other authors have no conflict of interest in regard to this study.

AUTHOR CONTRIBUTIONS

TF performed data analysis and wrote the draft. KT, KT, YK, MN, and HI performed immunoblotting. AT and SS performed ImmunoCAP. TY and HM performed ELISA. TF, KT, KT, TM, SS, NK, and KI performed patients' care. KI designed the study and finalize the manuscript. All authors have read and approved the final form of the manuscript.

ETHICAL APPROVAL

The study was conducted in accordance with the principles embodied in the Declaration of Helsinki (1965) and was conducted with the approval of the institutional ethical board (No. 201336). Written informed consent was obtained at each point for the performance of an OFC, ROIT, and Ex-P.

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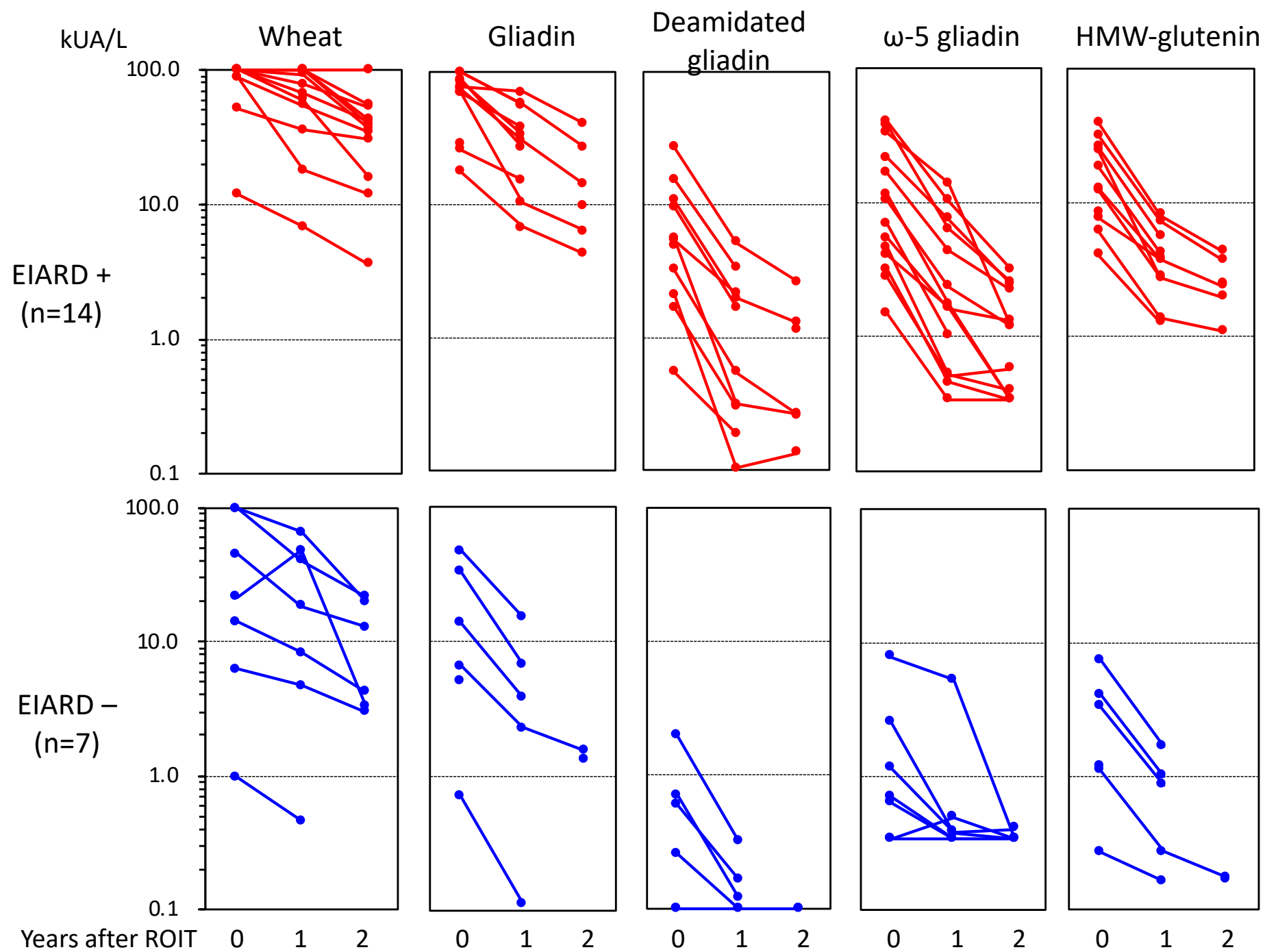
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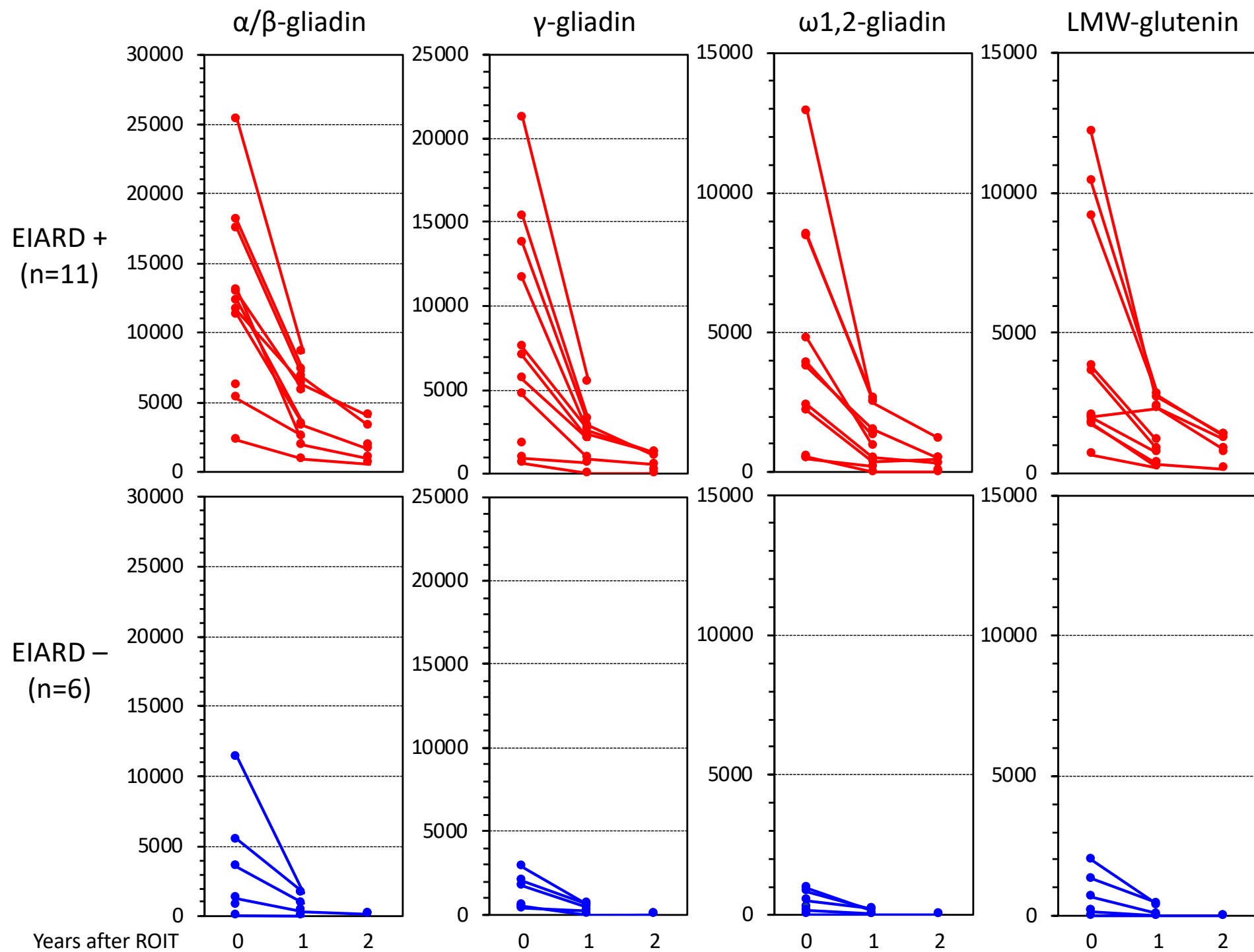
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Grade Score	0	Grade 1 1 point	Grade 1' 5 points	Grade 2 10 points	Grade 2' 20 points	Grade 3 40 points	Grade 4 60 points
<i>Respiratory</i> (subjective)	none	Itchy nose	Laryngeal discomfort	Nasal congestion Suffocating breath	Speech disturbance Difficulty in breathing	Loss of voice Choking	
(objective)		Sneeze	Mild transient coughing Runny nose	Intermittent coughing Mild wheeze SpO ₂ ≥ 98%	Frequent coughing Apparent wheeze Hoarseness SpO ₂ 97-95%	Continuous coughing Strong wheeze, Retraction Intentional breathing Inspiratory stridor SpO ₂ 94-91%	Weak breath sound Strong retraction Cyanosis SpO ₂ ≤90%
<i>Skin/Mucosa</i> (subjective)	none	Itch, around mouth Mild discomfort, Flush	Itch, local and mild	Itch, whole body	Forced scratching		
(objective) <area>		<Peri-oral> Hives, Erythema, Swelling, Vesicle	Eye edema, bloodshot <Local> Hives, Erythema, Swelling, Edema	<Multiple> Hives, Erythema, Swelling, Edema	<Spreading/ Generalized> Hives, Erythema, Swelling, Edema		
<i>Gastrointestinal</i> (subjective)	none	Oral or pharyngeal itch, hot taste, Gravelly throat	Mild nausea, Abdominal pain (FS1)	Nausea, Abdominal pain (FS2)	Strong abdominal pain (FS3)	Unbearable abdominal pain (FS4)	
(objective)			Increased bowel sound	Diarrhea, vomiting	Recurrent vomiting	Dehydration by vomiting	
<i>Psycho -neurological</i>	none	Refuse to eat Mild excitement	Loss of activity Irritation	Sleepy, Tendency to lay down Mild excitement	Sleep (not usual) Agitated •Crying	Tend to unconscious Uncontrolled panic	Unconsciousness
<i>Cardiovascular</i>	none					Pale, Tachycardia, Cold extremities Cold sweat, Mild BP↓	Bradycardia Low BP
<i>Blood pressure</i>						<1 year: 70≤BP <10 years:70+(2×age)≤BP ≥10 years:90≤BP	< 1 year : 50≤BP <10 years : 60≤BP ≥10 years : 70≤BP





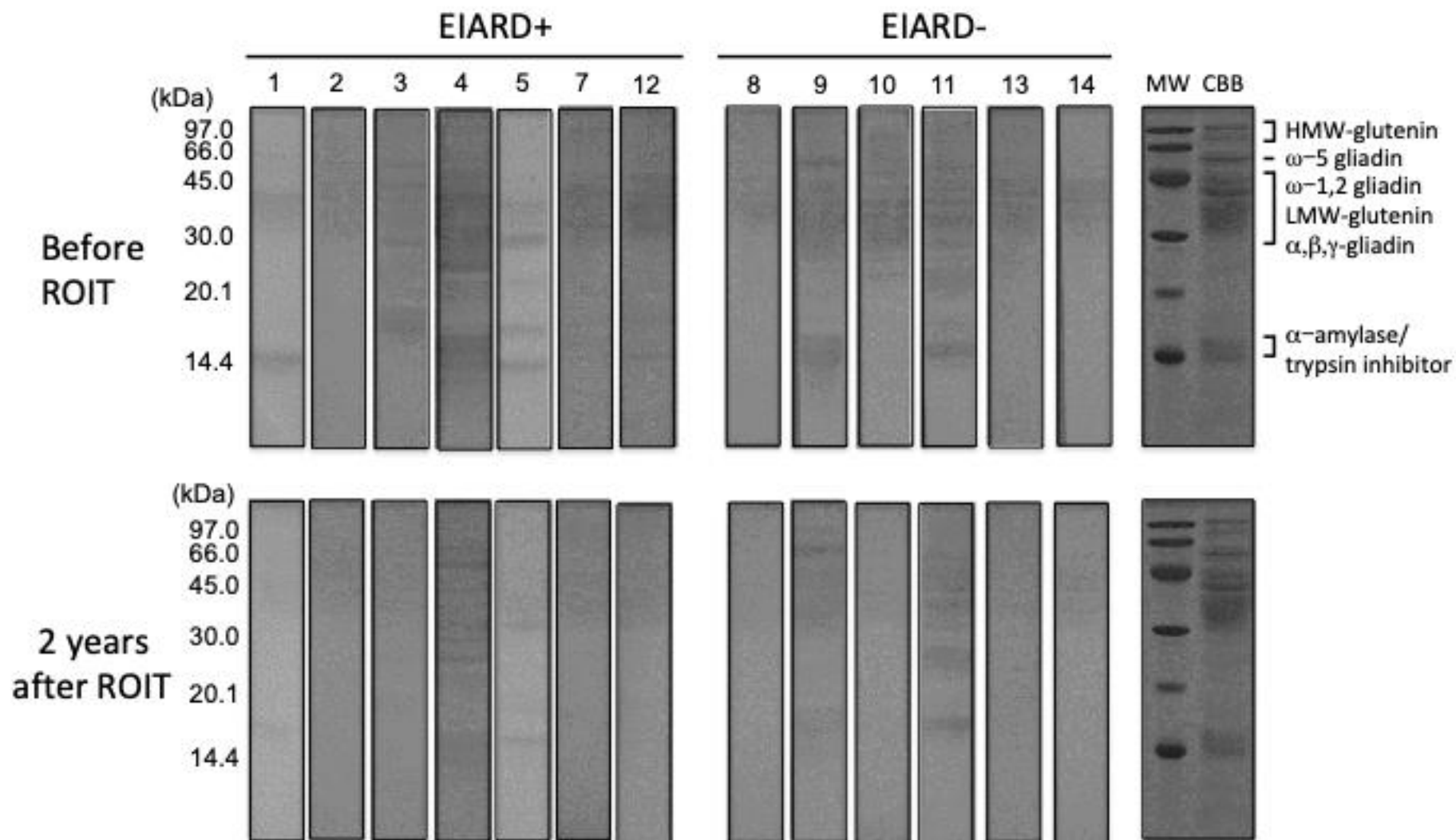


Table S1. Typical dose increment plan during the rush phase

0.1-0.15-0.2-0.25-0.3-0.4-0.5-0.65-0.85-1.0-
1.3-1.7-2.0-2.5-3.0-4.0-5.0-6.5-8.5-11.0-
14-18-23-30-39-50-65-85-100
Gram of udon noodles (2.6% protein)

Table S2. Specific IgE levels to wheat components during the course of oral immunotherapy detected by ELISA (OD)

Group Sample	EIARD+			EIARD-			EIARD+ vs. EIARD-		
	Before OIT	1 y	2 y	Before OIT	1 y	2 y	Before OIT	1 y	2 y
γ -Gliadin	7014 (607-21244)	2166 (0-5479)	797 (0-1238)	1133 (352-2865)	433 (1-619)	0 (0-0)	p<0.05	p<0.05	
α/β -Gliadin	12284 (2273-25279)	4631 (903-8591)	1761 (543-3974)	2361 (29-11317)	915 (0-1679)	144 (130-158)	p<0.01	p<0.01	p<0.05
ω 1,2-Gliadin	3750 (465-12948)	1094 (0-2631)	395 (0-1203)	39 (0-944)	122 (0-216)	0 (0-0)	p<0.05	p<0.05	
$\gamma+\alpha/\beta+\omega$ - Gliadin	24081 (3419-49067)	7361 (903-15264)	3252 (543-4989)	3967 (595-14999)	1469 (270-2515)	144 (130-158)	p<0.01	p<0.01	p<0.05
LMW-glutenin	2024 (656-12147)	998 (194-2788)	1034 (148-1377)	423 (0-1997)	75 (0-452)	0 (0-0)	p<0.01	p<0.05	p<0.05

Data are expressed as median (range).

Mann-Whitney U test

FIGURE S1. Anaphylaxis Scoring Aichi (ASCA)

The ASCA lists allergy symptoms in the 5 organ systems (respiratory, skin/mucosal, gastrointestinal, psycho-neurological, and cardiovascular). Each organ score of 0–60 points is given according to the severity. The total score (TS) is the sum of the highest 5 organ scores observed throughout the course of an OFC (maximum 240 points).

FIGURE. S2 Time course of individual specific IgEs to wheat and its components detected by the ImmunoCAP[®] system.

EIARD: Exercise-induced allergic reaction on desensitization

ROIT: Rush oral immunotherapy

FIGURE. S3 Time course of individual specific IgEs to wheat components detected by ELISA system.

EIARD: Exercise-induced allergic reaction on desensitization

ROIT: Rush oral immunotherapy

FIGURE S4 IgE immunoblotting before and after rush oral immunotherapy (ROIT)

SDS-PAGE and IgE immunoblotting of crude extract of wheat flour are shown. The serum samples (x12.5 dilution) obtained before ROIT (upper column) and 2 years after ROIT (lower column) were treated in the same condition. The patient numbers are

21 matched with Table 2; #1-7, 12 are EIARD+ patients, and #8-11, 13, and 14 are
22 EIARD- patients. Gluten (gliadin and glutenin) bands are present at approximately
23 30-100 kDa, and water-soluble fractions are observed at approximately 14-20 kDa.
24 EIARD: Exercise-induced allergic reaction on desensitization
25 ROIT: Rush oral immunotherapy

26

