Exposure-Sensitization Relationship for α-Amylase Allergens in the Baking Industry

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Fungal α-amylase is an important occupational allergen in the bakery industry. Epidemiologic studies focusing on the relationship between α-amylase allergen exposure and work-related respiratory allergy, however, have not been reported yet. In this cross-sectional study, sensitization to occupational allergens and work-related symptoms were studied in 178 bakery workers and related to allergen exposure. Alpha-amylase allergen concentrations were measured in personal dust samples, using a sandwich enzyme immunoassay. All workers were categorized into groups on the basis of their job histories and the α-amylase exposure levels of their job titles. Of all workers 25% had one or more work-related symptoms. As much as 9% of the bakery workers showed a positive skin prick test reaction to fungal amylase, and in 8% amylase-specific IgE was demonstrated. Alpha-amylase exposure and atopy appeared to be the most important determinants of skin sensitization, with prevalence ratios for atopy of 20.8 (95% CI, 2.74 to 158) and for medium and high α-amylase exposure groups of 8.6 (95% CI, 1.01 to 74) and 15.9 (95% CI, 1.95 to 129), respectively. Furthermore, a positive association was found between positive skin prick tests to α-amylase and work-related respiratory symptoms. In conclusion, this study has shown that there is a strong and positive relationship between α-amylase allergen exposure levels in bakeries and specific sensitization in bakery workers. Houba R, Heederik DJJ, Doekes G, van Run PEM. Exposure-sensitization relationship for α-amylase allergens in the baking industry.

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Work-related respiratory allergy is highly prevalent among bakery workers, occurring in approximately 10 to 20% of all bakers (1–4). In several countries, baker's asthma is one of the most frequent occupational lung diseases (5–8). The majority of patients with baker's asthma is sensitized to wheat and rye flour, but the last 10 to 15 yr have shown an increased interest in the role of baking additives, especially α-amylase. Fungal α-amylase (1,4-α-D-glucan glucanohydrolase), usually derived from *Aspergillus oryzae*, is a glycoprotein that catalyzes the hydrolysis of internal α(1,4)-glycosidic linkages in various polysaccharides. It is routinely added to baking flour (in milligrams per kilogram of flour) to hasten the baking process and improve bread quality.

The first cases of α-amylase allergy have been described in the enzyme-processing industry in the 1970s (9). Since then, several other studies have reported respiratory allergies in bakery workers caused by α-amylase, often in the absence of demonstrable reactivity to wheat allergens (7, 10–15). Baur and coworkers (5) showed in a study of 140 bakers who visited a clinic with work-related symptoms that 24% were sensitized to α-amylase (5).

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Although the number of studies on α-amylase allergy in patients with baker's asthma is growing, epidemiologic studies in bakery workers focusing on relationship between α-amylase exposure and type I sensitization or work-related respiratory symptoms have not been reported yet. Some recent cross-sectional studies showed that in a population of bakery workers 5 to 7.5% were sensitized to α-amylase, as shown by skin prick testing (16, 17). In neither of these studies, however, was information available on airborne α-amylase exposure levels.

Exposure to α-amylase in airborne dust has been measured by assessing enzyme activity of the dust in a protein-processing factory (18), and bakeries (19, 20). For two reasons, however, measurements of enzyme activity may have a limited validity in quantifying allergen exposure. First, inactive or denaturated enzymes are not measured, but they possibly still act as allergens. Second, these methods measure the amylase activity of both fungal and cereal origin, and are therefore not specific. For these reasons, measurement methods of fungal α-amylase based on immunochemical techniques are to be preferred. Although allergens of proteolytic enzymes have been measured before in personal air samples (21, 22), immunochemical measurements of airborne α-amylase in bakeries have only recently been presented (23).

To our knowledge no study has been published before in which α-amylase allergen exposure data were applied in an epidemiologic survey. In this report results are presented of the relationship of α-amylase allergen exposure in bakeries with sensitization to fungal amylase and respiratory symptoms. The results of this cross-sectional study are part of a large prospective study among a few hundred bakery workers in the Netherlands. Primary aim of the whole project is to study exposure-incidence

relationship between allergen exposure and work-related asthma. In a previous report (24), an enzyme immunoassay (EIA) for measuring wheat antigens for the same population was presented.

METHODS

Population

The health survey was carried out between October 1992 and July 1993 and comprised 203 production workers in 14 Dutch bakeries. Maintenance workers were excluded from the analysis because of potential other exposures (e.g., welding fumes), leaving 178 bakery workers in the study. All workers completed a short self-administered Dutch version of an internationally accepted respiratory questionnaire (25), supplemented with questions on work-related symptoms. Symptoms were considered to be work-related if they were reported by the subject as being provoked by contact with flour or process-related products (e.g., baking additives) during work ("Do you have any of the following allergic symptoms during work after contact with certain agents at work?"). Work-related rhinitis was defined as the presence of sneezing or running nose (production of nasal secretions) during work. Work-related conjunctivitis was defined as the presence of itchy or teary eyes. Symptoms reported to be caused by irritants (smoke, potash, etc.) were not included. Additionally, smoking habits and job histories were obtained. Forced expiratory lung function measurements were conducted as described previously (26). Population characteristics are given in Table 1. Venous blood samples were taken from 169 workers for analysis of IgE antibodies, and 169 workers underwent skin prick tests (SPT), as described below. For 167 workers both serum samples and SPT results were available. Informed written consent was obtained from all subjects before the start of the study according to Dutch legal requirements.

Skin Prick Tests

Conventional SPTs were applied using a lancet with a 1-mm point, which was pressed at a 90-degree angle to the skin surface through a drop of allergen solution. All SPTs were performed by two trained technicians. The following occupational allergens were tested: wheat flour (ALK Benelux; the Netherlands) (ALK, 52.06), rye flour (ALK, 52.05), fungal α-amylase from *Aspergillus oryzae* (ALK, 52.52), bakers yeast (*Saccharomyces cerevisiae*) (ALK, 52.01), and storage mites (ALK, 35.00; 1,000 NE/ml). All extracts with occupational allergens had a concentration of 5 mg/ml, except where noted. Subjects were classified as atopic if they had at least one positive response to one of five tested common allergens: house dust mite (ALK, SQ 510), grass pollen (ALK, SQ 293), tree pollen (ALK, SQ 197), cat fur (ALK, SQ 555), and dog fur (ALK, SQ 553). Positive and negative controls consisted of phosphate-buffered saline (PBS) with or without histamine (10 mg/ml), respectively. All tests were read after 15 min and were considered positive if the mean wheal diameter was at least 3 mm greater than the negative control.

Specific IgE Determination

Sera were stored at −20° C until IgE analysis. Specific IgE determination to wheat flour was performed by a commercial immunoassay (AlaSTAT; DPC, Apeldoorn, the Netherlands) (27). Sera of Class 2 or higher (> 0.7 kU/L) were considered positive. Specific IgE to α-amylase was assessed with a modification of a previously described EIA (28). Microwells were coated overnight at 4° C with a semipurified preparation of α-amylase from *Aspergillus oryzae* (Fungamyl 1600S®; NOVO Nordisk, Bagsvaerd, Denmark; kindly provided by the Department of Occupational Medicine, Sahlgren Hospital, Göteborg, Sweden) at a concentration of 12.5 μg/ml of protein. Serum samples were incubated at a 1/5 dilution in PBS-Tween containing 0.2% gelatin (PBTG), and bound

TABLE 1

CHARACTERISTICS OF THE GROUP OF 178 BAKERY WORKERS

	Mean	SD	Range	Number	Percent
Age, yr	34.0	10.0	20–61		
Years in bakery industry	10.2	8.7	0.2–43		
Years smoked	11.2	10.6	0–42		
FVC, L	5.31	0.97	3.09–7.36		
FEV_1, L/s	4.25	0.86	2.17–6.37		
Tiffenau-index, %	80.1	7.8	46.8–95.4		
PEF, L/s	10.5	2.5	4.8–18.0		
Smokers				97	54
Ex-smokers				35	20
Nonsmokers				46	26
Male, %				157	88
Respiratory symptoms, n = 178					
Chronic cough				18	10
Chronic phlegm				13	7
Shortness of breath				7	4
Ever wheezing				38	21
Frequent wheezing				7	4
Chest tightness				18	10
Work-related symptoms, n = 178					
Rhinitis				26	15
Conjunctivitis				10	6
Chest tightness				9	5
Skin symptoms				19	11
Positive SPT reactions, n = 169					
House dust mite				41	24
Grass pollen				35	21
Tree pollen				15	9
Cat fur				28	17
Dog fur				28	17
Wheat flour				14	8
Rye flour				8	5
Fungal α-amylase				16	9
Bakers yeast				2	1
Storage mites				19	11
Positive IgE-response, n = 169					
Wheat flour				9	5
Fungal α-amylase				13	8

IgE was measured by subsequent incubations of 1 h at 37° C with monoclonal mouse antihuman IgE (1/16,000; Central Laboratory of the Blood Transfusion Service, Amsterdam, the Netherlands), biotinylated affinity-purified rabbit antimouse Ig (1/5,000; Dakopatts [DAKO], Copenhagen, Denmark) and avidin-peroxidase (1/2,000; DAKO), and, finally, an incubation for 30 min at room temperature with *o*-phenylenediamine (OPD). An OD_{492} exceeding the OD + 0.05 of the reagent blank (no serum control) was interpreted as a positive reaction.

Alpha-Amylase Allergen Exposure Measurements

In all bakeries, personal dust samples were collected in the workers breathing zone during full-shift periods of 6 to 8 h using polytetrafluoroethylene (Teflon) filters (pore size, 1.0 μm; Millipore Corp., Bedford, MA) and inhalable dust (PAS-6) sampling heads at a flow rate of 2 L/min (29). α-Amylase allergens were recovered from the filters by extraction with 2.5 ml PBS in a 10-ml centrifuge tube. Each tube was vortexed for 2 min, sonicated for 2 min, vortexed for 5 min, and sonicated for 2 min, successively. The extract was centrifuged at 5,000 *g* for 15 min, and the supernatant was collected and stored at −20° C. The α-amylase concentration in each sample was measured with a sandwich-EIA. Microtiter plates were coated with affinity-purified polyclonal rabbit anti-α-amylase antibodies. After washing, undiluted samples and 12 dilutions of a standard α-amylase antigen preparation (Fungamyl 1600S®) were added to the wells, and the plate was incubated for 1 h at 37° C. After another wash, wells were incubated with biotinylated affinity-purified rabbit anti-α-amylase antibodies, and subsequently with peroxidase-conjugated avidin and OPD. The reaction was stopped by adding HCl, and the absorbance at 492 nm of each well was measured with an EIA-reader. The dose-response curve for the standard preparation was obtained by four-parameter curve fitting, and allergen concentrations of samples were determined by interpolation in this curve. Only samples with an optical density higher than the optical density of the reagent blank (no α-amylase) + 5 SD were considered, resulting in a detection limit of the sandwich assay of 100 pg/ml. This resulted in a detection limit for personal allergen measurements of 250 pg/m³. Samples with high concentrations (> 1.5 ng/ml) were retested at higher dilutions. The validity and specificity of the assay have been investigated extensively. The amylase assay appeared to be highly specific for fungal amylase, and immunoblotting revealed a very similar reaction profile for IgE from sensitized bakery workers and the rabbit antibodies (24).

Statistical Analyses

All statistical analyses were performed using SAS software (version 6.09). Differences in mean exposure levels between groups were tested using a *t* test (PROC ANOVA). Exposure-response relationships were studied with univariate, stratified, and multiple regression analysis. As proposed by several investigators (30, 31), prevalence ratios (PR) were calculated, by using Cox's proportional hazards model (32), as modified by Breslow (33) with SAS software (PROC PHREG). Differences at $p < 0.05$ (two-sided) were considered significant.

RESULTS

Symptoms, SPTs, and IgE Measurements

An overview of the prevalence of respiratory symptoms, work-related symptoms, and positive IgE and SPT reactions is presented in Table 1. Forty-four workers (25%) had one or more work-related symptoms, with the highest prevalence for rhinitis and the lowest for chest tightness. Of the 28 workers (16%) who reported work-related rhinitis or chest tightness, 19 (68%) had rhinitis only, 2 (7%) had asthma symptoms only, and seven (25%) reported these symptoms simultaneously.

Sixty-five (38%) of the workers had a positive skin test to at least one common allergen, and they were defined as atopic. Twenty-five (15%) had a positive skin test to one or more occupational allergens (wheat flour, rye flour, α-amylase, or baker's yeast). There was a reasonable agreement between α-amylase sensitization measured by SPT and IgE analysis. Of the 13 IgE positive workers 77% also had a positive SPT to α-amylase; 63% of the SPT-positive workers were also IgE-positive. At our department, another occupational asthma cohort study was performed in laboratory animal workers. In that study SPTs were also performed with the fungal amylase extract as a control for the bakery study. Seven of 416 laboratory animal workers (1.7%) had a positive reaction to α-amylase. This was significantly different from the prevalence rate in bakery workers.

α-Amylase Exposure

A total of 546 personal dust samples were available. On the basis of dust exposure levels, production workers were classified in eight job titles (24). Because exposure data were lognormal distributed, the geometric means (GM) and geometric standard deviations (GSD) were computed next to the arithmetic mean (AM). The geometric means of full-shift airborne dust levels varied from 3.0 mg/m³ for doughmakers of large industrialized bakeries to 0.4 mg/m³ for slicers, packers, and transport workers.

In 480 personal samples from production workers, α-amylase exposure levels were determined. The other samples were taken in maintenance workers, which were excluded from this study (n = 27), were used during the development of the α-amylase assay, other assays or for endotoxin analysis (n = 23), or were lost before filter elution (n = 16). Alpha-amylase exposure levels varied considerably depending on job title and type of bakery. Bakers involved in dough production had a much higher α-amylase exposure than did other bakery workers (oven staff, packers). In confectioneries and rye bread production sites, however, no amylase was used in the production process, and α-amylase exposure levels in these bakeries were all close to the detection limit. Four exposure categories were defined based on frequency of measurements above detection limit and level of α-amylase exposure in each job title and each type of bakery (*see* Table 2). Category I consisted of all doughmakers working in the factory producing crispbakes (a kind of toast), where α-amylase exposure was very high. Category II consisted of all other production workers handling α-amylase frequently (doughmakers and all-round staff from wheat-bread-producing bakeries and bread and mixed bakers from small bakeries), and category III of all workers handling α-amylase only occasionally. Although the range in α-amylase allergen exposure levels in each category was large, mean exposure levels were significantly different. Workers in these categories can be seen as more or less homogeneously exposed groups. For workers in one job category, however, α-amylase exposure was difficult to determine. In all-round staff in the crispbakes-producing bakery, full shift personal α-amylase exposure levels varied strongly (GSD = 8.2), depending on the type of work performed on the day of sampling. In fact, this job title was a heterogeneous group of workers, of which some could be classified as high exposed and some as low exposed, depending on the number of days worked as doughmaker, packer, etc. Because detailed information on these determinants was not available, workers in this exposure category could not be divided into high or low exposed. Therefore, this group was treated as a separate exposure category (Category IV in Table 2). All workers were classified in one of these categories on the basis of their total job history (highest exposed category ever worked in).

Exposure-Response Relationship

The relationship between α-amylase allergen exposure and prevalence of a positive SPT for this enzyme is shown in Figure 1. In the entire population, sensitization rate increased from 1.4% in the low exposed workers, and 12.8% in the medium exposed workers, to 30.4% in the high exposed workers. Positive SPT reactions were more common among atopic workers and in this group especially a strong exposure-response relation was found, with more than 50% sensitization in the high exposed atopic workers. No clear relationship was found in nonatopic workers.

TABLE 2

EXPOSURE CATEGORIES FOR α-AMYLASE ALLERGENS

Exposure Category	Personal Samples (*n*)	α-Amylase Exposure (ng/m^3) Below Detection Limit (*n*)	(%)	Above Detection Limit (*n*)	AM	GM	GSD	(*range*)	Number of Workers in Category (*n* = 169)
I High*	27	0	0	27	39.4	18.1	4.6	0.2–221.8	23
II Medium*	152	109	72	43	3.4	1.3	3.8	0.2–33.1	39
III Low*	275	268	98	7	1.9	0.7	4.0	0.2–8.8	71
IV Indistinct/strong varying*	26	11	42	15	25.1	6.1	8.2	0.2–150.2	36

Definition of abbreviations: n = number of personal samples below or above detection limit; AM = arithmetic mean of samples above detection limit; GM = geometric mean of samples above detection limit; GSD = geometric standard deviation of samples above detection limit.

* Exposure significantly different from all other categories ($p < 0.05$).

In a multiple regression analysis, α-amylase exposure and atopy appeared to be the most important determinants of skin sensitization (Table 3), with significant prevalence ratios (PR) for atopy (PR = 20.8) and for high and medium α-amylase exposure groups (PR = 15.9 and 8.6, respectively). Several other potential determinants were tested, but none of them was significantly associated with a positive SPT for α-amylase: current smoker (PR, 0.62; 95% CI, 0.23 to 1.68), ever smoker (PR, 0.76; 95% CI, 0.26 to 2.18), age (PR, 1.01; 95% CI, 0.97 to 1.06), and years in bakery industry (PR, 1.00; 95% CI, 0.95 to 1.06). Addition of these variables to the multiple regression model hardly changed the prevalence ratios for atopy and α-amylase exposure.

Similar analyses were performed with IgE sensitization, as illustrated in Figure 2 and Table 3. In the univariate analysis, the prevalence of positive IgE tests tended to increase with intensity of exposure. After stratification for atopy, however, there was no clear exposure-response relation. The multivariate analysis showed that the only statistically significant determinant of positive IgE was atopic status (Table 3). Prevalence ratios of positive IgE to α-amylase by exposure group were elevated but not statistically significant. No association could be found between positive IgE and some other potential determinants: current smoker (PR, 0.93; 95% CI, 0.31 to 2.76), ever smoker (PR, 1.09; 95% CI, 0.30 to 3.94), age (PR, 0.99; 95% CI, 0.93 to 1.05), and years in bakery industry (PR, 0.98; 95% CI, 0.91 to 1.05). Addition of any of these to a multivariate model with atopy and exposure did not change the results shown in Table 3.

The relationship between SPT sensitization to α-amylase and the prevalence of work-related respiratory symptoms is shown in Table 4. For the total population a strong and positive association was found. Prevalence of work-related rhinitis was almost five times higher in α-amylase SPT-positive workers, and the prevalence of work-related chest symptoms was almost 12 times higher. The most important potential confounder in this relationship was atopic status, and because all but one of the positive SPT reactions to α-amylase were found in the atopic workers, the analysis was also performed for this group of workers only. Although prevalence ratios were smaller, there was still a statistically significant association between positive α-amylase SPT and work-related respiratory symptoms. Similar results were obtained for the relation between positive α-amylase IgE and work-related symptoms (also statistically significant).

Only part of the workers with positive SPTs to α-amylase had work-related respiratory symptoms (eight of 16). Most of the

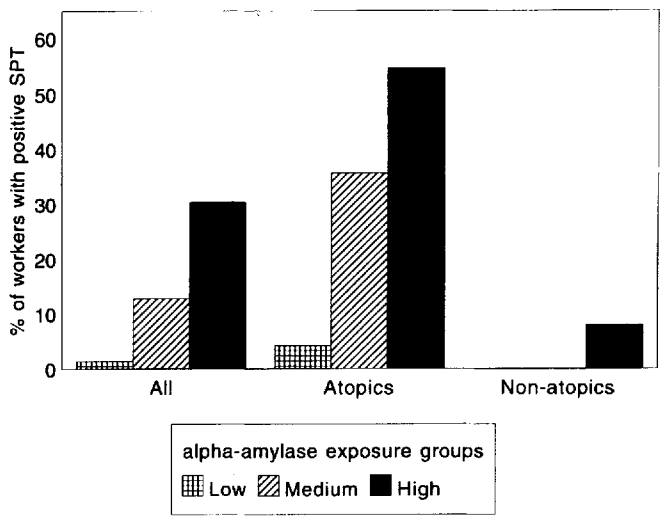

Figure 1. Prevalence of positive skin prick test to α-amylase by exposure group and atopic status. The number of workers in each group are: High exposed, 23 bakery workers (12 nonatopics and 11 atopics); Medium exposed, 39 bakery workers (25 nonatopics and 14 atopics); Low exposed, 71 bakery workers (47 nonatopics and 24 atopics).

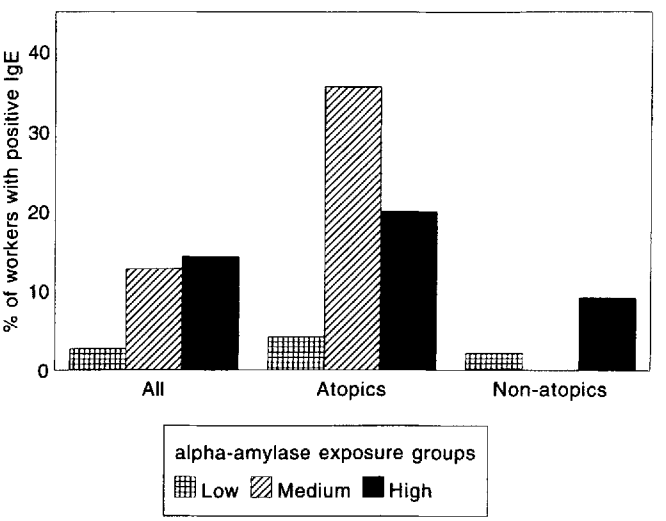

Figure 2. Prevalence of positive IgE to α-amylase by exposure group and atopic status. For numbers of workers per group, *see* legend in Figure 1.

TABLE 3

MULTIVARIATE ANALYSIS OF SENSITIZATION TO α-AMYLASE IN RELATION TO ATOPY AND α-AMYLASE EXPOSURE CATEGORY IN A GROUP OF BAKERY WORKERS

	PR†	95% Confidence Interval	p Value
Model 1: skin prick tests, n = 169			
Atopy	20.8	2.74–158	< 0.01
High α-amylase exposure*	15.9	1.95–129	< 0.01
Medium α-amylase exposure*	8.6	1.01–74	< 0.05
Low α-amylase exposure	1.0		
Indistinct α-amylase exposure*	4.6	0.48–45	0.18
Model 2: IgE, n = 167			
Atopy	8.3	1.84–38	< 0.01
High α-amylase exposure*	3.9	0.65–24	0.13
Medium α-amylase exposure*	4.6	0.85–22	0.08
Low α-amylase exposure	1.0		
Indistinct α-amylase exposure*	2.4	0.40–14	0.33

* Low exposed group is reference category.
† Prevalence ratio.

workers without symptoms (five of eight) worked in the high exposed group. We therefore looked at the degree of anti-α-amylase reactivity by comparing the mean wheal diameters of SPT-positive workers within each exposure category, as given in Table 5. Although positive SPT reactions were most prevalent in the high exposed group of workers, the largest wheal diameters were found in the medium exposed category and the smallest in the high exposed group. The difference was statistically significant. Anti-α-amylase IgE reactivity of the IgE-positive workers in the high exposed group was also lower compared with the IgE reactivity in the medium exposed group. This difference, however, was not statistically significant.

DISCUSSION

Fungal amylase appeared to be an important occupational allergen in the bakery industry. As much as 9% of the bakery workers in this study showed a positive SPT to this enzyme, and 8% had a positive IgE test. In our study considerable effort has been put into measuring and modeling α-amylase allergen exposure of the bakery workers. The number of bakery workers in our study was relatively small. Nevertheless, a strong and positive association was found between α-amylase allergen exposure and SPT reactivity to this enzyme. For IgE sensitization, there was also a positive trend with α-amylase exposure, but this association did not reach statistical significance in the multivariate analysis. Atopic status was another important determinant of α-amylase sensitization. For skin prick test sensitization, the clearest exposure-response relationship was found within the

TABLE 5

MEAN WHEAL DIAMETER OF SPT TO α-AMYLASE FOR SKIN-PRICK-TEST-POSITIVE BAKERY WORKERS

Exposure Category	Positive Skin Prick Tests (*n*)	Mean Wheal Diameter	Range
I High	7	3.9	3.2–5.1
II Medium	5	9.2	6.6–14.9
III Low	1	4.1	–
IV Indistinct/strong varying	3	7.2	5.5–10.0

group of atopic workers. No clear association was found in nonatopic bakers. However, prevalence of positive SPT reactions in this group was low (only one bakery worker), and an exposure-response relationship might have been found if a larger population had been studied. It might also be that nonatopic workers become sensitized at higher allergen exposure levels than do atopic workers. No association was found between α-amylase sensitization and smoking habits, age, or years in industry.

Alpha-amylase exposure occurred mainly in doughmaking activities. Bakers working in these occupational titles were also exposed to other allergens, especially wheat flour. In a previous study, an EIA for measuring wheat flour antigens was presented, and wheat flour exposure of all bakery workers was measured (24). Although there was a low correlation between wheat flour allergens and α-amylase allergens if individual measurements were considered (r = 0.19), some undesirable correlations existed when grouping workers in exposure categories (r = 0.30). Therefore, wheat flour allergen exposure should be considered as a potential confounder, when estimating exposure-response relationships for α-amylase in bakery workers. The relationships described in this study, however, are most likely to be caused by α-amylase exposure and not by wheat flour exposure. First, in this study we looked at specific end points for α-amylase (SPT and IgE). It is not very likely that exposure to wheat flour caused sensitization to fungal amylase. Second, if we restricted the analyses to workers with high exposure to wheat flour (all doughmakers and all workers in small bakeries; n = 96), the high and medium exposed α-amylase categories were not changed, but the low exposed group was restricted to doughmakers from confectioneries and the rye-bread-producing bakeries (oven staff, packers, etc. excluded). In this subgroup, the exposure-response relation presented in Figure 1 remained unchanged. Third, in another analysis we calculated the mean α-amylase and wheat flour allergen exposure for each individual worker for whom we had exposure measurements. We then performed a multivariate analysis with SPT sensitization as the dependent variable and either the mean personal wheat flour exposure or the mean personal α-amylase

TABLE 4

PREVALENCE RATIOS OF WORK-RELATED RESPIRATORY SYMPTOMS IN RELATION TO SKIN PRICK TEST SENSITIZATION TO α-AMYLASE IN A GROUP OF BAKERY WORKERS

	Total		Positive SPT α-Amylase		Negative SPT α-Amylase		Prevalence Ratio	95% Confidence Interval
Symptom	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)		
All workers	(*n* = 169)		(*n* = 16)		(*n* = 153)			
Rhinitis	24	14	8	50	16	10	4.78	2.05–11.2
Chest tightness	9	5	5	31	4	3	11.95	3.21–44.5
Atopic workers only	(*n* = 65)		(*n* = 15)		(*n* = 50)			
Rhinitis	14	22	7	47	7	14	3.33	1.17–9.50
Chest tightness	7	11	4	27	3	6	4.44	1.00–19.9

exposure as the continuous independent variable. A significant association could be found for α-amylase exposure. Wheat flour exposure also showed an elevated prevalence ratio, although not significant. When both α-amylase and wheat flour were entered into the model, the relation for wheat flour exposure disappeared, whereas the prevalence ratio for α-amylase exposure remained unchanged. This shows that α-amylase exposure is the best explanatory variable in our exposure-response analyses. In all models, atopic status was included as covariate.

Another potential confounder is exposure to fungi in the bakeries. In a study of wheat millers, Moneo and coworkers (34) suggested that sensitization for fungal α-amylase could have been caused indirectly by *A. oryzae* growing on milled wheat because of cross-reactivity between this fungus and α-amylase (14). It is our opinion, however, that this phenomenon has not biased our study results. Measurements of airborne fungi in the bakeries revealed only low concentrations of fungi, and only a small proportion appeared to be *Aspergillus* spp. (1 to 2%; data not shown). It is unlikely that these exposure levels caused sensitization to α-amylase, but if they did, α-amylase sensitization would also be expected in doughmakers with similar exposure to fungi, but without exposure to fungal α-amylase (doughmakers from confectioneries and rye-bread-production sites). However, no α-amylase sensitization was observed in this group.

In this study α-amylase exposure was related to specific sensitization, and subsequently α-amylase sensitization was related to work-related respiratory symptoms. Fungal amylase allergen exposure was not directly related to respiratory symptoms. Work-related respiratory symptoms are not specific health end points for α-amylase exposure. The role of α-amylase allergens in the development of symptoms cannot be studied without taking into account the role of exposures to other allergens in the workplace such as cereal allergens. Wheat flour especially contains a large number of proteins that appear to be allergenic, and this cereal flour is an important factor in baker's asthma. At this moment, analyses are under way in which work-related symptoms are related to both α-amylase allergen and wheat flour allergen exposure.

Exposure-response relationships found in our study may have been influenced by selection bias. Bakers with respiratory allergies may have switched to other job titles with low α-amylase exposure, or they may have exchanged the bakery work for another job without allergen exposure. The removal to lower exposed jobs has been taken into account to some extent since we modeled α-amylase exposure for each baker as the highest exposure category ever worked in. Control of selection bias caused by workers who have left the bakery industry was not possible, but it may have played a role in our study. In the highest exposed group of workers, the highest prevalence of positive SPT and IgE reactions to α-amylase were found, but the prevalence of work-related symptoms and the degree of *in vitro* and *in vivo* reactivity to fungal amylase was low (measured, respectively, as wheal diameter of the SPT and the optical density in the IgE analysis). It is possible that, especially in this category, workers who develop respiratory symptoms are likely to leave because of the high allergen exposure levels. Personal communications with employers in the bakeries confirmed this impression. Because this selection is dependent on α-amylase exposure, it is likely that underestimation of the observed exposure-response relationship has occurred. This selection bias, however, can be studied in detail only in a longitudinal study. At this moment most of the bakery workers in this study are part of a longitudinal study.

Two other epidemiologic studies focused on α-amylase sensitization. Cullinan and coworkers (16) found positive SPT reactions in 5% of British mill and bakery workers. In this study, a positive SPT response was significantly and independently associated with atopy, but no independent effect could be found for smoking, dust exposure, or wheat allergen exposure. Amylase exposure was not measured in this study. In an Italian study, 7.5% of the bakery workers were sensitized to α-amylase, derived by skin prick testing (17). Atopy appeared to be the most important risk factor for sensitization to occupational allergens (α-amylase and wheat flour), and, additionally, positive associations were found for smoking habits and years worked in the industry. Again, measurements of α-amylase allergens were not performed. Prevalence of positive SPT in these two studies are difficult to compare with our results because the concentration of the fungal α-amylase extract was different in each study. Furthermore, differences in α-amylase exposure may be another important explanation for differences in SPT response. As mentioned, no information was available on α-amylase allergen exposure levels in both studies, so the role of exposure could not be established.

Exposure-response relationships among workers in bakeries have been reported before, for flour dust exposure and wheat aeroallergen exposure (4, 16, 35). No exposure-response relationships have been reported for α-amylase exposure yet. Our study results might have implications for the management and prevention of occupational asthma in the bakery industry. This study suggests that reduction of α-amylase allergen exposure levels may lead to a reduction of the number of sensitized bakery workers. If this finding is confirmed by other (longitudinal or preferably by intervention) studies, this type of information can be used to establish exposure limits for α-amylase allergen exposure below which sensitization and development of occupational asthma are less likely to occur. Before that, however, much work has to be done in the development of a standardized assay for measuring α-amylase exposure. In our study we used polyclonal serum, and although this has no influence on the validity of our study results, the development of an assay based on monoclonal serum and one common standard α-amylase allergen extract might be necessary not only for a good comparison of α-amylase allergen exposure levels in several epidemiologic studies but also for standard setting.

In conclusion, this study has shown that α-amylase allergen exposure, together with atopy, is a major determinant of allergen-specific type I sensitization. Sensitization to α-amylase is strongly associated with work-related respiratory symptoms indicative of allergy. In addition to cereal flours, fungal α-amylase allergens play an important role in the development of occupational allergy among bakery workers.

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