Wheat oral immunotherapy was moderately successful but was associated with very frequent adverse events in children aged 6-18 years

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Short title: Wheat oral immunotherapy

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Abstract (198 words)

Aim: This study investigated oral immunotherapy for children aged 6-18 years with wheat allergies.

Methods: Well-cooked wheat spaghetti was given to 100 children with wheat allergies every day for 17 weeks, increasing from 0.3mg to 2,000mg of wheat protein, followed by three-month and nine-month maintenance phases. Blood samples were taken before therapy and at follow-up visits. The study was carried out in 2009-2015 in four Finnish paediatric allergology units.

Results: The children (67% male) had a mean age of 11.6 years (range 6.1 to 18.6) and 57 were using wheat daily 16 months after the initiation of therapy. Allergic symptoms occurred in 94/100 children: mild in 34, moderate in 36 and severe in 24. Specific immunoglobulin E for ω-5-gliadin was significantly higher in patients who did not reach the target dose and were related to the intensity of reactions.

Conclusion: The majority (57%) of children with wheat allergies could use wheat in their daily diet 16 months after the initiation of oral immunotherapy, but 94/100 had adverse reactions and 60 were moderate or severe. Specific immunoglobulin E to omega-5-gliadin may provide a biomarker for how much wheat can be tolerated and the intensity of the reactions to immunotherapy.

Keywords: Children, desensitisation, food allergy, oral immunotherapy, wheat

Keynotes:

- This study investigated oral immunotherapy for children aged 6-18 years with wheat allergies.

- We found that 57/100 of the children could use wheat in their daily diet 16 months after the initiation of oral immunotherapy, but 94/100 had adverse reactions and 60 were moderate or severe.
- Specific immunoglobulin E to omega-5-gliadin may provide a biomarker for how much wheat can be tolerated and the intensity of the reactions to immunotherapy.

INTRODUCTION

Wheat is the major food grain in countries with mild temperatures. Wheat-related morbidity is common and potentially related to the specific physicochemical properties and immunogenicity of various wheat proteins (1,2). The clinical phenotype of classical immunoglobulin E (IgE) mediated wheat allergy varies from mild gastrointestinal discomfort to severe life-threatening anaphylaxis (3,4).

A large number of clinical trials and a few well-designed landmark studies have shown that oral immunotherapy (OIT) is potentially disease-modifying treatment for milk, egg and peanut allergies (5-7). However, the consensus that the safety issues have been insufficiently determined and that there is a lack of evidence on the long-term effectiveness of OIT have led to recommendations that this approach to food allergies should not be routinely used in clinical settings (8).

Little is known about OIT in patients with an IgE-mediated wheat allergy. The initial report on wheat desensitisation therapy in 2005 demonstrated successful outcomes in a seven-year-old girl with a wheat allergy who originally presented with abdominal pain, diarrhoea, asthma and facial angioedema after eating wheat (9). Only a few small studies on the use of OIT for children with wheat allergies have been published since then (10-17). We conducted a multicentre, prospective open-label study of oral wheat immunotherapy in a series of 100 children with an IgE-mediated allergy to wheat.

PATIENTS AND METHODS

Study population

The Oral Desensitization to Wheat in School Aged Children study was a prospective open-label multicentre study that investigated oral wheat immunotherapy in children aged 6-18 years children (ClinicalTrials.gov Identifier: NCT01755884). The primary outcome of the study was the number of patients who would be able to eat wheat on a daily basis 16 months after the start of the intervention, which comprised a 17-week build-up phase followed by three-month and nine-month maintenance phases. The secondary outcomes were the number of patients with adverse events during each phase of the study and the changes in the levels of specific IgE. The study was carried out between August 2009 and February 2015 in four paediatric allergology units in the Helsinki, Oulu, Kuopio and Tampere University Hospitals and in one private unit at Pihlajalinna Medical Centre, Tampere, Finland. Written informed consent was obtained from the patients and their parents. The study protocol was approved by the ethics committees of all the participating centres.

A total of 100 children aged 6-18 years with a known wheat allergy were recruited to the study. The inclusion criteria were that they needed to be aged between six and 18 years, have a history of immediate reactions when they ate wheat, a positive wheat-specific IgE test result (>3.5 kU/L) and be on a diet that excluded wheat and the related cereals of barley and rye. The diagnosis and current immediate reactivity was confirmed with an open oral food challenge (OFC), which was performed by attending physicians according to the regular wheat OFC protocol in clinical practice (18). An OFC was not performed in 13 cases, as there had been a definite immediate reaction after accidental wheat ingestion in the last three months before they entered the study. The exclusion criteria were uncontrolled asthma or any significant systemic disease or poor compliance.

Desensitisation protocol

The desensitisation protocol included three phases: the build-up phase of 17 weeks, initial maintenance phase of three months and a long-term maintenance phase of nine months (Fig. 1). Wheat spaghetti that had been well-cooked, at 100°C for 15 minutes, was given to the patients every day starting from a minimum portion of one millimetre of cooked spaghetti, corresponding to 0.0003g of wheat protein, and the dose was increased every 1-2 weeks until they received 24 single strands of spaghetti measuring 24mm each, corresponding to 2,000mg of wheat protein, at 17 weeks. Table 1 provides the detailed desensitisation protocol. Patients received standard doses of antihistamine each day during the build-up phase, namely 2.5mg of desloratadine for children under 12 and 5mg for children over the age of 12, and on an as needed basis after that. The daily use of spaghetti or other wheat products with an obtained maintenance dose continued for an additional three months during the first maintenance phase. Thereafter, the patients were encouraged to continue to eat wheat products each day with no restrictions for an additional nine months during the second maintenance phase. Clinical follow-up visits were scheduled at three and 12 months after reaching the maintenance dosage. The adverse reactions and medication were recorded using a symptom diary and patients contacted the study centre or local emergency unit if they had significant reactions. Adverse events were documented in the hospital patient records during these personal contact visits, at the scheduled build-up visits and at the three and nine-month follow-up visits.

The intensity of the symptoms and the overall classification of reactions were defined as no, mild, moderate or severe, according to the principles of a classification system proposed in 2016 (19) and taking into account those described in the American Academy of Allergy, Asthma and Immunology workgroup report in 2009 (20). Mild reactions were defined as just subjective symptoms or mild objective symptoms, including all local reactions. Moderate reactions were defined as generalised objective symptoms with one or more of the following -

generalised urticaria or angioedema, flushing, generalised itching, acute rhinoconjunctivitis, moderate vomiting or acute diarrhoea - without respiratory or cardiovascular involvement. This category also included mild respiratory symptoms without other symptoms or intensive subjective symptoms, such as discomfort and stomach pain or tiredness, combined with mild or moderate objective symptoms. Severe reactions were defined as objective respiratory symptoms, such as extensive coughing, inspiratory stridor or expiratory wheezing, and, or, cardiovascular symptoms such as unconsciousness, lethargy, collapse, drop in blood pressure or tachycardia, alone or in combination with other symptoms.

Blood samples and laboratory testing

Peripheral venous blood samples were taken before therapy and at the three-month and nine-month visits. Eosinophil counts, total serum IgE levels and specific IgE to wheat, gluten and omega-5-gliadin were measured using the CAP-FEIA fluorescent enzyme immunoassay (ThermoFischerScientific, Uppsala, Sweden). Skin prick tests for wheat were carried out by trained local laboratory personnel or nurses, using an in-house formulated allergen of powdered whole grain wheat diluted with 0.9% sodium chloride with 1:10 weight/volume and a positive control of 10 mg/mL histamine dihydrochloride (ALK-Abelló, Horsholm, Denmark) (4).

Statistical evaluation

The Student's t-test was used to analyse normally distributed continuous variables and the Mann-Whitney U test or Kruskal-Wallis analysis was used for skewed distributions. Spearman's nonparametric correlation analysis was used to analyse the relationship between laboratory parameters, age and wheat dosage. Differences in the distribution of individuals among the groups were tested with chi-square statistics unless any expected value was less than five. In those cases Fisher's exact test was used. Logistic regression was used to analyse the

relationship between baseline variables, symptoms and reactions, the achievement of target dose and discontinuation of therapy. The Friedman test and Wilcoxon signed rank test were used to analyse temporal changes in laboratory parameters. A two-tailed *p* value of 0.05 was considered to indicate statistical significance. All the data were analysed using SPSS Statistics software for Windows, version 22 (IBM Corp, Armonk, New York USA).

RESULTS

Outcome of therapy and amount of wheat tolerated

The baseline characteristics of the patients are shown in Table 2. The majority were male (67%) and their mean age was 11.6 years (range 6.1 to 18.6). Out of the 100 patients, 77 completed the build-up phase and went on to the first three-month maintenance phase, 72 completed that phase and went on to the second nine-month maintenance phase and 57 patients were still using wheat daily at the end of the study, 16 months after the intervention started (Fig. 1). We found that 64 patients reached the target dose of 2,000mg of wheat protein per day at the end of the 17-week build-up period. Of the 36 patients who did not reach the target dose, the median maximum tolerated dosage was 5.5 strands of spaghetti, corresponding to 445mg of wheat protein and ranging from 1-1,760 mg. At the end of the first, three-month, maintenance period, 47/72 patients were still using the target amount of 2,000mg wheat protein daily, whereas the median daily amount of wheat consumed by the other 25/72 patients was 330mg (range 5-1,750mg). At the end of the second, nine-month, maintenance period, 18/57 patients still on therapy were consuming a median of 500mg (range 83-1,000mg) wheat protein, whereas the number of patients still eating the target amount of wheat or more was 39/57 at the time of the final follow-up visit. Out of these, 29 were using the target dose in form of the form of spaghetti or pasta, bread, biscuits, porridge or other wheat products, corresponding to 2,000mg of wheat protein per day. The remaining 10 patients had more than 2,000mg of wheat in their daily diet, varying from 3g to no

restriction, in the form of 5-6 slices of bread, a normal size pasta meal or other non-specified wheat products. Reaching the target dose or the daily amount of wheat were not related to the age or sex of the patients (data not shown).

The initial ω-5-gliadin specific IgE levels were significantly higher in the patients who did not reach the target dose, with a median and interquartile range (IQR) of 6.02 kU/L (2.34-16.2) versus 1.81 kU/L (0.14-7.14) (p=0.005). The differences were not significant for wheat or gluten IgE (data not shown). Furthermore, 22/26 (85%) of the patients negative for ω-5-gliadin specific IgE reached the target dose compared to 41/72 (57%) of the patients who were initially positive for ω-5-gliadin IgE (p=0.016). The initial skin prick test result for wheat did not differ between subjects not reaching and reaching the target dose, with a median (IQR) of 10mm (5-20mm) versus 9mm (IQR 5-20) (p=0.474).

Symptoms and reactions

Only six of the 100 patients did not have any reactions during any phase of the study, whereas 34% experienced mild, 36% moderate and 24% severe reactions during some phase of the study. The symptoms and reactions that occurred in patients during each phase of the study are shown in the Table 3. We found that 70/100 (70%) patients experienced symptoms related to wheat ingestion during the build-up phase and these reactions were moderate or severe in 43 (43%) patients. Similarly, symptoms occurred in 60/77 (78%) patients during the first three-month maintenance phase, of which 28 (35%) were moderate or severe, and in 52/72 (72%) patients during the second nine-month maintenance phase, of which 17 (24%) were moderate or severe. The reactions were related to physical exercise 1-4 hours after wheat ingestion in five patients during the build-up phase, in two patients during the first maintenance phase and in four patients during the second maintenance phase. Other co-existing factors, such as viral infections, were not recorded. We found that 12 patients used intramuscular epinephrine

during the entire study period, one of them for two separate reactions and the remaining 11 for single reactions. In the logistic regression analysis, the intensity of reactions at the initial food challenge were not related to the likelihood of reaching the target dose (data not shown), whereas the intensity of reactions during the build-up period and the likelihood of reaching the target dose were inversely related (Table 4a). Of the 72 patients who successfully completed the first maintenance phase, 30 were using antihistamine daily and 42 were only using it occasionally when they visited their clinics at the end of this phase. The corresponding numbers at the end of the second nine-month maintenance phase were 8/57 for daily use and 49 for occasional use..

The initial skin prick test result or the initial levels of wheat or gluten specific IgE were not statistically significantly related to the severity of reactions experienced by each individual patient during the entire study period (Fig. 2). In contrast, the initial ω-5-gliadin specific IgE were related to the intensity of reactions, in that higher initial IgE levels were significantly associated with more intensive reactions during some phase of the study (Fig. 2). However, it should be noted that some patients with very high initial levels of these antibodies only had mild or even no reactions during the study.

Temporal changes in laboratory parameters

We had three wheat, gluten and ω-5-gliadin IgE samples available for 62, 61 and 58 patients, respectively, and these showed that the specific IgE levels decreased significantly as with desensitisation therapy progressed and the individual *p* values obtained using the Friedman test were less than 0.001 for wheat, gluten and ω-5-gliadin IgE (Fig. 3). Furthermore, when the first and second samples were compared – namely the sample obtained before therapy and at the end of the three-month maintenance phase or therapy was discontinued – the decrease was only significant for ω-5-gliadin levels ($p<0.001$) using the Wilcoxon signed rank test.

However, when the samples were analysed separately, this change was significant for those who successfully completed the therapy ($p<0.001$). There was no significant change for any of the samples that were available for the 27/43 subjects who discontinued the therapy at any stage ($p=0.085$).

Characteristics of the patients with unsuccessful therapy

The characteristics of the patients with positive and negative outcome are shown in the Table 5. This showed that 43/100 patients discontinued therapy at some point during the study (Fig. 1), with 23/43 (53%) of the drop outs occuring during the build-up phase, 5/43 (12%) during the maintenance phase and 15/43 (35%) during the follow-up phase. Of the 43 patients who discontinued the therapy 34 (79%) had objective or objective and subjective symptoms and eight (16%) only had subjective symptoms. In the logistic regression analysis, the intensity of reactions during the initial food challenge were not related to discontinuing the therapy (data not shown), but the likelihood of discontinuing the therapy was significantly increased by the intensity of reactions during the build-up phase (Table 4B).

DISCUSSION

In this large series of children with IgE-mediated wheat allergy, we report that 57 of 100 patients were eating wheat daily after desensitisation therapy and follow-up, including 17 weeks of build-up and three and nine months of maintenance. However, only 39 of these were eating the target amount of wheat at the end of the study, namely least 2,000mg of wheat protein. Virtually all of the subjects (94%) experienced some wheat related allergic symptoms during the study and these reactions were moderate in 36% and severe in 24% of the individual children during the entire study period. None of the baseline characteristics were able to identify patients who discontinued therapy during the study. The initial levels of ω-5-gliadin specific IgE might be useful as an inverse biomarker for the amount of wheat tolerated

and for the intensity of reactions experienced during OIT. Although the design of the study does not allow us to draw any definite conclusions on the efficacy of wheat OIT, it provides valuable data, especially on the side-effects associated with this form of therapy for children who are allergic to wheat.

The direct comparison of the safety and efficacy profiles between various studies is challenging, due to variable OIT protocols and clinical phenotype of the target populations and between various foods. Many previous studies on OIT for egg, milk and peanut allergies have emphasised that the vast majority of patients only experience mild local reactions (21-23). However, this might lead to a false sense of security, as other studies have shown that moderate or severe systemic reactions seem to be fairly common during OIT (24,25). With regard to wheat OIT, most of the observed reactions were mild in the Sato et al study (15), although three of the reactions were classified as severe during the maintenance phase. In a Spanish study (13), two of the six patients experienced mild adverse events during phase when the doses were increased. Our study showed a high rate of adverse reactions during wheat OIT, with 94/100 of patients experienced symptoms related to wheat consumption at some point during the study. These reactions were moderate in nearly 30% and severe in nearly 15% of the patients during the build-up phase. On the other hand, the proportion of patients with mild reactions increased up to 40-50% and the proportion of those with severe reactions decreased to less than 10% during the maintenance phases. However, having no reactions during the build-up phase did not guarantee non-reactivity later on maintenance, as even 80% of those 30 patients who did not have any adverse reactions during the build-up period experienced mild to severe reactions during the maintenance phases. In our view, it seems evident that the risk of moderate and severe allergic reactions is real and unpredictable in children with IgE-mediated wheat allergy who are receiving OIT. This risk, combined with the

discomfort related to less severe adverse events, which was sometimes continuous, in most patients during OIT needs to be carefully weighed against the risk, severity, anxiety and fear of reactions related to unintentional exposure to wheat protein while on an avoidance diet. In addition, the comparative health-related quality of life between OIT and avoidance should be evaluated. We are currently planning to survey the long-term outcome of the current study population in terms of wheat use and quality of life issues.

Previous OIT studies have shown a short-term and long-term failure rate of 30-70% (26,27). We found that 43% of the patients discontinued therapy at some point during the present study. None of the demographic factors could discriminate between those with successful and unsuccessful therapy. One important factor that may lead to the discontinuation or continuation of OIT might be the level of motivation, especially in those not reaching the target dose. Abdominal pain often reported during all phases of our study is a disturbing symptom that can affect a patient's motivation to continue OIT. Also the adherence to the therapy might decrease with less frequent follow-up visits. Another factor that could have potentially affected the outcome of the study might be related to the form of wheat used and, or, the steep increasing dosing regimen used during the build-up phase of the study, especially among the patients with the severe phenotype. Furthermore, it is possible that extensively cooking the spaghetti at 100°C for 15 minutes might have changed the tolerogenic potential of the wheat proteins and could have contributed to the outcome of our study. It was shown in a transgenic OVA23-3 mouse model that the intensity of heat treatment significantly affected the allergenic properties of the antigen and that egg white proteins aggregated markedly upon extensive heating in retort pouch conditions (28). In our opinion, all of these findings emphasise the currently inadequate knowledge about how to define an optimal OIT protocol and the successful target population for wheat OIT.

High initial levels of specific IgE have been reported to be related to lower tolerated doses, higher numbers and severities of reactions and unsuccessful OIT outcomes (24,26,29,30). An important finding in the present study was that the initial levels of specific IgE for ω-5-gliadin were significantly higher in patients who did not reach the target maintenance dose. Furthermore, the initial and follow-up levels of ω-5-gliadin specific IgE were related to the intensity of objective symptoms and the severity of reactions. This might have been related to the specific properties of ω-5-gliadin as an antigen (4). However, the initial or follow-up levels of specific IgE did not differ between those who discontinued OIT and those who succeeded with the therapy. The only recognisable difference was that the levels of specific IgE only decreased significantly among those with successful therapy. Randomised controlled studies are needed to verify whether the stability or increase in the component-specific IgE levels could be used as a predictive marker of wheat OIT success.

The major limitation of the current study was the non-randomised, uncontrolled design. Therefore, we cannot draw any definite conclusions on the efficacy of wheat desensitization therapy. However, it is very unlikely that close to 60% of the patients would have outgrown their wheat allergy during the short study period. For practical reasons, the diagnosis and the initial immediate reactivity was confirmed using an open OFC, performed by attending physicians according to the regular wheat OFC protocol in clinical practice, instead of using a double-blind placebo-controlled food challenge. Furthermore, no food challenge test was performed after the therapy to define the reactivity threshold or to test the sustained unresponsiveness after any therapy-free period. Thus, in theory, the study population might have included some false positive patients who initially showed a placebo-like reactivity to wheat during the therapy and after the maintenance. However, as stated earlier, almost all of the patients had a previous clinical history of severe or intensive reactivity to wheat, were

highly sensitised to wheat allergens and showed typical subjective and objective symptoms during the initial OFC.

CONCLUSION

Nearly 60% of the children who were allergic to wheat were able to use wheat products daily 16 months after the initiation of OIT. However, wheat OIT was associated with a high rate of adverse reactions and a significant proportion of these reactions were moderate or severe. Furthermore, non-reactivity during the build-up phase did not guarantee non-reactivity during the maintenance phases. Our study showed that ω-5-gliadin IgE had some potential as a biomarker for the tolerated dose and the intensity of reactions. Based on the results reported here, we think that wheat OIT using cooked spaghetti is not ready for routine clinical practice. The utility of wheat in other forms, such as other forms of pasta, wheat bread, wheat flour, hypoallergenic wheat extracts, should be investigated for OIT. Future trials should also focus on better patient selection, dosing protocols and adjunctive treatments in randomised controlled studies that address the immunologic mechanisms, safety and health-related quality of life issues of wheat OIT.

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Conflicts of Interest

No conflicts of interest.

Abbreviations

IgE, immunoglobulin E; OFC, open oral food challenge; OIT, oral immunotherapy

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Table 1. The detailed protocol for wheat desensitisation used in this study

	Daily dose of boiled wheat spaghetti		**Amount of wheat protein (mg)**
Week number	**Length in millimetres**	**Number of single strands of spaghetti**	
1	1 *		0.3
2	2 *		0.7
3	3		1.0
4	4		1.4
5	5		1.7
6	10 *		3.5
7	15		5.0
8	20 *		7.0
9	40 *		14.0
10	80		28.0
11	160		60.0
12	(240, equals 1 strand)	1 *	80.0
13		2	170.0
14		4 *	330.0
15		8	670.0
16		16 *	1,300.0
17		24	2,000.0

The first dose of the dosage step-ups marked with asterisk (*) were given in the hospital outpatient clinic. All the other dosage step-ups and daily doses occurred at home. The daily use of spaghetti or other wheat products, the amount of wheat protein corresponding to 2,000mg in maximum, continued with the amount of achieved maintenance dosage for an additional three months as the first maintenance phase. Thereafter, the patients were encouraged to continue to eat spaghetti or other wheat products daily with no restrictions for an additional nine months as the second maintenance phase.

Table 2. Baseline characteristics of the study population (n=100)

Age (years), mean (range)	11.6 (6.1-18.6)
Male sex, n	67
Laboratory findings at the beginning of the study, median (range)	
Total serum IgE (kU/L) (n=95)	1,096 (44-7,323)
Blood eosinophils (% of leukocytes) (n=99)	9 (2-30)
Wheat IgE (kU/L)	
All patients, values at or above 100 kU/L defined as 100 kU/L	100 (2.6-100)
Patients with defined absolute values available (n=76)	201 (2.6-2,810)
Gluten IgE (kU/L)	
All patients, values at or above 100 kU/L defined as 100 kU/L	100 (1.8-100)
Patients with defined absolute values available (n=65)	146 (1.8-2,610)
Omega-5-gliadin IgE (kU/L) (n=98), absolute values	2.9 (0-100.0)
Wheal diameter (mm) on skin prick test with wheat, median (range) (n=87)	10 (5-20)
Initial wheat challenge (open OFC or accidental ingestion)	
Patients with OFC done in hospital outpatient clinic, n (%)	87
Cumulative symptom eliciting wheat dosage (mg), median (IQR)	300 (0.1-600)
Use of intramuscular epinephrine, n (%)	29 (35)
Patients with recent accidental ingestion (<3 months), n (%)	13 (13)
Estimated symptom eliciting wheat dosage (mg), median (IQR)	100 (0.1-900)
Use of intramuscular epinephrine, n (%) (data available for 12 patients)	3 (25)
Symptoms presented during OFC or at accidental wheat ingestion (n=100)	
Subjective symptoms, n	
Pruritus	12
Oral itching	64
Abdominal pain	39
Nausea, discomfort	29
Weakness, dizziness	8
Objective symptoms, n	
Urticaria	42
Erythema	31
Nasal congestion and/or rhinitis	28
Conjunctival symptoms	14
Laryngeal symptoms	2
Bronchial wheezing	18
Emesis	25
Acute diarrhea	2
Drop in blood pressure	3
Cardiovascular collapse	1
Overall classification of reaction, n	
No reaction	0
Mild	15
Moderate	57
Severe	28

Table 3. Wheat ingestion related symptoms, medication and drop-outs, i.e. discontinuation of therapy, during oral wheat desensitization therapy in 100 school-aged wheat allergic children. For patients with multiple reactions, the most intensive reaction is reported during each phase of the study.

	Build up phase (17 weeks)	*Maintenance phase 1 (3 months)*	*Maintenance phase 2 (9 months)*
Number of patients entering the phase	100	77	72
Total number of drop outs during the phase, n (%)	23 (23)	5 (6.6)	15 (21)
Overall classification of reactions, number of patients (%)			
No reaction	*30*	*17 (22)*	*20 (28)*
Drop outs	1 (3.3)	0 (0)	1 (5.0)
Use of intramuscular epinephrine	0 (0)	0 (0)	0 (0)
Mild reaction	*27*	*32 (42)*	*35 (49)*
Drop outs	7 (26)	0 (0)	7 (22)
Use of intramuscular epinephrine	0 (0)	0 (0)	0 (0)
Moderate reaction	*29*	*22 (29)*	*11 (15)*
Drop outs	8 (29)	4 (18)	6 (33)
Use of intramuscular epinephrine	0 (0)	2 (9.1)	1 (5.6)
Severe reaction	*14*	*6 (7.8)*	*6 (8.3)*
Drop outs	7 (50)	1 (17)	1 (50)
Use of intramuscular epinephrine	6 (43)	2 (33)	2 (100)
Subjective symptoms, number of patients (%)			
Pruritus	4	6 (8)	6 (8.3)
Oral itching	18	25 (32)	25 (35)
Abdominal pain	37	24 (31)	15 (21)
Nausea, discomfort	13	5 (6.5)	9 (13)
Weakness, dizziness	2	2 (2.6)	1 (1.4)
Objective symptoms, number of patients (%)			
Urticaria	19	13 (17)	12 (17)
Angioedema	7	5 (6.5)	5 (6.9)

Erythema	9	5 (6.5)	5 (6.9)
Nasal congestion and/or rhinitis	13	11 (14)	7 (9.7)
Conjunctival symptoms	4	7 (9.1)	7 (9.7)
Coughing	8	4 (5.2)	3 (4.2)
Laryngeal stridor	1	1 (1.3)	0 (0)
Bronchial wheezing	23	19 (25)	16 (22)
Emesis	13	13 (17)	3 (4.2)
Acute diarrhoea	6	1 (1.3)	0 (0)
Drop in blood pressure	0	0 (0)	0 (0)
Cardiovascular collapse	0	0 (0)	0 (0)

Table 4

A. Logistic regression analysis of the relation between the intensity of symptoms during the build-up phase and achievement of target dose.

	Target dose (n=64)	**Less than target (n=36)**	**OR**	**95% CI for OR**	**p value**
Intensity of subjective symptoms (n)					
No subjective symptoms	37	10	1.00		
Mild	14	5	1.32	0.38-4.55	0.659
Moderate	11	17	5.72	2.04-16.0	*0.001*
Intensive	2	4	7.40	1.18-46.4	*0.033*
Intensity of objective symptoms (n)					
No objective symptoms	36	10	1.00		
Mild	8	4	1.80	0.45-7.23	0.407
Moderate	16	14	3.15	1.16-8.59	*0.025*
Intensive	4	8	7.20	1.79-28.9	*0.005*
Overall classification of reactions (n)					
No reaction	28	2	1.00		
Mild reaction	15	12	11.20	2.21-56.8	*0.004*
Moderate reaction	16	13	11.38	2.27-56.9	*0.003*
Severe reaction	5	9	25.20	4.15-153.0	*<0.001*

B. Logistic regression analysis of the relation between the intensity of symptoms during the build-up phase and discontinuation of the therapy at any phase.

	Succesful therapy (n=57)	**Unsuccesful therapy (n=43)**	**OR**	**95% CI for OR**	**p value**
Intensity of subjective symptoms (n)					
No subjective symptoms	33	14	1.00		
Mild	12	7	1.38	0.45-4.22	0.578
Moderate	11	17	3.64	1.36-9.73	*0.010*
Intensive	1	5	11.79	1.26-110.3	*0.031*
Intensity of objective symptoms (n)					
No objective symptoms	29	17	1.00		
Mild	8	4	0.85	0.22-3.26	0.816
Moderate	14	15	1.95	0.77-4.97	0.161
Intensive	6	6	1.71	0.47-6.14	0.414

Overall classification of reactions (n)					
No reaction	23	7	1.00		
Mild reaction	14	13	3.05	0.98-9.48	0.054
Moderate reaction	14	15	3.52	1.15-10.8	*0.027*
Severe reaction	6	8	4.38	1.13-17.0	*0.033*

Table 5. Characteristics of the patients with unsuccessful and successful therapy.

	Successful therapy n=57	*Unsuccessful therapy n=43*	*p value*
Age (yrs), median (range)	11.4 (6.3-16.5)	12.9 (6.1-18.6)	0.067
Male sex, n (%)	39 (68)	28 (65)	0.831
Target dose reached during the build-up phase, n (%)	49 (86)	15 (35)	*<0.001*
Reason for discontinuation of the desensitisation therapy, n (%)			
Subjective symptoms	-	8 (19)	
Objective symptoms	-	7 (16)	
Subjective and objective symptoms	-	27 (63)	
Other reason	-	1 (2.3)	
Intensity of the most intensive reaction during the entire study period, n (%)			0.053
No reactions	5 (8.8)	1 (2)	
Mild reaction	24 (42)	10 (23)	
Moderate reaction	15 (26)	21 (49)	
Severe reaction	13 (23)	11 (26)	
Initial laboratory values before therapy, median (IQR)			
Total serum IgE (kU/L)	1,168 (477-2249)	852 (44-5310)	0.159
Blood eosinophils (% of leukocytes)	9 (6-13)	8.5 (2-30)	0.310
Wheat IgE (kU/L)	145 (67.9-554.59	100 (91.1-280)	0.447
Gluten IgE (kU/L)	100 (64.5-225.5)	100 (93.8-214)	0.636
Omega-5-gliadin IgE (kU/L)	3.0 (0.2-15.4)	2.8 (1.1-8.2)	0.782
Wheal diameter on skin prick test with wheat (mm)	10 (8-12)	9 (7-12)	0.269

Figure legends

Figure 1. Primary outcomes of the study. The diagram illustrates the course of the study, the amount of wheat tolerated and the number of the patients with successful and unsuccessful, i.e. discontinuation, therapy during each phase of the study.

Figure 2. Comparison of the initial levels of allergen specific IgE and skin prick test results to the intensity of the most intensive reaction in each individual patient during the entire study period of wheat desensitization therapy. *p-value* indicates the level of significance on Kruskal-Wallis test between all the groups. **A.** Wheat specific IgE **B.** Gluten specific IgE **C.** ω-5-gliadin specific IgE **D.** Skin prick test. In dot plots, each dot represents single patient and the black dotted line indicates median value.

Figure 3. Temporal changes in the antigen specific IgE levels among patients with all three samples available. Wheat specific IgE **B.** Gluten specific IgE **C.** ω-5-gliadin specific IgE. In dot plots, each dot represents single patient. Box plots show interquartile range and median (black line inside the box) and the whiskers indicate 5% and 95% values. Statistical comparison was performed using Friedman test.