

Buckwheat flour hypersensitivity: an occupational asthma in a noodle maker

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Summary

Background There have been very few reports of occupational allergies caused by inhalation of buckwheat flour. In this paper, we present a case of occupational asthma and rhinitis caused by buckwheat flour inhalation.

Methods and results The patient had strong positive responses to grass and ragweed pollens as well. The bronchoprovocation test showed early asthmatic response to buckwheat flour extracts. Serum specific IgE antibody to buckwheat flour was detected by enzyme-linked immunosorbent assay (ELISA). In order to further identify the allergenic component of the extracts, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting studies were performed. Eight IgE binding components (9–55 kDa) were detected within the buckwheat flour extracts.

Conclusion These results suggest that inhalation of buckwheat flour can caused IgE mediated bronchoconstriction.

Keywords: occupational allergy, rhinitis, buckwheat flour

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Introduction

Buckwheat flour (*Fagopyrum esculentum*, Moench) is a common food, and is known to have been a cause of asthma and serious anaphylactic reactions following ingestion in Japan and United States [1,2]. Some cereal flours such as wheat and rye flour have induced occupational asthma and their specific IgE antibodies have been found in the affected workers [3,4]. There was a report of occupational asthma and contact urticaria caused by buckwheat flour in a subject who worked in a creperie [5]. This clinical report did not look at what specific antibodies might be involved in causing this condition.

In this paper, we report on a case of occupational asthma and rhinitis caused by buckwheat flour, as confirmed by bronchoprovocation tests in a patient running a buckwheat flour noodle shop. Serum specific IgE antibody and IgE binding component were identified. The antigenic relationship with grass and weed pollens was also evaluated.

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Subjects and methods

The patient was a 26-year-old non-smoking woman, who was admitted to the Allergy Clinic of the Ajou University Hospital, Suwon, Korea. Seven years earlier, she had married and began to work which involved making noodles with buckwheat and wheat flours. Her job included mixing and pouring the buckwheat flour. She experienced profuse rhinorrhoea and sneezing for 3 years and began to feel shortness of breath 2 months before admission. She complained that these symptoms were aggravated after handling buckwheat flour. On admission, physical examination showed a clear breathing sound without wheezing.

A skin-prick test showed immediate positive responses to alder, oak, ragweed, timothy grass, blue grass, rye grass pollens, and buckwheat and wheat flour extracts. Radio-allergosorbent test (RAST) revealed positive responses as shown in Table 1. Total IgE level by PRIST was 400 IU/mL. Her chest x-ray was normal. Methacholine bronchial challenge test revealed a positive response at 0.62 mg/mL. Ingestion of buckwheat and wheat-flour confining foods provoked no symptoms.

Table 1. Skin-prick test and RAST

Allergens	Skin-prick test		RAST (class)
	Weal	Erythema	
Alder	2	19	0
Oak	2	14	ND
Cochroach	4	29.5	3
<i>D. farinae</i>	2.5	17	0
Ragweed	2	6.5	2
Timothy grass	2	9	0
Blue grass	2	13	2
Rye grass	2	6	ND
Wheat flour (HM)	5	26	3
Buckwheat flour (HM)	4	13.5	NA
Rye grain (B)	3	26.5	3
Wheat grain (B)	2	7	3
<i>Chironomus plumosus</i>	2	13.5	NA
Histamine	2	13.5	

B. Bencard extracts. HM. Home made extracts. ND. Not done. NA. Not available. Skin reactivity was presented as mean weal and erythema diameter.

Preparation of buckwheat and wheat flour

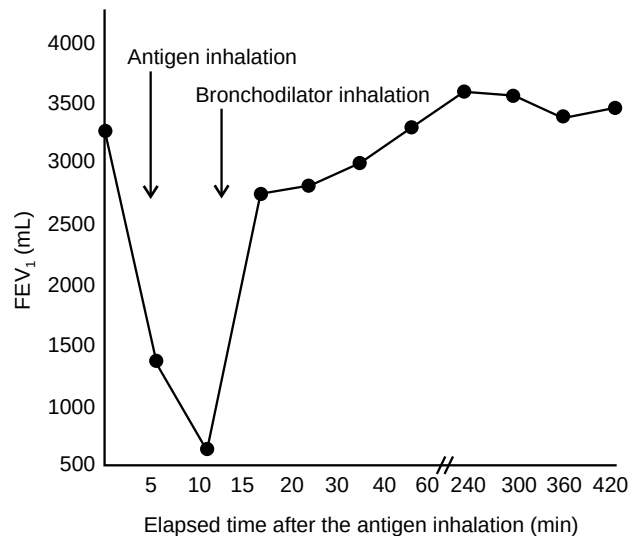
Buckwheat and wheat flour were obtained from her shop. They were extracted in a phosphate-buffered saline (PBS, pH 7.5), 1:10 w/v, 4°C for 24 h, followed by centrifugation at 10 000 *g* at 4°C for 1 h. The supernatant was dialysed against distilled water and lyophilized at -70°C. For the skin-prick test, 1:5 w/v extracts were mixed with an equal amount of sterile glycerine.

Bronchoprovocation test with buckwheat flour

Bronchoprovocation tests were performed in a similar manner to those in other occupational asthma studies [6,7]. Lactose, buckwheat or wheat flour-containing capsule was inhaled through a spinhaler (Intal, Fison, UK). The forced expiratory volume in 1 second (FEV₁) and peak expiratory flow rate (PEFR) were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 h after the challenge.

ELISA for specific IgE and IgG4 antibodies to buckwheat flour

The presence of specific IgE antibodies to buckwheat flour was determined by ELISA. Microtitre plates (Dynatech, USA) were coated with 100 µL extracts of buckwheat flour (10 µg/well) dissolved in Carbonate buffer (pH 9.6). After incubation overnight at 4°C, each well was washed three times with 0.05% Tween phosphate buffered saline

**Fig. 1.** Result of bronchoprovocation test with buckwheat flour.

(PBS-T), and the remaining binding site was blocked by incubation with 250 µL of 10% newborn calf serum for 1 h at room temperature. The wells were then incubated for 2 h at room temperature with 100 µL of undiluted serum from either the patient, or control sera from five patients who showed negative skin-prick test responses to common inhaled allergens as well as to buckwheat flour. After washing three times with PBS-T, 100 µL of 1:1000 w/v biotin-labelled goat anti-human IgE antibody (Sigma Chemical Co., USA) were added to the wells and incubated for 2 h at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1000 w/v streptavidin-peroxidase (Sigma Chemical Co., USA) for 30 min before another washing step followed by incubation with

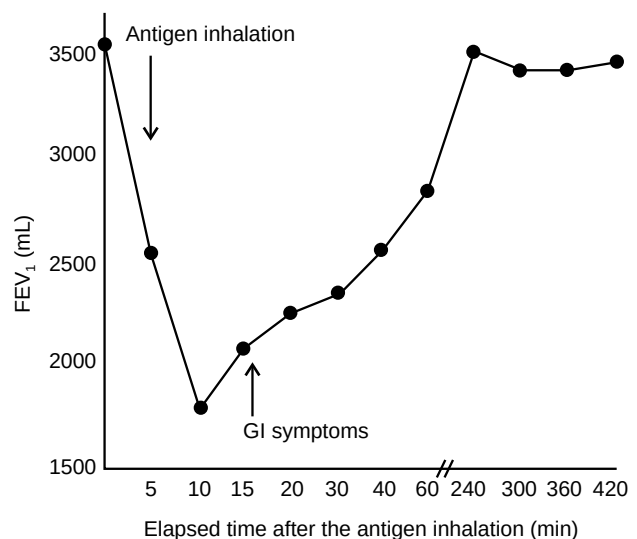
**Fig. 2.** Result of bronchoprovocation test with wheat flour.

Table 2. Specific IgE binding to buckwheat flour in sera of patient and control according to antigen concentration

Subject \ Absorbance ($\times 100$)	Antigen coated (μg)		
	10	50	100
Patient	100	150	150
Control 1	35	40	42
Control 2	48	42	43
Control 3	48	60	43

100 μL ABTS (2,2-azino-ethylbentazoline sulfonic acid in citrate phosphate buffer, for 10 min at room temperature. The reaction was stopped by addition of 50 μL 2 M sodium azide and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate.

ELISA inhibition assay

To observe for possible crossreactivity with ragweed, rye grass, and wheat flour extracts, the patient serum was preincubated with the above extracts for 1 h at room temperature. ELISA was then performed in the same manner.

SDS-PAGE and immunoblotting study

Buckwheat (150 μg) was dissolved in a buffer (10 μg) and boiled. Ten microlitres of standard markers (4, 6, 17, 22, 30, 42, 60, 148, 250 kDa, Novex, San Diego, USA) and antigen solution were applied to a Novex precast tris-glycine gradient gel (4–20% acrylamide) for the separation antigens. Electrophoresis was performed with a Novex Mini-cell for 90 min at 125 constant voltage. The gel was fixed and stained with Coomassie brilliant blue. For the immunoblotting, the gel was soaked in transfer buffer (12 mM tris, 96 mM glycine in 205 methanol) for 10 min. Electroblotting was carried out for 90 min at 200 mM using a Novex

Western transfer apparatus. The blotted nitrocellulose membrane was then treated with 0.5% tris-bovine serum albumin solution for 1 h to block non-specific protein binding. The non-diluted patient's serum and control sera were incubated with membrane for 2 h at room temperature. They were then washed with 0.1% triton in TBS-T for 10 min, and 0.5% NaCl in TBS-T for a further 10 min each. Alkaline phosphatase-conjugated anti-human IgE antibody (Sigma, USA) was diluted to 1:500 with TBS-T and used as a second antibody solution. The membrane was incubated with the second antibody for 1 h at room temperature and washed again as above. A substrate solution (NBT/BCIT kit, Pierce, USA) was applied to the membrane until the positive band appeared. After the reaction, the membrane was washed with distilled water and observed.

Result

Bronchoprovocation test with buckwheat flour

Lactose powder capsule as a placebo was inhaled and observed for 7 h. There was no significant change in FEV_1 during the observation period. As shown in Fig. 1, early asthmatic reaction was noted after inhalation of 10 mg buckwheat flour in a capsule. Figure 2 shows the bronchoprovocation test using wheat flour in a capsule. An early asthmatic reaction was observed after the 10 mg inhalation. The patient did not complain of any gastrointestinal symptoms during the challenge procedure.

Detection of specific IgE and IgG₄ antibodies to buckwheat flour

Tables 2 and 3 show the specific IgE and IgG₄ binding to buckwheat flour extracts in the patient's serum and control sera, which were derived from five patients who showed all negative results on skin-prick test with 50 common inhalant allergens including buckwheat flour extracts. Highly specific IgE binding to buckwheat flour was noted in the patient's serum in comparison to those of the controls. Moreover, the patient had a high specific IgG₄ binding. One of the controls also had a high binding. Figure 3 reveals the buckwheat flour ELISA inhibition results with addition of four different pollen extracts as well as buckwheat flour extracts. Significant inhibition was noted with serial addition of rye grass, wheat flour, and ragweed antigens as well as buckwheat flour extracts.

Gel electrophoresis

In order to understand the protein components of buckwheat flour extracts, the extracts were analysed by 4–20%

Table 3. Specific IgG₄ binding to buckwheat flour in sera of patient and control according to antigen concentration

Subject \ Absorbance ($\times 100$)	Antigen coated (μg)		
	10	50	100
Patient	118	157	138
Control 1	64	102	78
Control 2	78	74	78
Control 3	84	100	79

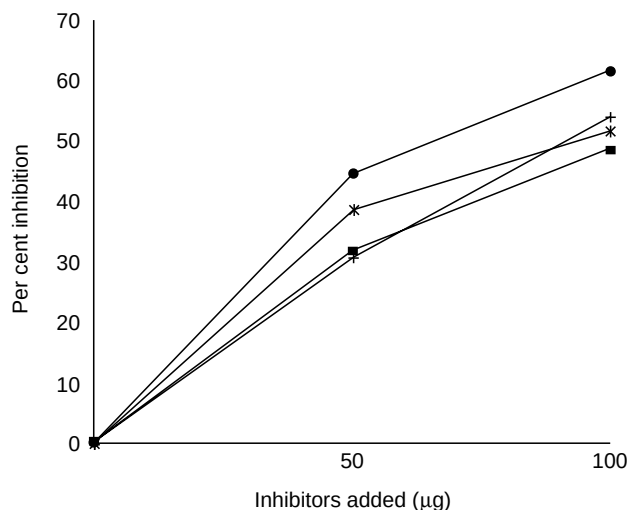


Fig. 3. Buckwheat flour ELISA inhibition result with addition of buckwheat (●), rye grass (■), ragweed (*) and wheatflour (+) extracts.

gradient SDS-PAGE. Figure 4a reveals the resolution of the buckwheat flour extracts into more than 10 bands ranging 9–67 kDa (9, 14, 17, 20, 27, 30, 32, 34, 36, 38, 41, 45, 55, 67 kDa).

Immunoblot analysis

Figure 4b shows the binding of specific IgE in patient's and control sera on a blotted membrane. There were eight bands with molecular weights of 9, 14, 17, 20, 24, 34, 45, 55 kDa bound to IgE antibody.

Discussion

This study reveals that two commonly used flour, buckwheat flour and wheat flour, can induce occupational asthma in an exposed worker. There have been a few reports of allergy to buckwheat flour in this country [7a, 7b]. Specific IgE has been implicated as the antibody involved in mediating these reactions. This study demonstrates that the pathogenetic mechanism of occupational asthma caused by buckwheat flour may

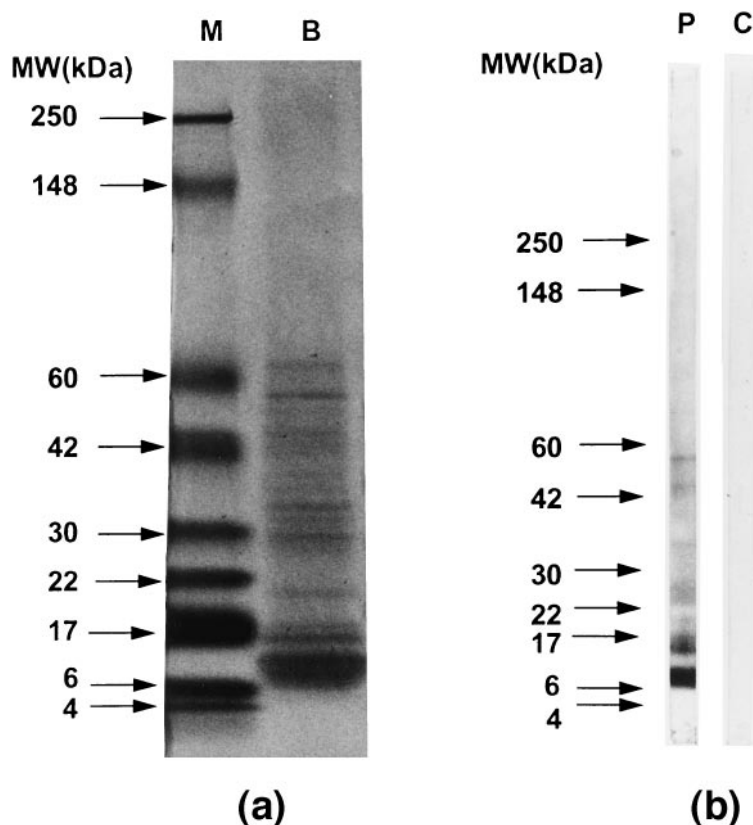


Fig. 4a. Gradient SDS-PAGE (4–20%) of buckwheat flour extracts. M. Marker protein. B. Buckwheat flour extracts.

Fig. 4b. Immunoblotting of buckwheat flour extracts and patient (P) and control (C) sera.

be an IgE-mediated reaction, since the skin-prick and bronchoprovocation tests with buckwheat flour all showed a positive response to the buckwheat flour extracts. Furthermore, its specific IgE antibody was detected by ELISA, whose inhibition result suggested specificity of IgE binding to buckwheat flour antigen. The immunoblotting study also showed eight IgE binding components, which was different from an immunoblot study with buckwheat seed [8]. They stressed that 24 kDa protein was a major allergen in buckwheat-allergic patients whose symptoms were provoked by buckwheat ingestion. These results show that occupational exposure to buckwheat flour through inhalation could induce IgE mediated bronchoconstriction.

Some investigators [9,10] have stressed that specific IgG₄ antibody could present as an exposure marker in workers exposed to laboratory animals. Lee *et al.* [7a] noted the presence of specific IgG₄ in sera of negative control patients. In their study, asymptomatic control patients who showed a negative skin-prick test had high specific IgG₄ without specific IgE. These results suggest that frequent exposure to buckwheat flour-containing food may induce production of specific IgG₄, which seems not to be directly involved in the pathogenesis of respiratory symptoms.

There have been several reports that atopy plays an important role in the development of IgE sensitization to occupational allergen with high molecular weight [11,12]. The patient had atopy and showed positive responses to pollens and other inhalant allergens. Atopy may have contributed to the development of occupational asthma in this patient.

The possibility of crossreactivity between pollens and cereal grains has been discussed [13,13b]. Another group reported little crossreactivity between buckwheat flour and wheat flour [5]. Buckwheat is a *polygonaceae* belonging to the weed group, and it is taxonomically unrelated to cereals such as wheat, rice, barley and rye. This study suggested the possibility of cross-allergenicity between buckwheat and wheat flour, and between buckwheat flour and pollens such as rye grass and ragweed. Further investigations are needed to clarify the common IgE binding epitope among them.

In conclusion, this study demonstrated that buckwheat flour can induce occupational asthma in an exposed patient through inhalation. IgE-mediated reaction may be involved in the development of bronchoconstriction. Cross-allergenicity between buckwheat and wheat flour, and some pollens, remains a possibility to be confirmed.

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