

DR SHOHEI KUBOTA (Orcid ID : 0000-0002-2005-890X)

DR TERUAKI MATSUI (Orcid ID : 0000-0002-3660-1823)

DR SHIRO SUGIURA (Orcid ID : 0000-0003-1048-2704)

Article type : Original Article

Exercise-induced allergic reactions after achievement of desensitization to cow's milk and wheat

Shohei Kubota¹, Katsumasa Kitamura¹, Teruaki Matsui¹, Yoshihiro Takasato¹, Shiro Sugiura¹,
Komei Ito¹

¹ Department of Allergy, Aichi Children's Health and Medical Center, Obu, Japan

Corresponding author:

Komei Ito

Department of Allergy, Aichi Children's Health and Medical Center 7-426, Morioka, Obu, Aichi
474-8710, Japan

E-mail: koumei_itoh@mx.achmc.pref.aichi.jp

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/PAI.13479](#)

This article is protected by copyright. All rights reserved

Conflicts of interest

The authors have no conflicts of interest directly relevant to the content of this article.

Financial support

No financial support was received for this study.

Abstract

Background: We have previously reported that more than half of the patients who achieved desensitization after wheat rush oral immunotherapy (OIT) developed exercise-induced allergic reaction on desensitization (EIARD). However, data on EIARDs after slow OIT are lacking. Therefore, this study aimed to investigate the results of exercise provocation tests (EPTs) in patients after slow OIT for cow's milk and wheat allergies.

Methods: This was a retrospective chart review of 87 EPTs in 74 patients. The EPTs were performed in patients who were desensitized to at least 6,600 mg cow's milk protein or 5,200 mg wheat protein with slow OIT and were identified to be at a high risk of EIARDs. EPTs were performed after ingestion of the maximum desensitization dose. The patients' clinical characteristics and symptoms were analyzed.

Results: The EPT results were positive for cow's milk in 49% (21/43) of the patients and for wheat in 48% (15/31) of the patients. There was no significant difference in the clinical characteristics between the EIARD-positive and EIARD-negative groups. The specific IgE (sIgE) levels before OIT and the reduction rates of sIgE before and after OIT did not correlate with the outcomes of the EPTs. Among the EIARD-positive patients, 13 patients (cow's milk, n=7; wheat, n=6) underwent a second EPT, and the EIARD disappeared in 8 patients (cow's milk, n=4; wheat, n=4).

Conclusion: EIARDs were observed after slow OIT for cow's milk and wheat. Further research into the predictive factors of EIARDs in these patients is needed to understand its clinical manifestations.

Keywords: food allergy, oral immunotherapy, exercise provocation test, food-exercise-induced anaphylaxis, ω -5 gliadin, desensitization, slow oral immunotherapy

Key message

This is the first case series of exercise-induced allergic reaction on desensitization (EIARD) after the implementation of slow oral immunotherapy (OIT) for wheat and cow's milk. Although this study was conducted in a retrospective manner, the strength of this study is that the diagnosis of EIARD was based on the exercise provocation tests (EPTs). We found that EIARDs occur not only after rush OIT for wheat¹¹, but also after slow OIT for cow's milk and wheat.

Introduction

Cow's milk (CM) and wheat are the second and third most common causes of childhood food allergies in Japan, respectively.¹ Most children with these allergies acquire tolerance spontaneously with age. However, some do not^{2–5} and thus undergo oral immunotherapy (OIT). OIT for CM or wheat effectively achieves desensitization,^{6–9} but exercise after ingestion of the causative antigen can cause immediate allergic symptoms.^{10–14} Manabe et al. suggested two types of exercise-induced allergic symptoms: food-dependent exercise-induced anaphylaxis¹⁵ and desensitization status and exercise-induced anaphylaxis.^{10,16}

We previously reported on 14 cases of exercise-induced allergic reactions among 21 patients who were desensitized to 5,200 mg wheat protein after rush OIT.¹¹ We designated this status as “exercise-induced allergic reaction on desensitization” (EIARD). There are also several

case reports of EIARD after both slow and rush OIT.^{12–14} However, there are no case series or case control studies of EIARD after the implementation of slow OIT. Therefore, this study aimed to analyze the results of exercise provocation tests (EPTs) for diagnosing EIARD after slow OIT and to identify the factors associated with EIARD.

Methods

Study design and population

This was a retrospective study of 74 patients who achieved desensitization to 6,600 mg CM protein (n=43) or 5,200 mg wheat protein (n=31) after slow OIT and who underwent EPTs between March 2012 and December 2019. All EPTs were performed at the Aichi Children's Health and Medical Center. EPTs with acetylsalicylic acid used as a cofactor¹⁷ were excluded from the analysis.

Oral immunotherapy

All the patients had completely eliminated the causative foods from their diets before initiating slow OIT for one antigen (either CM or wheat). The initial dose was based on the total doses and severity of allergic symptoms in oral food challenges.^{18,19} For patients whose symptom-provoked threshold dose was high (>66 mg CM protein or 52 mg wheat protein), the dose was increased by 10–20% after every 5th–10th asymptomatic ingestion. The dose was increased more slowly for the patients whose threshold was low (<66 mg CM protein or 52 mg wheat protein), as previously reported.¹⁸ All patients were instructed to avoid exercising and bathing one hour before and two hours after taking the dose to minimize the risk of exercise-induced symptoms. Bronchial asthma was well controlled in all patients throughout OIT implementation in accordance with the Japanese childhood asthma guidelines.²⁰ After achieving desensitization with the full dose (6,600

mg CM protein or 5,200 mg wheat protein)¹, all patients were instructed to exercise after allergen consumption at home to ensure the safety of the routine consumption. EPTs were applied to the high-risk patients who had displayed unstable or suspicious exercise-induced symptoms during OIT (e.g., allergic symptoms due to unintended exercise after ingestion, unexpected anaphylaxis or frequent allergic symptoms during OIT).

Exercise provocation tests

Patients ingested the maximum desensitization dose and underwent either a free running, treadmill ergonometric stress test, or a stepping exercise test after 30 minutes. The exercise was performed for at least 15 minutes, with a target heart rate of >180 beats/minute. Negative EPT results were confirmed by repeated exercises after ingesting the causative food, such as free running for 15 minutes, at home. Patients with positive EPT results underwent follow-up EPTs after a period of time (usually about 2 years) according to the physicians' and families' discretions.

Risk factors of EIARD

The following clinical information was collected as potential factors associated with EIARD: age at the start of OIT and at EPT; history of other allergic diseases; average pace of increasing the amount of antigen during OIT (defined as the difference between the full dose and the starting dose, divided by the duration of OIT); and specific IgE (sIgE) titer (milk and casein, or wheat and ω -5 gliadin) before starting OIT and within 1 year before the EPT (after OIT). sIgE levels were detected using ImmunoCAP[®] (Thermo Fisher Scientific, Uppsala, Sweden). A level of ≤ 0.34 kUA/L was fixed at 0.34 kUA/L, indicating no sensitization. Levels >100 kUA/L were fixed at 100 kUA/L.

EIARD-free patients at home

A case-control study was performed to compare the characteristics of the high-risk patients with those of other patients. We reviewed the charts of the patients who started OIT in 2016 and achieved full-dose desensitization with no suspicious episodes of EIARD in their daily life including exercises after ingestion. We found 33 CM- and 27 wheat-allergic patients (EIARD-free patients).

Statistical analysis

Between-group comparisons were performed using the Mann–Whitney U test or Wilcoxon rank sum test for continuous variables and using the Fisher's exact test for categorical variables. All statistical analyses were conducted using R-3.6.0 program (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). *P* values of <0.05 were considered significant.

Ethical Considerations

This study was approved by the institutional ethical board of Aichi Children's Health and Medical Center (No. 2017001). and was conducted in accordance with the Declaration of Helsinki. Informed consent to participate was obtained from the parents of all patients.

Results

Patient and oral immunotherapy characteristics

Among the patients with CM allergy, the initial OIT dose was high in 26 (60%) CM and 11 (35%) wheat OIT (Table 1). In total, four (9%) CM allergy patients and five (16%) wheat allergy patients experienced anaphylaxis during OIT without exercise. Of them, one patient with a CM allergy

required an adrenaline injection. Fatigue and the common cold were the frequent co-factors of anaphylaxis. Among the CM allergy patients, 5% achieved full-dose desensitization by 1 year, 35% by 2 years, and 60% by 3 years after the start of OIT. Among the wheat allergy patients, 17% achieved full-dose desensitization by 1 year, 42% by 2 years, and 79% by 3 years after the start of OIT. The remaining patients took more than 3 years to reach the full dose.

Exercise provocation tests

At the first CM-EPTs (n=43), 20 patients developed allergic symptoms (Figure 1). One EPT-negative patient experienced a convincing event of EIARD at home. Therefore, 21 patients (49%) were diagnosed to be EIARD positive (Table S1). Overall, eight (38%) patients were administered antihistamines and eight (38%) were administered β 2 stimulants, of whom one received both antihistamines and β 2 stimulants at the first EPT. Two patients received intramuscular injection of adrenaline for anaphylactic shock (cases 17 and 19). Further, seven patients (33%) underwent a second EPT within a median of 1.5 (range: 0.9–3.4) years after the first EPT, of whom four patients tested negative (Figure 1).

Among the 31 patients with a wheat allergy, 14 were EPT-positive (Figure 1). One wheat-EPT-negative patient experienced EIARD at home. Thus, 15 patients (48%) were diagnosed to be EIARD positive (Table S2). Overall, seven (47%) patients were administered antihistamines and six (40%) were administered β 2 stimulants, of whom five received both antihistamines and β 2 stimulants at the first EPT. None of the patients received adrenaline. In total, six (40%) patients who tested positive during the first EPT underwent a second EPT after a median of 2.1 (range: 0.4–5.9) years, of whom four tested negative (Figure 1). The results of the CM and wheat allergy patients who tested negative on the second EPT were confirmed by multiple exercise tests after ingestion at home.

Risk factors of EIARDs and specific IgE levels

There was no significant difference in the characteristics and median age of starting OIT between the EIARD-positive and EIARD-negative groups (Table 2). There was also no significant difference in the average pace of increasing the amount of antigen in the OIT between the two groups for CM ($P=0.52$) and wheat ($P=0.053$) allergies. Further, the average frequency of causative food intake during OIT was not significantly different between the EIARD-positive and EIARD-negative groups for either the CM or wheat allergy patients (Table S3).

With respect to the sIgE levels, there was no significant difference between the EIARD-positive and EIARD-negative groups before OIT (CM: $P=0.51$, casein: $P=0.96$, wheat: $P=0.93$, ω -5 gliadin: $P=0.23$; Table 2). In both groups, the sIgE levels of CM, casein, wheat, and ω -5 gliadin were lower after OIT than before OIT ($P<0.001$ for all comparisons, Figure 2). There was also no significant difference in Δ sIgE calculated using the formula $([\text{pre OIT} - \text{post OIT}] / \text{pre OIT})$ between the groups. One of the two patients who developed anaphylactic shock in response to CM during EPT had CM-sIgE levels of 0.43 kUA/L and casein-sIgE levels of 0.49 kUA/L (case 19). Eight patients in the wheat EIARD-positive group showed negative sIgE to ω -5 gliadin measured after OIT (Table 2).

Characteristics of high-risk patients compared to the EIARD-free patients

We enrolled 60 EIARD-free patients and compared their clinical characteristics to those of the high-risk patients. The high-risk group had significantly higher IgE antibody levels to CM and casein, were older at the start of OIT, and took a longer time to achieve the full OIT dose. Further, the high-risk group included a higher proportion of boys than did the EIARD-free group. (Table 3).

Discussion

Although EIARDs after rush OIT to wheat allergy have been reported,¹¹ evidence on EIARD after slow OIT has been scarce. In this study, 36 of the 74 high-risk patients (48.6%) who underwent an EPT after slow OIT were diagnosed as remaining EIARD. Compared with EIARD-free patients who had no suspicious episodes during successful slow OIT, the high-risk patients were older at the commencement of OIT, were likely to be boys, and took a longer time to achieve the full dose desensitization. Although we could not identify the discriminating factors between positive- and negative-EPTs in the high-risk patients, to the best of our knowledge, this is the first case-control study of EIARD after slow OIT.

With respect to the pre-CM OIT sIgE levels, the high-risk patients had significantly higher CM- and casein-sIgE levels than did EIARD-free patients. However, sIgE did not discriminate the results of EPTs. In wheat OIT, no difference of sIgE to wheat or ω -5 gliadin was observed between the high-risk and EIARD-free patients. Although the sIgE levels decreased after OIT,²¹ there were no significant differences in the sIgE levels after OIT or Δ sIgEs between the EIARD positive- and negative-groups. Moreover, some patients in the EIARD-positive group had negative sIgE levels to the antigen after OIT. These findings support that sIgE levels are not predictive of EIARDs.

Sustained unresponsiveness (SU) is considered an indicator of the therapeutic effectiveness of OIT.⁸ However, we could not evaluate the relationship between SU and EIARDs in our study because we instructed the patients to introduce CM and wheat products in their daily diet, preferably to enable routine consumption rather than to evaluate SU. One institution in Japan reported that 41% of patients who have achieved SU, evaluated by 2-week cessation of intake, experienced allergic symptoms including anaphylaxis within 4 years after confirmation of SU.²²

Importantly, the most common trigger of the symptoms was exercise. Therefore, even patients who achieved SU may remain at risk of EIARDs.

This study found that EIARDs may remain for several years even in the patients who continued to consume daily amounts of antigen. Considering the patient's daily life after OIT, EIARDs may be used as an indicator of the effectiveness of OITs, in addition to SU, to estimate the acquisition of tolerance.

In the case-control study, we identified an average of 30 patients per year who achieved EIARD-free desensitization to CM and wheat after 3 years of slow OIT. On the other hand, the high-risk patients were picked up in more than 7-years of EPT records. Furthermore, there were also patients who failed to achieve full-dose desensitization after long-term slow OIT. Thus, it was difficult to estimate the prevalence of remaining EIARDs after slow OIT, but it might be roughly 5%–10% in our hospital. However, because our hospital is a specialized facility with a biased number of refractory patients, it does not suggest the real ratio of remaining EIARD in the population.

A nationwide survey in Japan found that OITs were performed more frequently in 2015 than in 2011.²³ Consequently, the occurrence of allergic symptoms during exercise in desensitized children, who might have eliminated the causative food in the past, has become a significant issue in schools. In Japan, all elementary schools and most junior high schools provide school lunch. The guideline recommends complete elimination of the allergic food until the student completely achieved tolerance including the absence of exercise-provocation, because the physical education class is sometimes scheduled after lunch time. Physicians are expected to evaluate the exercised-induced symptoms before permitting patients to consume the target food in their daily meals, especially at school. In our facility, we do not allow patients to eat the causative food in the school lunch until the absence of EIARD is confirmed.

In general, exercise is prohibited within 2 hours after ingestion of the antigen during OIT, even in the maintenance phase.²⁴ Therefore, evaluation of the absence of EIARDs after OIT is important to introduce daily consumption of the causative food. The evaluation using an EPT is desirable in patients who have an unstable occurrence of allergic symptoms during OIT, or in those who have a suspected episode of EIARD after OIT.²⁵ However, performing an EPT requires medical resources. Furthermore, it is burdensome for the patients and is accompanied by the risk of anaphylaxis. Therefore, EPTs are performed in limited number of specialized facilities in Japan. Given the increasing number of OITs performed, the need for performing an EPT after OIT is also expanding, at least for the high-risk patients. Further studies are needed to identify the risk factors of EIARD and develop alternative diagnostic methods.

EPTs generally have a low sensitivity and high specificity.²⁶ The former means that one negative EPT is not enough to rule out the possibility of EIARD. In the present study, we instructed the EPT-negative patients to confirm the result at home, and 5% (2/40) of them experienced a convincing EIARD event at home. In case of a positive EPT that provoked isolated wheezing without skin or mucosal symptoms, we excluded the possibility of exercise-induced bronchospasm with an exercise-provocation test without intake. EIARD-positive patients sometimes experienced an EIARD event and realized their EIARD did not disappear yet. For those who had no repeated episodes of EIARD, we performed the second EPT (n=13), and eight (62%) disappeared EIARD several months to years later.

This study has some limitations. First, we could not calculate the actual frequency of EIARD after OIT because of the retrospective nature of the study. Second, we found EIARD-positive patients only in those who had undergone an EPT. However, there have been some patients who experienced exercise-induced symptoms during the OIT and refused to perform an EPT. Further prospective cohort studies are warranted to determine the true prevalence and

associated factors of EIARDs.

In conclusion, EIARDs were observed after slow OIT for CM and wheat. Evaluation of EIARDs after OIT should be prioritized because it impairs the safety and quality of life of the patients who have successfully achieved full-dose desensitization to the allergen. Further research into the mechanisms of EIARDs is needed for its management.

Author contributions

SK designed the paper, processed the data, performed the analysis, and drafted the manuscript. KK, TM, and YT contributed to the conception and interpretation of the study and reviewed the paper. SS and KI conceptualized the study, analyzed and interpreted the data, and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Acknowledgements

We appreciate the help of our colleagues in Aichi Children's Health and Medical Center. We would also like to thank Editage (www.editage.com) for English language editing.

References

- 1) Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2017. *Allergol Int* 2017;66:248-264.
- 2) Elizur A, Rajuan N, Goldberg MR, Leshno M, Cohen A, Katz Y. Natural course and risk factors for persistence of IgE-mediated cow's milk allergy. *J Pediatr* 2012;161:482-487.
- 3) Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. *J Allergy Clin Immunol* 2007;120:1172-1177.

- 4) Keet CA, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The natural history of wheat allergy. *Ann Allergy Asthma Immunol* 2009;102:410-415.
- 5) Kotaniemi-Syrjänen A, Palosuo K, Jartti T, Kuitunen M, Pelkonen AS, Mäkelä MJ. The prognosis of wheat hypersensitivity in children. *Pediatr Allergy Immunol* 2010;21:e421-e428.
- 6) Longo G, Barbi E, Berti I, et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol* 2008;121:343-347.
- 7) Kaneko H, Teramoto T, Kondo M, et al. Efficacy of the slow dose-up method for specific oral tolerance induction in children with cow's milk allergy: comparison with reported protocols. *J Investig Allergol Clin Immunol* 2010;20:538-539.
- 8) Sato S, Utsunomiya T, Imai T, et al. Wheat oral immunotherapy for wheat-induced anaphylaxis. *J Allergy Clin Immunol* 2015;136:1131-1133.e7.
- 9) Rodriguz del Rio P, Diaz-Perales A, Sanchez-Garcia S, et al. Oral immunotherapy in children with IgE-mediated wheat allergy: outcome and molecular changes. *J Investig Allergol Clin Immunol* 2014;24:240-248.
- 10) Manabe T, Oku N, Aihara Y. Food-dependent exercise-induced anaphylaxis among junior high school students: a 14-year epidemiological comparison. *Allergol Int* 2015;64:285-286.
- 11) Furuta T, Tanaka K, Tagami K, et al. Exercise-induced allergic reactions on desensitization to wheat after rush oral immunotherapy. *Allergy* 2020;75:1414-1422.
- 12) Caminiti L, Passalacqua G, Vita D, Ruggeri P, Barberio G, Panjo GB. Food-exercise-induced anaphylaxis in a boy successfully desensitized to cow milk. *Allergy* 2007;62:335-336.
- 13) Calvani M, Sopo SM. Exercise-induced anaphylaxis caused by wheat during specific oral tolerance induction. *Ann Allergy Asthma Immunol* 2007;98:98-99.
- 14) Kusunoki T, Mukaida K, Hayashi A, et al. A case of wheat-dependent exercise-induced anaphylaxis after specific oral immunotherapy. *J Investig Allergol Clin Immunol*

2014;24:358-359.

- 15) Kidd JM 3rd, Cohen SH, Sosman AJ, Fink JN. Food-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1983;71:407-411.
- 16) Manabe T, Oku N, Aihara Y. Food-dependent exercise-induced anaphylaxis in Japanese elementary school children. *Pediatr Int* 2018;60:329-333.
- 17) Matsuo H, Morimoto K, Akaki T, et al. Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2005;35:461-466.
- 18) Sugiura S, Kitamura K, Makino A, et al. Slow low-dose oral immunotherapy: threshold and immunological change. *Allergol Int* 2020;69:601-609.
- 19) Ito K. Diagnosis of food allergies: the impact of oral food challenge testing. *Asia Pac Allergy* 2013;3:59-69.
- 20) Arakawa H, Hamasaki Y, Kohno Y, et al. Japanese guidelines for child asthma 2017. *Allergol Int* 2017;66:190-204.
- 21) Longo G, Barbi E, Berti I, et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol* 2008;121:343-347.
- 22) Manabe T, Sato S, Yanagida N, et al. Long-term outcomes after sustained unresponsiveness in patients who underwent oral immunotherapy for egg, cow's milk, or wheat allergy. *Allergol Int* 2019;68:527-528.
- 23) Sato S, Sugizaki C, Yanagida N, et al. Nationwide questionnaire-based survey of oral immunotherapy in Japan. *Allergol Int* 2018;67:399-404.
- 24) Arasi S, Caminiti L, Crisafulli G, et al. The safety of oral immunotherapy for food allergy during maintenance phase: Effect of counselling on adverse reactions. *World Allergy Organ J* 2019;12:1-3.

- 25) Rigbi NE, Goldberg MR, Levy MB, Nachshon L, Golobov K, Elizur A. Changes in patient quality of life during oral immunotherapy for food allergy. *Allergy* 2017;72:1883-1890.
- 26) Kohno K, Matsuo H, Takahashi H, et al. Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2013;62:229-238.

Tables

Table 1. Baseline patient characteristics

	Cow's milk allergy patients (n=43)	Wheat allergy patients (n=31)
Male sex, n (%)	34 (79%)	28 (90%)
History of atopic dermatitis, n (%)	31 (72%)	23 (74%)
History of bronchial asthma, n (%)	22 (51%)	13 (42%)
History of allergic rhinitis, n (%)	34 (79%)	21 (68%)
History of anaphylaxis, n (%)	22 (56%)	22 (71%)
Age at starting slow OIT (years)	5.6 (1.6–11.5)	5.6 (1.1–13.8)
OIT initial dose, n (%)		
≥66 mg cow's milk protein or ≥52 mg wheat protein	26 (60%)	11 (35%)
<66 mg cow's milk or <52 mg wheat protein	15 (35%)	16 (52%)
Unknown	2 (5%)	4 (13%)
Duration from starting OIT to the full dose (years)	2.6 (0.3–6.9)	2.2 (0.7–3.8)
Duration from starting OIT to the EPT (years)	4.1 (0.7–10.4)	3.6 (1.7–9.7)
sIgE (crude) before OIT (kUA/L)	20 (1.33–100)	22.2 (4–100)
sIgE (component) before OIT (kUA/L)	17.3 (1.36–100)	0.9 (0.34–16.7)

Values are presented as the number (%) or median (range).

sIgE (crude), specific immunoglobulin E titer to cow's milk, and wheat

sIgE (component), specific immunoglobulin E titer to casein (cow's milk), and ω -5 gliadin (wheat).

Levels of ≥ 100 kUA/L and ≤ 0.34 kUA/L were set to 100 kUA/L and 0.34 kUA/L, respectively.

The full dose was set to 6600 mg cow's milk protein and 5200 mg wheat protein.

Abbreviations: EPT: exercise-provocation test, OIT: oral immunotherapy

Table 2. Factors associated with EIARD positivity

	Cow's milk			Wheat		
	EIARD+	EIARD-	<i>p</i>	EIARD+	EIARD-	<i>p</i>
	(n=21)	(n=22)		(n=15)	(n=16)	
Age at EPT (years)	10.2 (6.5–15.7)	10.6 (6.2–14.4)	0.52	9.4 (5.5–16)	9.3 (6.3–13.4)	0.94
Male sex, n (%)	16 (76%)	18 (82%)	0.72	12 (80%)	16 (100%)	0.10
History of atopic dermatitis, n (%)	15 (71%)	16 (73%)	1	9 (60%)	14 (88%)	0.11
History of bronchial asthma, n (%)	12 (57%)	10 (45%)	0.55	7 (47%)	6 (38%)	0.72
History of allergic rhinitis, n (%)	17 (81%)	17 (77%)	1	12 (80%)	9 (56%)	0.25
Age at starting slow OIT (years)	5.1 (2.2–11.5)	5.9 (1.6–10.8)	0.95	6 (1.7–13.8)	5.2 (1.1–10.6)	0.4
OIT initial dose (mg of cow's milk or wheat protein)	165 (3–1650)	49.5 (7–330)	0.713	26 (2–260)	26 (5–260)	0.51
OIT initial dose, n						
≥66 mg cow's milk protein or ≥52 mg wheat protein	16	10	0.11	6	5	1
<66 mg cow's milk protein or <52 mg wheat protein	5	10		8	8	
Duration from starting OIT to the full dose (years)	2.7 (0.6–6.9)	2.6 (0.3–5.1)	0.49	2 (0.7–7)	2.9 (1.1–6)	0.053
Average pace of increasing intake (mg/years)	2422	2570	0.52	3349	2314	0.053
Duration from the full dose OIT to EPT (years)	1.0 (0.1–4)	0.6 (0.1–5)	0.59	0.8 (0.1–1.8)	0.8 (0.1–5)	0.66

sIgE (crude) before OIT (kUA/L)	15.7 (3.22–100)	20.2 (1.33–100)	0.51	21.9 (1.36–100)	17.3 (4.22–100)	0.93
sIgE (component) before OIT (kUA/L)	14 (4–100)	22.4 (5.47–100)	0.96	3.22 (0.34–16.7)	0.54 (0.34–5.95)	0.23
Δ crude-sIgE	0.88	0.89	0.65	0.76	0.83	0.15
Δ component-sIgE	0.89	0.89	0.75	0.08	0.15	0.44

Values are presented as the number (%) or median (range).

Blood sampling occurred within 1 year of commencing consumption.

sIgE (crude), specific immunoglobulin E titer to cow's milk, and wheat sIgE (component), specific immunoglobulin E titer to casein (cow's milk), and ω -5 gliadin (wheat)

Δ crude-sIgE and Δ component-sIgE were defined as the rate of change between sIgE before slow OIT and the first exercise.

Levels of $100 \geq \text{kUA/L}$ and $\leq 0.34 \text{ kUA/L}$ were set to 100 kUA/L and 0.34 kUA/L, respectively.

The average pace of increasing intake was defined as the full dose minus the initial dose, divided by the number years of consumption

The full dose was set to 6600 mg cow's milk protein and 5200 mg wheat protein.

Continuous and categorical variables were analyzed using the Mann-Whitney *U* test and the Fisher's exact test, respectively.

Abbreviations: EIARD: exercise-induced allergic reaction on desensitization, EPT: exercise-provocation test,

OIT: oral immunotherapy

Table 3. Characteristics of the high-risk patients and EIARD-free patients

	Cow's milk			Wheat		
	High risk (n=43)	EIARD free (n=33)	<i>p</i>	High risk (n=31)	EIARD free (n=27)	<i>p</i>
Male sex, n (%)	34 (79%)	15 (45%)	0.004	28 (90%)	17 (63%)	0.025
History of atopic dermatitis, n (%)	31 (72%)	21 (64%)	0.46	23 (74%)	21 (78%)	1
History of bronchial asthma, n (%)	22 (51%)	7 (21%)	0.009	13 (42%)	4 (15%)	0.04
History of allergic rhinitis, n (%)	34 (79%)	2 (6%)	<0.001	21 (68%)	3 (11%)	<0.001
History of anaphylaxis, n (%)	22 (56%)	12 (36%)	0.24	22 (71%)	12 (44%)	0.061
Age at starting slow OIT (years)	5.6 (1.2-16.0)	3.3 (1.6-11.5)	<0.001	5.6 (1.1-13.8)	3.6 (1.1-10.2)	0.004
OIT initial dose (mg of cow's milk or wheat protein)	66 (3-1650)	165 (3-660)	0.23	26 (1.8-260)	130 (1.8-260)	0.23
Duration from starting OIT to the full dose (years)	2.6 (0.3-6.9)	1.5 (0.6-4.2)	0.001	2.2 (0.7-7)	1.4 (0.3-4.2)	0.026
sIgE (crude) before OIT (kUA/L)	20.0 (1.33-100)	6.3 (0.68-70)	0.0047	22.2 (4-100)	18.3 (0.7-100)	0.33
sIgE (component) before OIT (kUA/L)	17.3 (1.36-100)	5.28 (0.34-50.1)	<0.001	0.9 (0.34-16.7)	0.72 (0.34-50)	0.77

Values are presented as the number (%) or median (range).

sIgE (crude), specific immunoglobulin E level to cow's milk, and wheat

sIgE (component), specific immunoglobulin E level to casein (cow's milk), and ω -5 gliadin (wheat).

Levels of ≥ 100 kUA/L and ≤ 0.34 kUA/L were set to 100 kUA/L and 0.34 kUA/L, respectively.

The full dose was set to 6600 mg cow's milk protein and 5200 mg wheat protein.

Continuous and categorical variables were analyzed using the Mann-Whitney *U* test and the Fisher's exact test, respectively.

Abbreviations: EPT: exercise-provocation test, OIT: oral immunotherapy

Figure legends

Figure 1. Flowchart of the exercise provocation test for cow's milk and wheat.

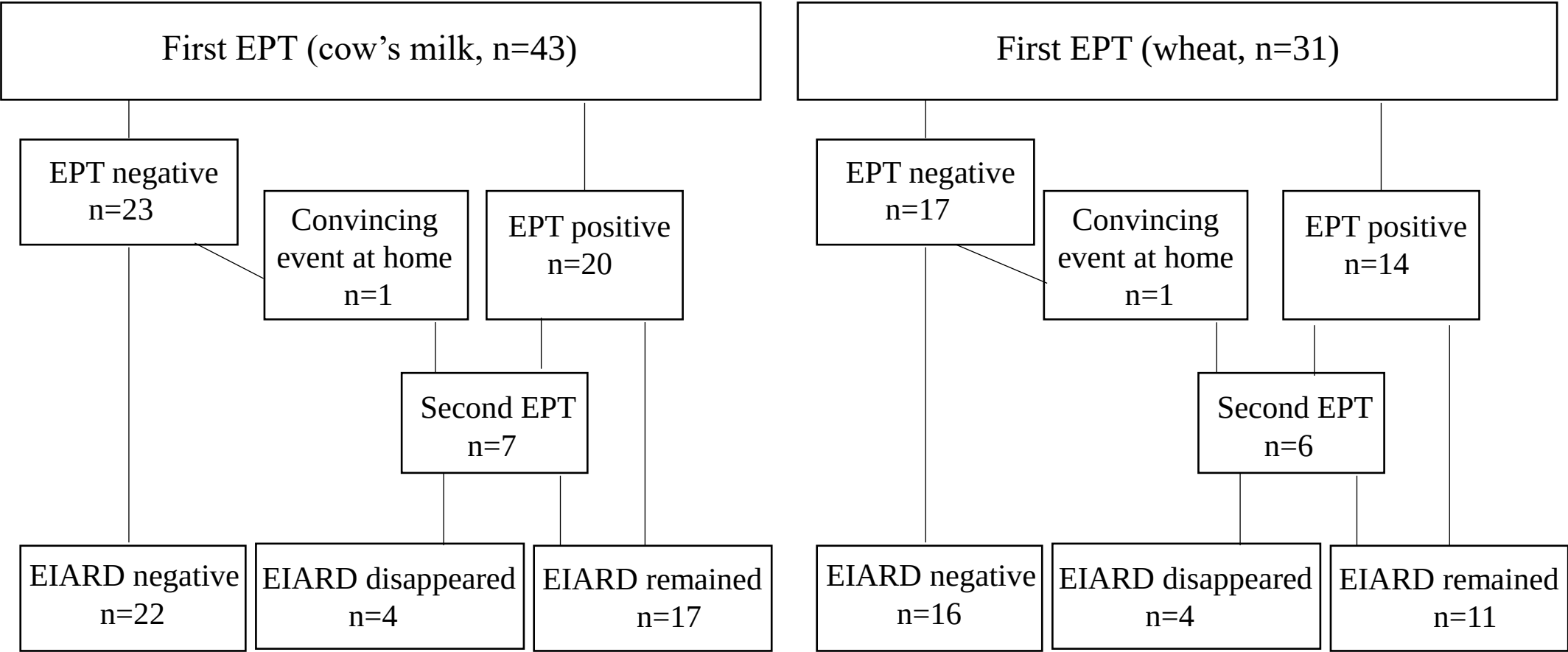
After slow oral immunotherapy (OIT) to cow's milk, 43 patients underwent the first exercise provocation test (EPT). Of them, 20 patients' results were negative. One negative patient experienced a convincing event of exercise-induced allergic reaction to desensitization (EIARD) at home. Ultimately, 21 and 22 patients were diagnosed as EIARD-positive and EIARD-negative, respectively. Seven patients underwent a second EPT, of which four tested negative. After slow oral immunotherapy (OIT) to wheat, 31 patients underwent the first exercise provocation test (EPT). Of them, 14 patients' results were negative. One negative patient experienced a convincing event of exercise-induced allergic reaction on desensitization (EIARD) at home. Finally, 15 and 16 patients were diagnosed as EIARD-positive and EIARD-negative, respectively. Six patients underwent a second EPT, and four of them tested negative.

Figure 2. Time course of the specific IgE detection in response to cow's milk, wheat, and each component in the ImmunoCAP® system.

In the EIARD-positive and EIARD-negative groups, sIgE levels of cow's milk, casein, wheat, and ω -5 gliadin measured after slow oral immunotherapy (OIT) were lower than those measured before the start of OIT.

Appendices: NA

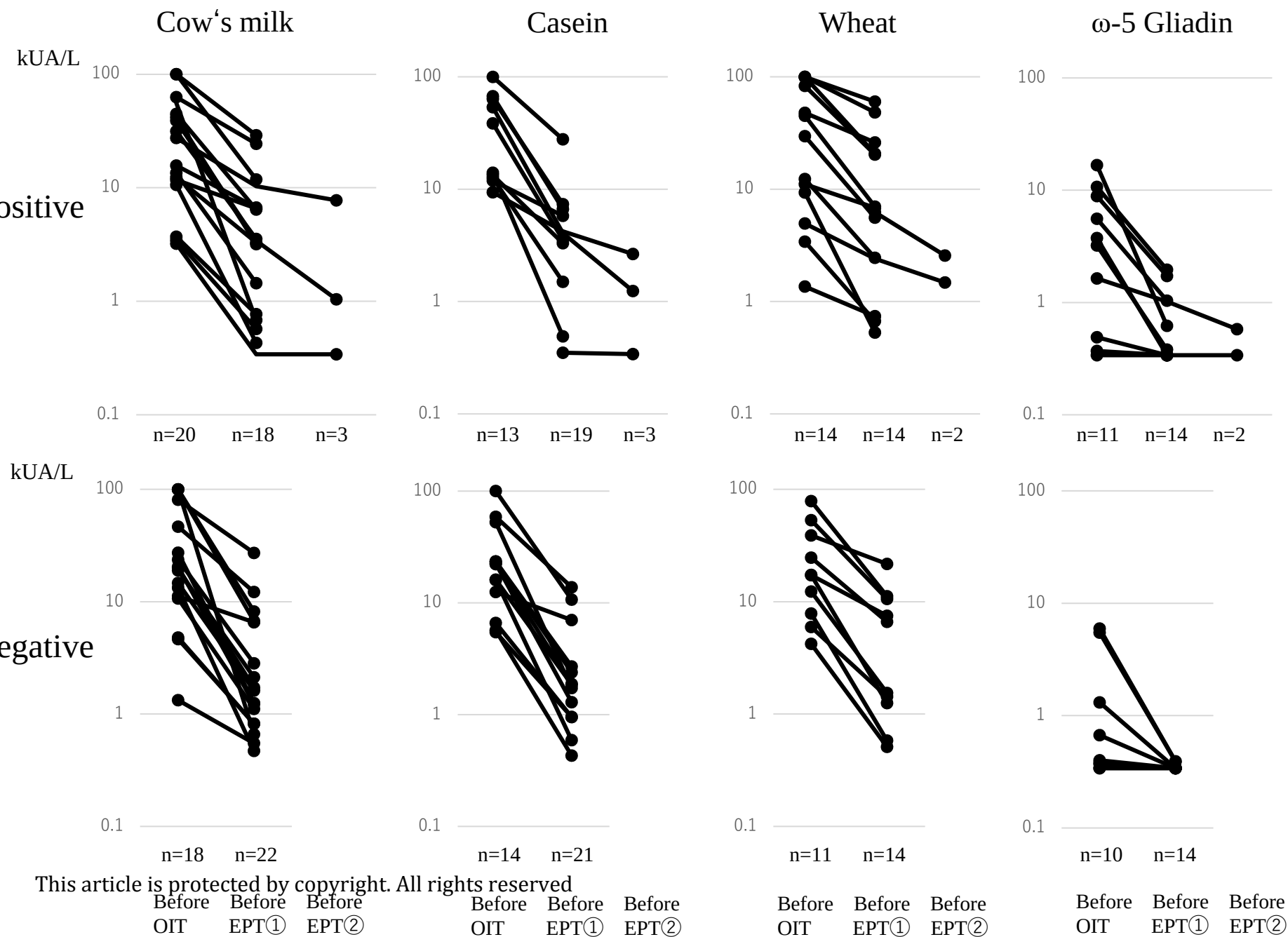
Fig. 1



December
2019

Fig. 2

pai_13479_f2.pdf



1 **Table S1. Characteristics of the patients with positive cow's milk EIARD**

2

3

Case no.	Sex	Age, years	EPT no.	sIgE before OIT		sIgE before EPT		CM protein (mg) intake at EPT	EPT symptoms	Age at starting OIT	OIT starting dose CM protein (mg)	Duration from OIT to the full dose (years)
				CM	Casein	CM	Casein					
1	W	6.5	1st	100	100	29	27.8	9900	Intermittent coughing, hives	4.8	6.6	1.8
2	M	6.6	1st	12.4	13.3	3.29	3.3	6600	Local hives	4.3	165	0.8
3	M	6.7	1st	3.45	ND	0.57	0.52	13200	Negative †	3.4	330	1.3
4	M	7.4	1st	27.4	9.37	10.3	4.22	7900	Wheezing	2.5	165	2.9
		8.3	2nd			7.74	2.65	6600	Wheezing			
5	W	8.6	1st	44.7	63.6	6.41	7.34	6600	Apparent wheezing	5.6	66	2
		10.2	2nd			3.09	3.52	6600	Negative			
6	M	8.7	1st	31.5	38.6	6.41	7.34	6600	Wheezing	4	66	3.7
7	M	8.9	1st	ND	ND	2.37	2.62	9900	Multiple itch, transient coughing, nausea, mild decrease of blood pressure	4.7	66	1.9
8	M	9.3	1st	39	53.6	3.41	4.06	6600	Abdominal pain, wheezing	4.5	330	0.4
		12.7	2nd			1.04	1.24	6600	Local hives			
9	M	9.4	1st	3.56	4	ND	ND	6600	Local hives	5.6	66	2.2
		11.9	2nd			4.17	4.28	11600	Negative			
10	M	9.6	1st	100	67.2	11.8	6.66	6600	Wheezing, itch(local)	4.2	33	4.6
11	M	10.2	1st	8.84	8.25	ND	ND	6600	Apparent wheezing, local hives	4.3	165	ND
12	W	11.5	1st	ND	ND	6.57	5.8	9900	Local hives	8.6	330	2.1

13	M	11.5	1st	15.7	ND	6.68	6.26	11600	Local hives	9.3	165	1.4
14	W	11.6	1st	41.7	ND	3.18	2.63	10800	Local hives, runny nose	6.1	5	3.4
		12.6	2nd			1.14	0.91	10800	negative			
15	M	11.8	1st	ND	ND	24.4	26.8	6600	Eye edema, hives	11	1650	0.1
16	W	12.4	1st	3.71	ND	0.77	0.76	6600	Wheezing, local erythema	2.2	660	6.7
		13.7	2nd			ND	ND	6600	Negative			
17	M	13.2	1st	22.1	28.1	ND	ND	6600 plus a bread roll‡	Spreading hives, low blood pressure, de-saturation	10.9	66	1.4
18	M	13.9	1st	3.22	ND	0.34	0.35	6600	Multiple hives	9.8	165	1.1
		15.4	2nd			0.34	0.34	6600	Spreading hives, runny nose			
19	M	13.9	1st	10.6	14	0.43	0.49	6600	Loss of voice, unbearable itch, Change in activity, low blood pressure	5.1	165	2.7
20	M	15.5	1st	13.5	12.6	1.44	1.5	10800	Local hives	11.5	ND	2.2
21	M	15.7	1st	55.8	ND	0.68	0.71	6600	Multiple hives	5.3	3.3	3.2

4

5 The full dose is 6600 mg CM protein. The allergens consumed during the EPT were cow's milk (3.3% milk protein) or drinking yogurt

6 (3.6% milk protein).

7 †The EPT was negative, however the patient subsequently developed hives when he exercised after ingestion of allergens at home.

8 ‡Exercise after the consumption of bread rolls gave no reaction.

9 Abbreviations: CM: cow's milk, EPT: Exercise provocation test, OFC: oral food challenge, ND: no data, OIT: oral immunotherapy,

10 sIgE: specific IgE (kUA/L)

11

12 **Table S2. Characteristics of the patients with positive wheat EIARD**

13

14

Case no.	Sex	Age, years	EPT No	sIgE before OIT		sIgE before EPT		Wheat protein (mg) intake at EPT	EPT symptoms	Age at starting OIT	OIT starting dose wheat protein (mg)	Duration from OIT to the full dose (years)
				wheat	ω-5G	wheat	ω-5G					
1	M	5.5	1st	83.3	3.22	20.3	0.38	5200	Transient cough, local hives	2.5	52	0.6
2	M	6	1st	ND	ND	6.23	0.34	8400	Local hives	3.6	ND	ND
		9.7	2nd			2.57	ND	11200	Multiple hives			
3	M	6.6	1st	9.36	3.75	0.53	0.34	10800	Multiple hives	1.7	26	ND
		7.2	2nd			0.4	0.34	11200	Negative			
4	M	6.6	1st	100	8.86	60.1	1.72	5600	Cough, multiple hives	4.3	130	ND
5	M	7.5	1st	45	0.49	6.98	0.34	5200	Wheezing, multiple hives	3.9	26	ND
6	W	7.6	1st	100	10.7	48.3	1.97	11200	Wheezing, multiple hives	4.2	1.8	2.6
		8	2nd			48.3	1.97	5600	Negative			
7	M	8.4	1st	11.1	ND	6.85	0.34	5200	Wheezing	6	26	1.5
		14.3	2nd			6.62	0.34	5200	Negative			
8	M	9.4	1st	47.9	ND	26.1	0.34	8100	Wheezing, multiple hives	7.4	26	0.8
		11.7	2nd			20.2	0.34	11100	Negative			
9	M	9.5	1st	29.6	5.57	5.58	1.04	5600	Local hives	5.4	26	0.6
10	M	9.6	1st	4.96	1.64	2.41	1.02	5200	Wheezing, multiple hives	6.7	52	2.3
		11.5	2nd			1.48	0.58	5200	Wheezing, local hives			
11	M	13.7	1st	12.3	0.37	2.45	0.34	10800	Negative †	9.2	260	1.2

12	M	13.9	1st	3.41	0.34	0.67	0.34	11200	Local hives	8	52	0.5
13	W	14.4	1st	1.36	ND	0.74	0.34	5400	Transient cough, multiple hives	10.6	260	0.5
14	W	14.6	1st	100	16.7	20.7	0.62	5600	Transient cough, multiple hives	6.8	26	2
15	M	16	1st	14.2	0.34	ND	ND	9100	Multiple hives	13.8	13	1.6

The full dose is 5200 mg wheat protein. Ingredients used in EPT are udon noodles (2.6% wheat protein), pastas (5.4% wheat protein), piece of bread (9.3% wheat protein), or bread roll (10.1% wheat protein).

† EPT was negative, but patient subsequently observed hives and difficulty in breathing when he exercised after ingestion at home.

Abbreviations: EPT: exercise provocation test, ND: no data, OFC: oral food challenge, OIT: oral immunotherapy, sIgE: specific IgE (kUA/L), UN: udon noodles, ω-5G: ω-5 gliadin

Table S3. Average frequency of causative food intake during OIT

	Cow's milk			Wheat		
	EIARD+	EIARD-	<i>p</i>	EIARD+	EIARD-	<i>p</i>
Seven times a week	9	5	0.45	6	4	1
Five-six times a week	7	9		2	2	
Three-four times a week	3	5		3	3	
One-two times a week	0	0		1	1	
Unknown	2	3		2	5	

P-value based on fisher exact test.

Abbreviations: EIARD: exercise-induced allergic reaction on desensitization