

Short communication

Clinical responses to ingested fungal α -amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*?

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α -Amylase and hemicellulase, derived from culture of *Aspergillus* species, are commonly added to flour as improvers during baking. Two cases of women occupationally sensitized to α -amylase who developed allergic symptoms after eating baked bread have been reported. With a randomized, controlled study design, we have investigated whether similar responses occur in those sensitized to *Aspergillus* species. Seventeen subjects with positive skin prick tests to *Aspergillus fumigatus* were studied. Symptomatic and physiologic responses after ingestion of bread baked with α -amylase and hemicellulase were compared, in a crossover fashion, with those after ingestion of bread baked without enzymes. No increase in respiratory or other symptoms, lung function, or nonspecific bronchial hyperreactivity was reported after ingestion of the enzyme-containing bread. We conclude that important clinical reactions to α -amylase and hemicellulase in baked bread do not frequently occur in those sensitized to *Aspergillus* species.

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α -Amylase and hemicellulase, produced by large-scale culture of *Aspergillus oryzae* or *A. niger*, are widely used as improvers in the baking industry. Those working in this industry may acquire symptoms of respiratory and, it has been suggested, skin hypersensitivity in association with specific IgE to the enzyme (1, 2). Two single case reports of young women, occupationally sensitized to α -amylase, who experienced allergic symptoms after eating bread prepared with the enzyme, have been published (3, 4). A further report, published only in abstract form, described a series of 58 persons with suspected food allergy and raised levels of specific α -amylase IgE, in 47 of whom clinical symptoms improved during an enzyme-free diet (5). It is unclear to what extent these observations were controlled.

The prevalence of sensitization to α -amylase or hemicellulase in the general population is unknown but is believed to be rare; probably a larger number are sensitized to *Aspergillus* species, particularly *A. fumigatus*. Some cross-reactivity between *Aspergillus*

species and *Aspergillus*-derived enzymes has been observed *in vitro* (T. Sorenson, Novo Nordisk, personal communication), and the development, in persons with fungal sensitization, of allergic reactions to ingested bread baked with enzymes of fungal origin cannot be excluded. We have carried out a controlled trial to investigate this issue.

Material and methods

Subject selection

Subjects with immunologic evidence of sensitization to *Aspergillus* species were selected from a hospital database and invited to participate in the study; each had, on at least two occasions, a positive (3-mm wheal) response to skin prick testing with a 5 mg/ml solution of *A. fumigatus* (Bencard). Duplicate measurements of serum specific IgE antibodies to crude extracts of *A. fumigatus* and to α -amylase were made using a standard CAP-RAST

assay (Pharmacia, Upjohn) with a lower limit of detection of 0.35 kU/l. Skin prick testing with the following solutions was also carried out: negative (saline) and positive (histamine) controls, mixed grass pollens, cat fur, house-dust mite (Bencard), *A. niger* (5 mg/ml Laboratorium Diephuis), *A. oryzae* (5 mg/ml Laboratorium Diephuis), α -amylase (100 μ g protein/ml, Novo Nordisk), hemicellulase (100 μ g protein/ml, Danisco Ingredients Ltd), and a mixed wheat extract (5000 pnu/ml, Biodiagnostics).

Study protocol

Subjects were admitted to hospital having abstained from all cereal-containing foods for 48 h; those taking antihistamine or inhaled anti-inflammatory medication were asked to forego this for 5 days before hospitalization. On admission, they completed a baseline questionnaire enquiring into previous and current respiratory and other symptoms including those consistent with allergic reactions to bread and other foods. Baseline measurements of pulse rate, blood pressure, temperature, FEV₁ (bellows spirometer, Vitalograph), and histamine PC₂₀ (Cockcroft method) were made.

Two types of bread, identical in appearance and taste and baked under standard conditions, were used for the study – one (“active”) prepared with enzymes according to the producers’ standard recommendations (3 ppm, or 3 mg/kg flour of α -amylase resulting in approximately 0.075 μ g/slice and 50 ppm, or 1.25 mg/slice of hemicellulase) and another without (“control”). Each was frozen before use in the study. Subjects ate two slices (50 gm) of each bread type, in random order, on consecutive days. Neither they nor the investigators were aware of the nature of the bread consumed. Responses were measured by a symptoms questionnaire, self-completed at 2-h intervals, which enquired into upper and lower respiratory, skin, and abdominal symptoms, and headache. Sixteen symptom types were scored on a scale of zero (no symptoms) to four (severe symptoms) and categorized as lower respiratory, nasal, ocular, oral, abdominal, or headache. The best of three measurements of FEV₁ (six times in the first hour and hourly thereafter); measurements of pulse rate, temperature, and blood pressure every 2 h; and, 24 h after ingestion of the bread, measurement of histamine PC₂₀ were recorded. During the study, subjects did not eat any other cereal-containing products and took no anti-inflammatory medication.

The study was approved by the ethical committee of Royal Brompton Hospital and was conducted according to standard guidelines of good clinical practice.

Analysis

Reactions to the active and control challenges were compared by methods suitable for the analysis of serial measurements (6). Physiologic measurements at each time point were averaged and the mean values used as a summary measure of outcome. These means were regressed individually against variables denoting pre-exposure measurements, the individual subject, and the bread type. Average symptom scores before and after each exposure were compared and the differences analysed by McNemar’s test for paired data with the calculation of exact *P* values. Bronchial reactivity before and after ingestion was compared by paired *t*-test. All statistical analyses, by SAS software and two-tailed levels of significance, were conducted without knowledge of the exposure type.

Subject characteristics

Seventeen subjects were studied. Their median (range) age was 42 (25–66) years, and 10 were men. Each had a positive (≥ 3 mm) skin prick test response to *A. fumigatus* (Table 1), and circulating, specific IgE (0.35 kU/l) was detected in the serum of 10 (59%). Four had positive skin reactions to α -amylase – each of these also had positive tests to the other *Aspergillus* species. Six had positive reactions to hemicellulase; five of these also reacted to *Aspergillus*. Thirteen were considered to be atopic, having a positive skin test (≥ 3 mm) to at least one common aeroallergen. All but one (subject 15) had a current diagnosis of asthma, in each case starting in childhood; baseline histamine PC₂₀ values ranged from 0.18 to more than 16 mg/ml, and were below 0.50 mg/ml in seven subjects. Three had an additional clinical diagnosis of allergic bronchopulmonary aspergillosis. None had been employed in the baking or detergent industries or in enzyme manufacture. All regularly ate bread. Thirteen reported that they had an “allergy”; six to various foods – but not bread.

Another subject, a baker with occupationally-induced sensitization (and asthma) to α -amylase, was studied in an identical manner. Skin prick testing in him revealed wheal diameters of 10 and 3 mm to α -amylase and *A. fumigatus*, respectively.

Results

In 10 subjects, the active challenge was administered on the first day of the study. Results of the regression analyses, summarized in Table 2, do not demonstrate consistent or statistically significant exposure effects for any of the physiologic variables.

Table 1. Characteristics of studied subjects

Subject no.	Age (years)	Sex	FEV ₁ (l)	Atopy*	Mean wheal diameter after skin testing (mm)						CAP (kU/l)	
					<i>A. fumigatus</i>	<i>A. oryzae</i>	<i>A. niger</i>	α -Amylase	Hemicellulase	Wheat	<i>A. fumigatus</i>	α -Amylase
1	25	F	1.70	+	3	0	0	0	0	0	15.6	0.0
2	66	M	1.18	+	8	3	1	0	0	2	2.4	0.0
3	60	F	0.60	-	6	4	5	4	5	0	36.6	1.7
4	54	F	2.07	+	3	0	0	0	3	2	0.3	0.0
5	30	M	4.45	+	3	0	0	0	0	0	0.0	0.0
6	48	M	3.90	+	4	0	0	0	0	0	2.0	0.0
7	48	M	0.56	+	4	3	3	3	4	3	8.1	0.0
8	39	M	2.84	+	5	6	5	6	7	0	87.0	6.3
9	42	F	2.80	+	4	3	3	3	4	3	0.0	0.0
10	28	M	3.05	+	3	0	3	0	0	4	0.0	0.0
11	42	F	3.00	+	3	3	3	0	0	0	0.0	0.0
12	45	M	1.12	+	5	0	0	0	0	0	9.5	0.0
13	25	M	3.87	+	7	0	0	0	0	0	0.4	0.0
14	35	M	5.15	+	3	0	0	0	0	0	0.0	0.0
15	56	F	1.10	+	4	6	4	0	3	4	14.3	0.0
16	29	F	2.95	+	3	0	0	0	0	2	0.0	0.0
17	55	M	2.50	-	3	5	4	0	0	0	2.9	0.0

* Defined by positive (≥ 3 mm) skin prick test to at least one common inhalant allergen.

Table 2. Results of statistical analyses; regression (β) coefficients indicate mean changes (per unit of measurement) in physiologic outcome variables resulting from α -amylase exposure

Physiologic variables	β (regression coefficient)	<i>P</i> value
FEV ₁ – early*	0.016	0.55
FEV ₁ – late**	–0.002	0.94
Pulse rate	–0.423	0.76
Temperature	0.045	0.52
Blood pressure (systolic)	1.437	0.29
Blood pressure (diastolic)	–1.393	0.26
Symptoms	Subjects with increased symptoms (<i>n</i>)	
	After active exposure	After control exposure
Any	4	4
Lower respiratory	5	1
Nasal	1	3
Ocular	2	1
Oral	4	1
Abdominal	1	0
Headache	2	0

* Early: first hour after exposure.

** Late: subsequent measurements.

The mean (range) difference in histamine PC₂₀ before and after active exposure was -0.095 mg/ml (range: -11.8 to +11.7); after control exposure, it was -0.151 mg/ml (-11.7 to +13.2). These were not significantly different ($P=0.969$), nor was there any consistency in changes between the two exposure types. Only a minority of subjects reported changes in symptoms after either exposure; there were no significant differences in any symptom groups between the two exposure types. Five subjects reported increased chest symptoms after active exposure (compared to one after control expo-

sure), but in none of these was there a systematic decrease in FEV₁ or fall in histamine PC₂₀.

There were no consistent reactions to active challenge in the subjects with positive skin tests to α -amylase or hemicellulase. No clinical or functional reactions were recorded to either challenge by the α -amylase-sensitized baker.

Discussion

Clinical reactions to bread baked with α -amylase have been demonstrated, under controlled conditions,

in two patients with occupationally acquired specific IgE to the enzyme (3, 4); it is not known how frequent such reactions are in the general population, nor, since the community prevalence of sensitivity to α -amylase has not been estimated, is it known how large the potential problem is. The prevalence of sensitivity to *Aspergillus* species in the general population is low; among a cohort of 714 New Zealand children prick tested at the age of 13 years, the prevalence of positive tests (mean wheal diameter ≥ 2 mm compared to that of a negative control) was 2.4% (7) or, among atopic children ($n=320$), 5.3%. In a community study of 450 children and young adults in the UK, we have measured an overall frequency of positive tests of 2%; 40% of this population was atopic (unpublished data). In occupationally exposed populations, this figure may be higher. It is probable that *Aspergillus* sensitivity is more common than that to α -amylase, and, given the ubiquity of bread in Western diets, the issue of whether clinical reactions to the enzyme occur in those with *Aspergillus* sensitivity is an important one.

Baur et al. have demonstrated that baking does not fully inactivate the IgE-binding capacity of α -amylase from *A. oryzae* (8). The same group has also shown that this allergen (Asp o 2, a 55-kDa glycosylated protein) does not show homology with the immunodominant major allergen identified in *A. fumigatus*, Asp f 1, an 18-kDa, nonglycosylated cytotoxin (9). However, cross-reactivity between different species of *Aspergillus* has been demonstrated (10), though not, as far as we are aware, between *A. fumigatus* and *A. oryzae*; therefore, clinical reactions arising from bread baked with α -amylase and hemicellulase among *Aspergillus*-sensitized individuals would arise from cross-reactions between other, minor allergens. In our group, selected from a nonoccupational population on the basis of IgE antibodies to *A. fumigatus*, all those with a positive skin test to α -amylase also recorded positive tests to all the *Aspergillus* species – and those with measurable serum IgE to the enzyme were those with the strongest serum responses to *A. fumigatus* – as consistent with some cross-reactivity. Of the two subjects with positive reactions to hemicellulase, but not α -amylase, one had positive tests to all *Aspergillus* species.

Using a carefully blinded study design, in subjects who had respiratory disease in association with specific IgE to *A. fumigatus*, we did not find clear evidence of clinical reactions after active exposure. Any differences in symptoms after expo-

sure to either bread type were very small, and not statistically significant. In none of those reporting more chest symptoms after active exposure was there any change in pulmonary function, bronchial reactivity, or any other physiologic variables measured. We cannot, however, exclude the possibility of type II errors – that is, the failure to detect a statistically significant exposure effect due to the relatively small size of the trial; a formal analysis suggests that this study had, for example, 80% power to detect a 50% fall in FEV₁. The small differences in outcomes and the large size of the remaining *P* values, however, suggest that the probability of such errors is low.

Our findings, from a small but carefully controlled trial, suggest that important reactions to *Aspergillus*-derived α -amylase and hemicellulase used in baking are unlikely to occur in those with specific IgE antibodies to *A. fumigatus*.

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