

Bakers' asthma caused by alpha amylase

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*Two bakers with bronchial asthma and two with rhinoconjunctivitis are described. Prick and RAST tests were positive with wheat flour in all of them, but the challenge test (nasal or bronchial) with wheat flour extract was positive only in one asthmatic baker. The prick test, RAST, and nasal or bronchial challenge done with alpha amylase extract (a glycolytic enzyme obtained from *Aspergillus oryzae* and used as a flour additive) were positive in all four patients. Our results support previous data indicating that alpha amylase used in bakeries is an important antigen that could cause respiratory allergy in bakers. It can function as sole causative allergen or in addition with other allergens used in the baking industry.*

INTRODUCTION

Bakers' asthma has been known since ancient Rome. Slaves working in bakeries and grain mills put on gloves and masks before starting work.¹ Ramazzini gave the first scientific description in 1713 and De Besche in 1929 included it as an occupational allergic disease.² Thereafter, other authors have contributed to knowledge of the mechanisms of the disease's development.

Cereal flour has been considered the main allergen involved in bakers' asthma⁵⁻⁷ but there are other substances described as causing bronchial asthma: molds,⁸ mites,⁹ and dust flour obtained from non-cereal sources such as buckwheat¹⁰ and chick pea,¹¹ and enzymes used to improve the flour.

We describe the studies carried out on two bakers with bronchial asthma and another two with rhinoconjunctivitis, in whom we found the alpha amylase derived from *Aspergillus oryzae* and used as a flour additive to be the causative allergen.

CASE REPORTS

Case 1

Patient 1, a 27-year-old male smoker, has been a baker for 7 years.

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Received for publication March 31, 1993.
Accepted for publication in revised form October 20, 1993.

He had a 3-year history of rhinoconjunctivitis related to his job. He remained asymptomatic on weekends and holidays and became worse upon using flour additives. The clinical examination revealed nasal mucosal congestion. Total blood cell count and biochemical studies were normal. Total serum IgE was 507 IU/mL. Spirometry was normal.

Case 2

Patient 2 is a 40-year-old male ex-smoker, and baker since the age of 20. He had a 2-year history of ocular and nasal itching, rhinorrhea, and sneezing. His symptoms worsened when he worked with flour and improved on holidays. Clinical examination and biochemistry were normal. White blood cell count was 5500/mm³ with 330 eosinophils/mm³. Total serum IgE was 80 IU/mL and spirometry was normal.

Case 3

Patient 3 is a 46-year-old male baker with intolerance to nonsteroidal anti-inflammatory drugs for 22 years. During the last 3 years, he has had ocular and nasal itching, sneezing, rhinorrhea, wheezing, and dyspnea on exposure to flour dust at his work. He remained asymptomatic during holiday periods. Clinical examination, biochemical studies, and total blood count were all normal. Sinus radiographs disclosed opacity of both maxillary sinuses. Chest radiograph was normal. Spirometric values were 71% for FEV₁ and 81%

for FVC. Total serum IgE was 101 IU/mL.

Case 4

Patient 4 is a 25-year-old male baker. Four years ago, 2 months after starting his job, he had nasal congestion and rhinorrhea, ocular and nasal itching, tight chest, wheezing, and dyspnea after exposure to flour dust. Clinical examination revealed a pale nasal mucosa and expiratory wheezes upon hyper-ventilating. White blood cell count was 5,100 with an absolute eosinophil count of 969. Biochemistry and spirometric values were normal. Total serum IgE was 251 IU/mL.

MATERIAL AND METHODS

Preparation of Wheat Flour and Alpha Amylase Dust Extract

Four grams of alpha amylase (Sigma Chemical Co., St. Louis, Mo) were extracted in 40 mL of 0.01 mol/L of phosphate and 0.15 mol/L of NaCl, pH 7.4 (PBS), and the mixture (1:10 wt/vol) was stirred for 12 hours at 4 °C. The suspension was filtered through a filter paper (Whatman Ltd., Maidstone, England), and sterilized by 0.22 µm filtration (Millipore, Molsheim, France), having a final concentration of 18 mg/mL in protein as determined by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif). The solution was aliquoted and stored at -20 °C until used. The same procedure was used to prepare extracts with standard

wheat flour (Aristegui Laboratory, Madrid, Spain), and with patients' wheat flour.

Skin Tests

All skin tests were performed by the prick technique beginning with a dilution of 10^{-6} wt/vol of the alpha amylase extract and were progressively increased in tenfold steps until a positive reaction was obtained. A standard battery of common inhalants and food allergens (Abello Laboratory, Madrid, Spain) were tested by epicutaneous routes.

Five patients with hay fever and five normal individuals were tested with the alpha amylase and wheat extracts as controls. Histamine phosphate (10 mg/mL) was used as a positive control and 50% glycerol was used as a negative control. All skin test sites were read after 15 minutes and four to eight hours later. A positive reaction was defined as a wheal at least 3 by 3 mm in the presence of a negative reaction to the 50% glycerol and a positive test with histamine phosphate.

Precipitin Test

The patients' sera were tested for precipitating antibodies to undiluted alpha amylase and wheat flour extracts by Ouchterlony diffusion gels.¹²

Determination of Alpha Amylase and Wheat-Specific IgE

Circulating IgE-specific antibodies to alpha amylase and wheat flour in serum were determined by a commercially available immunoassay (Phadezym, Pharmacia, Uppsala, Sweden), following the instructions given by the supplier. Briefly, 50 μ L of undiluted sera were incubated (four hours at room temperature) with allergen-coated paper discs (Pharmacia). After a washing step, β -galactosidase-labeled monoclonal antibodies with specificity for human IgE were added (50 μ L) and incubated 20 hours at room temperature. After a final washing, 250 μ L of a substrate solution were added and the enzymatic reaction allowed to develop for two hours at 37 °C,

stopped and read at 405 nm. The results were expressed in Phadebas Rast Units (PRU) per milliliter of serum, by extrapolating optical density values from a curve established with specific standards (Phadexct Reference IgE, Pharmacia). Inhibition assays were performed as described,¹³ using wheat flour as inhibitor when detecting alpha amylase-specific IgE.

Bronchial Challenge Tests

Bronchial challenges with methacholine, standard wheat flour, and alpha amylase extracts were performed by the method of Cockcroft et al,¹⁴ and Juniper et al¹⁵ with some modifications as described previously.^{16,17} These tests were performed during an asymptomatic period, after seven days without exposure to wheat flour dust. The following modifications were used: the aerosol was generated in all instances by the same DeVilbiss 646 nebulizer (DeVilbiss Co, Somerset, Pa) with an output of 0.28 mL/min, delivered into a mask held loosely over the subject's mouth and with a noseclip over the nose. The patient inhaled the solution at tidal breathing for two minutes, starting with phosphate buffered saline (PBS) solution as a control followed by an increasing concentration of methacholine: 0.04, 0.09, 0.19, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, and 25 mg/mL, at 6-minute intervals. Measurements of FEV₁ and FVC were made using a precalibrated Vitalograph-PFT spirometer (Buckingham, UK) before the test, at 30 seconds, and every four minutes after each inhalation. The test was terminated when the FEV₁ had fallen by 20% or more from the post-PBS level. The result was expressed as the provocative concentration of methacholine required for the induction of a 20% fall in the FEV₁ (PC₂₀), which was interpolated from the dose-response curve.

Bronchial challenge testing with alpha amylase and standard wheat flour extracts were performed as for the methacholine inhalation tests

but in a double-blind protocol. A control test day, with exposure to a PBS inhaled for two minutes, was carried out to ensure that the patient's FEV₁ was stable over at least eight hours and that any possible physiologic changes observed on challenge days were not attributable to normal circadian changes in pulmonary function or changes in effort.¹⁸ Different dilutions of these extracts were inhaled by the patient. Phosphate buffered saline was used as placebo. The highest allergen extract concentrations prepared for the patient's inhalation tests was 1:100 wt/vol of alpha amylase and 1:10 wt/vol of wheat flour, so that there would not be a significant difference in color, odor, or taste between these dilutions and the PBS. A member of the staff randomized all challenges so that the investigators and patients were unaware of the content of any challenge. FEV₁ was measured before and at intervals of five minutes after each inhalation for the first 30 minutes, at one hour, and later on, hourly, for the next eight hours. Inhalation was stopped when there was a fall in FEV₁ of 20% or more from PBS inhalation control. The following inhalation tests were conducted on different days: alpha amylase or wheat flour extract from 1:1,000,000 to 1:100 or 1:10 wt/vol, respectively, in tenfold steps. Two unexposed subjects with asthma were also challenged with a single blind, 1:100 wt/vol dilution of alpha amylase extract after having obtained informed consent.

Nasal Challenge

Nasal challenges (NC) were performed by a method previously suggested^{19,20} but with some modifications. The patient's baseline nasal function was assessed in the laboratory for 20 minutes before depositing any material in the nose. Nasal patency was measured as nasal inspiratory peak flow using a mini-Wright peak flow meter connected to an anaesthetic mask. The patients were first trained to use the

equipment and when reproducible values were achieved, three consecutive measurements were performed and the highest value used to express patency. After nasal challenge with 0.1 mL PBS in each nostril, dilutions of α -amylase or wheat flour extract (from 1:100,000 wt/vol to highest concentration in tenfold steps) was sprayed in each nostril using a deVilbiss spray No. 15. Before challenge, the patient inspired maximally and held his breath to avoid inspiring allergen into the lung. The allergens were applied at 10-minute intervals and the procedure was continued to maximum strength or until a convincingly positive reaction was achieved. All tests were performed on separate days. The conditions for a positive reaction were achieved in at least two of the three parameters: ≥ 5 sneezes, ≥ 0.5 mL of secretion (collected in a funnel connected to a syringe) and $\geq 20\%$ decrease in peak flow from PBS control challenge.

Two nonexposed patients with allergic rhinitis were also challenged in a single blind manner with a 1:100 wt/vol dilution of alpha amylase extract.

RESULTS

Skin Tests

The patients exhibited a strong, immediate, positive reaction after skin prick testing with alpha amylase extract. All of them also showed a positive reaction to our wheat flour

extract (Table 1). The four patients also presented a strong reaction with their own wheat flour that contained alpha amylase as an additive (not shown). Patient 1 also had a positive skin reaction to cat dander, *L. destructor*, and grass and olive pollens. Patient 2 had a positive skin test to grass, olive, and Artemisia pollens. Patient 3 showed a positive response to mites. Patient 4 had a positive prick test to mites, danders, and grass pollens. None of the four patients experienced a late skin reaction.

The control subjects did not have reactions to alpha amylase or to wheat flour extracts. This proves the nonexistence of any nonspecific reaction in spite of the concentration used. Neither of the control subjects had positive skin reactions to the remaining commercial common allergen battery, except for the atopic control subjects who had reactions to several pollens and/or mites.

Precipitin Tests

Antibodies to alpha amylase and wheat flour extracts were not observed in our patients' sera as detected by double-diffusion gels.

Specific IgE Measurement

To confirm in these patients the presence of IgE antibodies to alpha amylase, we looked for specific IgE antibodies in vitro. As shown in Table 2, there was IgE activity to alpha amylase and also to wheat flour. IgE activity to these antigens was not detectable in serum samples

Table 2. IgE Reactivity with Alpha Amylase and Wheat Flour as Detected by EIA (Phadezym, Pharmacia)*

Patient	Alpha Amylase	Wheat Flour
1	1.98	3.18
2	>17.5	1.57
3	0.76	0.81
4	0.93	2.43
(Control†) 1	<0.35	
(Control†) 2	<0.35	

* Results are expressed in Phadebas RAST units per milliliter.

† Sera from two unexposed subjects with IgE serum levels of 86 and 371 IU/mL.

from two unexposed subjects with hay fever and serum IgE of 86 and 371 IU/mL (Table 2). IgE reactivity with alpha amylase could not be inhibited with high concentrations of (over 1 mg/mL) our wheat flour extract (not shown), indicating that it was essentially free of alpha amylase.

Bronchial Challenge Tests

Methacholine challenge showed slight bronchial hyperresponsiveness in patient 3 (methacholine PC₂₀ = 2.7 mg/mL). On the control day of exposure to PBS, there was no significant change in spirometry in patients 3 and 4 throughout the day. In contrast, both patients had immediate asthmatic responses after challenge with the 1:10,000 and 1:100,000 wt/vol dilutions of the alpha amylase extract, respectively.

In patient 3, as depicted in Figure 1, at ten minutes there was a 23% fall in FEV₁ that returned to the baseline approximately one hour later. No late reaction was observed. In contrast, no bronchial response was observed in this patient after the inhalation of 1:10 wt/vol dilution of wheat flour extract (Fig 1).

In patient 4, as shown in Figure 2, there was an immediate positive response also to the undiluted (1:10 wt/vol) wheat flour extract. The two unexposed asthmatic patients showed methacholine PC₂₀ of 0.9 and 1.3 mg/mL. In contrast, they did not react to the 1:100 wt/vol α -amylase extract.

Table 1. Skin Prick Test Results

Patient	Alpha-Amylase Extract		Wheat Flour Extract	
	Wheal* 1:10 wt/vol	End Point† Titration wt/vol	Wheal* 1:10 wt/vol	End Point† Titration wt/vol
1	13 × 12	1:10,000	3 × 2	1:10
2	17 × 11	1:100,000	2 × 1	1:10
3	22 × 15	1:1,000,000	3 × 3	1:100
4	18 × 12	1:100,000	3 × 3	1:10
Controls‡	<2	1:100	<2	1:10

* Wheal largest diameters in mm.

† Extract dilution enough to elicit a small wheal of 2 mm.

‡ Five patients with hay fever and five normal individuals.

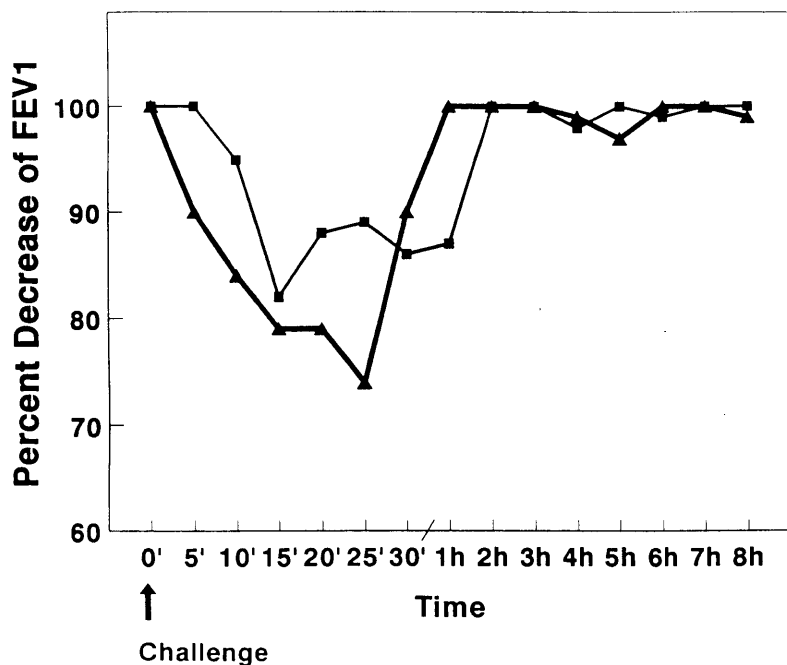


Figure 1. Bronchial provocation test with 1:10,000 wt/vol alpha amylase (▲) and with 1:10 wt/vol wheat flour (■) extracts in patient 3.

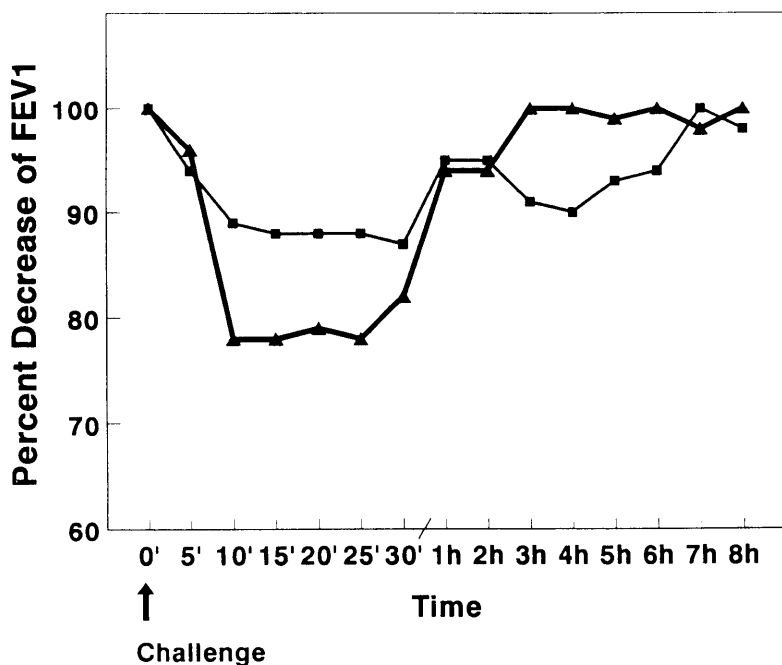


Figure 2. Bronchial provocation test with 1:100,000 wt/vol alpha amylase (▲) and with 1:10 wt/vol wheat flour (■) extracts in patient 4.

Nasal Challenge Tests

A marked decrease in PIFRN was seen in both patients 1 and 2 after challenge with alpha amylase ex-

tract (Fig 3). In addition, they had nasal pruritus and a measurable nasal secretion (Fig 3). This nasal response could not be induced in two

hay fever patients used as controls with the 1:100 wt/vol alpha amylase extract, indicating that our α -amylase extract lacked irritating effect on the nose.

Immediate onset of rhinitis response was not observed in patients 1 and 2, when they were nasal challenged with our wheat flour extract (Fig 3).

DISCUSSION

Alpha amylase used in baking is a glycolytic enzyme obtained mainly from *A. oryzae*. It has a molecular weight of 52,000 daltons and catalyzes carbohydrate hydrolysis. It is added to the flour in order to improve the bread's quality. In other words, it compensates for the low natural content of alpha amylase in flour, increases the quantity of carbohydrates fermentable by yeast, and stimulates the growth of *Saccharomyces cerevisiae*, which improves the ability of dough to rise.

Among the first descriptions of bakers' alpha amylase allergy is that of Baur,²¹ who studied bakers exposed to alpha amylase-contaminated flour. In some of these symptomatic bakers, he found specific IgE by prick and RAST tests and positive reactions to the bronchial challenges to the enzyme. He concluded that 2% of the exposed population and 34% of the asthmatic bakers were sensitized to this allergen. Subsequently, other authors²²⁻²⁵ have reported their investigations on this disease, and it seems that its importance is increasing due to the extensive use of this enzyme in the bread and food industries in some countries.

Our patients experienced rhinoconjunctivitis and asthma within a few months or years after starting work as bakers. Furthermore, the onset of nasal and respiratory symptoms within minutes of exposure to alpha amylase-treated flour dust and the noticeable improvement while away from work during holidays point to the symptom's occupational nature. It may be argued, however, that in view of their posi-

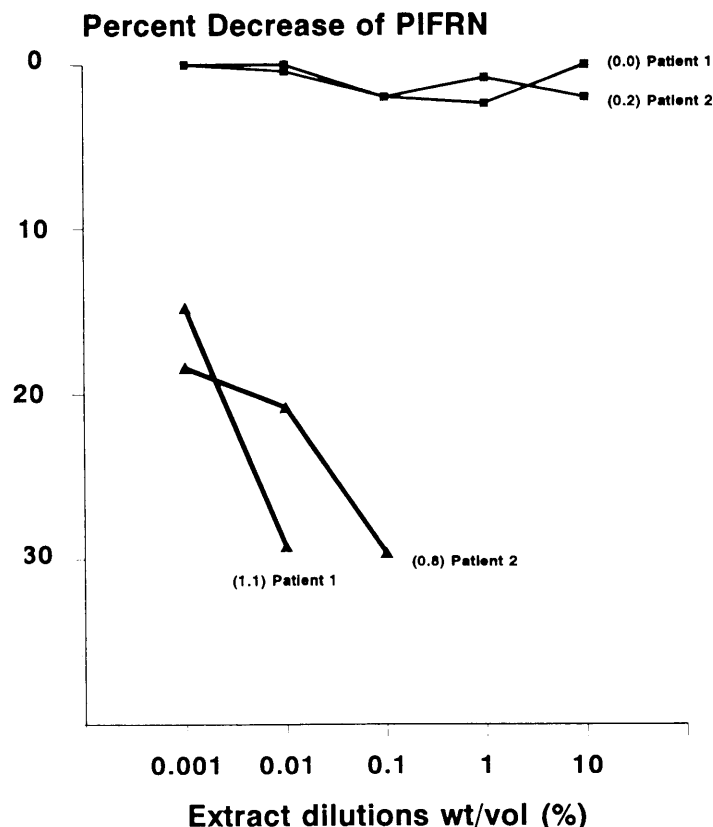


Figure 3. Nasal provocation test with 1:100 wt/vol (patient 1) and 1:100,000 wt/vol (patient 2) alpha amylase (▲) and with 1:10 wt/vol wheat flour (■) extracts in both patients. February 9, 1993

tive methacholine challenge, the subjects had pre-existing asthma and the dusty atmosphere at work simply aggravated their underlying condition. Bronchial asthma is known to be associated with airway hyperreactivity to various nonspecific stimuli and to drugs such as methacholine.²⁶⁻²⁸ The negative bronchial response, however, to PBS in the patients and the negative bronchial response to the highest concentration (1:100 wt/vol) of alpha amylase extract in two unexposed asthmatic patients with similar nonspecific bronchial hypersensitivity denote a true specific immediate bronchospastic reaction to the alpha amylase extract. These conclusions are supported by the presence of an immediate response in the prick tests and by the presence of specific IgE to the alpha amylase

extract. The negative results obtained in a group of unexposed normal and atopic subjects support the specificity of these findings.

On the other hand, the antigen(s) must be a macromolecule since it does not cross a dialysis membrane that should retain compounds with molecular weight larger than 6,000 to 8,000 daltons.

One of the patients, patient 4, also had positive prick, RAST, and bronchial challenge tests with wheat flour extract. Because of this and the positive prick and RAST tests to wheat flour obtained in the other patients, one might think that we used alpha amylase-contaminated flour. We could not, however, demonstrate alpha amylase in our wheat flour by inhibition assays, indicating the allergic independence of these substances. We therefore consider

that these findings are a manifestation of the polysensitization that could take place in bakers when they are exposed to additive treated flour.

In conclusion, the results of the investigation carried out support alpha amylase as an important antigen to be considered in bakers' induced asthma. It can function as the only cause or in conjunction with other allergens. An IgE-mediated immunologic mechanism appears to be operational in the underlying pathogenesis.

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HANTAVIRUS PULMONARY SYNDROME

The authors describe 17 patients with confirmed infection with a newly recognized strain of hantavirus. Ages ranged from 13 to 64 years; 72% were native American.

Prodromal symptoms included fever and myalgia in all; cough or dyspnea in 76%; nausea, vomiting, abdominal pain or diarrhea in 76%; and headache in 71%. The most common physical findings were tachypnea in 100%, tachycardia in 94%, and hypotension in 50%. There were leukocytosis, increased hematocrit, thrombocytopenia, prolonged prothrombin and partial thromboplastin times, elevated serum lactate dehydrogenase, decreased serum protein, and proteinuria. There was rapidly progressive acute pulmonary edema in 15 of the 17 patients. Thirteen patients died. All of those who died had had profound hypotension.

Infection with this newly described hantavirus causes the hantavirus pulmonary syndrome with a prodromal illness of 2 to 15 days (mean 5.4 days) followed by rapidly progressive, noncardiogenic pulmonary edema.

—RMS

Duchin JS, Koster FT, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. *N Engl J Med* 1994;330:949-55.