Glucoamylase: a current allergen in the baking industry

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Abstract

Background: Over a 10 year period a decline in the rate of sensitizations to α-amylase (Aspergillus oryzae) was observed in bakers investigated for allergic obstructive airway disease. At the same time, glucoamylase (Aspergillus niger) was identified as the currently the most relevant allergen in sensitizations to enzymes in the baking industry. The aim of the present study was to investigate whether, over a period of 10 years and in the case of new-onset disease, there had been any change in sensitization and exposure rates to enzymes used in the baking industry.

Methods: Total immunoglobulin-E (IgE) levels and specific IgE to baking enzymes were determined in 433 bakers investigated in the Baker’s Asthma prevention program (Bäckerasthma Präventionsprogramm, BAP) of the German Social Accident Insurance Institution for the foodstuffs and catering industry (Berufsgenossenschaft Nahrungsmittel und Gastgewerbe, BGN). At the same time personal dust exposure, including assessment of the level of α-amylase exposure in the area of exposure, was recorded.

Results and conclusions: Serological investigations revealed a significant decline in the rate of sensitization to α-amylase from 26 % to 13 %. At 28 %, the rate of sensitization to the baking enzyme glucoamylase was significantly higher than to cellulase (16 %) and α-amylase among subjects in 2010. Multiple sensitizations to all three baking agents are common. In total, 30 % of affected bakers are currently sensitized to at least one of the baking enzymes investigated. Data from individual dust measurements revealed a decline in α-amylase exposure while overall dust exposure remained almost unchanged. Today, 11 % fewer bakers are exposed to α-amylase compared with ten years previously and, at the same time, enzyme concentrations in exposed bakers have dropped significantly. The high sensitization rate to glucoamylase in affected bakers gives cause to investigate exposure levels in bakeries and to assess sensitizations in the context of occupational disease proceedings.

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respiratory disease who, for personal reasons, prefer to (or must) continue to work in the baking industry. The program comprises three modules: risk analysis (RA), an interdisciplinary health seminar, and continuous occupational medical and technical follow-up. RA includes a medical, biological, and technical examination during shift times at the workplace designed to record task-related exposure factors. α-Amylase from Aspergillus oryzae has been the most frequently described allergy-triggering enzyme since the 1980s. Using various test methods, the prevalence of sensitization to this enzyme was described as 24%–40% in cases of occupational respiratory diseases [1, 2, 3]. Enzymes are used in the baking industry to enhance dough processing properties, volume yield, browning, and shelf-life of baked products. They serve as a supplement for the enzymes naturally present in flour, the content of which can vary depending on the type of grain, soil conditions, and climate. Glucoamylase, cellulase, and xylanase are also described as baking allergens [4, 5, 6, 7]. The primary relevance of glucoamylase is in fermentation, a central process for loosening dough to ensure an efficient production process. RA includes the gravimetric analysis of personal dust levels and α-amylase exposure. An immunological test system to measure exposure levels to enzyme-containing dusts in baking industry employees during work hours have been in use as early as 1998 [8, 9]. Similar test systems have also been used by other investigators [10, 11] to measure levels of α-amylase exposure.

Collective

The collective consisted of 433 subjects from the baking industry first examined as new-onset cases of disease during work hours as part of the BAP (RA module). Measures in accordance with the German Ordinance on Occupational Diseases (§3 Berufskrankheiten-Verordnung, BKV) were justified in all subjects, i.e., they were affected either by allergic rhinitis and/or allergic bronchial asthma, or they were at real risk of being affected by occupational disease No. 4301.

299 of these affected subjects were examined between 1999 and 2001 (2000 group) and 134 between 2009 and 2011 (2010 group). Examinations during shifts at the workplace took place at the express wish of the subjects, who wished to continue in their present employment.

The 2000 group were aged 35 years on average and 9% of employees were female. The average age of the 134 bakers in the 2010 group was 37 years and 16% of employees were female. Thus, the 2010 group were 2.6 years older (p < 0.01) and the percentage of females 7% greater (p = 0.04) compared with the 2000 group.

In terms of lung function, no significant differences were seen between the two collectives in terms of airway resistance (R5Hz) measured using impulse oscillometry (comparable with airway resistance measured using whole body plethysmography) and spirometry.

Methods

Using ImmunoCAP RAST-FEIA (Thermo Fisher Scientific), the on-site occupational-medical examination involved measuring levels of total immunoglobulin-E (IgE) and specific IgE to wheat and rye flour, α-amylase and, since 2006, also to glucoamylase and cellulase at the Institute for Clinical Chemistry at the Mannheim University Hospital. Sensitization was considered present from a CAP class ≥ 2 (equivalent to ≥ 0.7 kU/l).

At the same time, personal total dust sampling using a PSS (PGP-GSP-System, GSA GmbH, Neuss, Germany) was performed. Sampling took place during times of maximum dust exposure. The mass concentration of the collected dust was measured by means of differential weighing in accordance with the IFA-workbook No. 7284 (Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung, IFA) [8]. To quantify α-amylase levels, filter extracts were analyzed with the α-amylase assay by eco-Luftsichtung und Raumklima GmbH (Cologne, Germany). Depending on the duration of sampling and volume flow rates, an average sample volume of 1.5 m³ resulted in a limit of quantification (LOQ) of 0.3 ng/m³ α-amylase. Data below the LOQ were calculated as zero. The methods and detection limit of the procedure are described by Grün et al. [12, 13].

Data analysis was performed using the statistical software IBM SPSS Statistics 19.0. Results were expressed either as prevalence and percentages, or as mean, standard deviation, median, and range. Difference hypotheses were tested using the χ² test or the Mann-Whitney U test. Statistical significance was defined as p = 0.05.

Results

Total IgE and sensitzations to flour and baking enzymes

The mean total IgE concentration in the 2000 group was 378 kU/l (median 168 kU/l) and in the 2010 group 548 kU/l (median 193 kU/l) (p = 0.06). Taking 120 kU/l as the normal value, the total IgE level in 62% of the 2000 group and 67% of the 2010 group was above normal (p=0.29). At the time of both investigations, between 71% and 78% of affected subjects were sensitized to wheat and rye flour (Tab. 1).

Whereas 26% of the 2000 group were sensitized to α-amylase, significantly fewer employees in the
2010 group (13 %) exhibited specific IgE to α-amylase (p < 0.01). At 28 % of subjects in the 2010 group, the sensitization rate to glucoamylase was significantly higher than the other baking enzymes cellulase (16 %) and α-amylase (Tab. 1 and Fig. 1).

With regard to the distribution of sensitizations according to CAP classes, a clear trend towards higher CAP classes was seen for α-amylase (k87) in the 2000 group compared with the 2010 group (U test: p < 0.01). CAP classes 5 and 6 were not observed in the latter group (Fig. 2).

Multiple sensitizations to these three baking enzymes are common: 12 participants were sensitized to all three, eight to two enzymes, and 18 to only one baking enzyme. Only four participants that tested positive for cellulase were not also sensitized to glucoamylase. All subjects sensitized to α-amylase were also sensitized to glucoamylase. In total, 30 % of the 2010 group was sensitized to at least one of the baking enzymes investigated.

The CAP class distribution for the three baking enzymes investigated in the 2010 group shows that sensitizations are distributed over CAP classes 2–4. Already in CAP class 3, the rate of α-amylase sensitization is only 2 %, whereas the rates of glucoamylase and cellulase sensitization in the same group are 11 % and 8 %, respectively (Fig. 3).

**Amylase exposure data and personal total dust levels**

Significantly more insured individuals (i.e., 64 %) were exposed to dust containing α-amylase in the time period from 1999 to 2001 compared with ten years later (p = 0.03). In this latter time period, we recorded an 11 % decline in the number of subjects exposed to α-amylase (Tab. 2). Moreover, the 2000 group was exposed to significantly higher levels of α-amylase compared with the 2010 group (5.7 vs. 1.1 ng/m³, p < 0.01; Fig. 4). A correlation between individual exposure and sensitization could not be established.

The decrease within the same time period from 3.8 to 3.0 mg/m³ (p = 0.09) of the time-weighted average of personal total dust levels in the inhalable fraction was not significant (Tab. 2).

**Discussion**

Until recently the enzyme α-amylase was one of the most potent allergens amongst enzymes used in the baking industry, with a sensitization rate of approximately 30 % in bakers affected by occupational allergic airways diseases [1, 2, 3]. The data from our collective of bakers affected by allergic airway disease show a 50 % reduction over the last ten years in the rate of sensitization to α-amylase, from approx. 26 % to 13 %. From a preventive point of view, these results are encouraging. In contrast, glucoamylase and cellulase sensitization rates were not significantly different in the two groups.

| Table 1 |
|---|
| The total above normal IgE concentration and rates of sensitization to flour and baking agent enzymes in subjects (CAP class ≥ 2) |
| | 2000 Group | 2010 Group | χ² Test (p) |
| Total IgE ≥ 120 kU/l | n = 299 | n = 134 | 0.29 |
| Wheat flour (f4) | 224 (74.9 %) | 95 (70.9 %) | 0.68 |
| Rye flour (f5) | 225 (75.3 %) | 104 (77.6 %) | 0.60 |
| α-Amylase (k87) | 79 (26.4 %) | 17 (12.8 %) | < 0.01 |
| Glucoamylase (rk302) | – | – | 38 (28.4 %) |
| Cellulase (rk305) | – | – | 20 (15.6 %) |

**Fig. 1:** Subject sensitization rates to baking agent enzymes (CAP class ≥ 2)

**Fig. 2:** Percentage distribution of the CAP classes for α-amylase-sensitized (CAP class ≥ 2) subjects in the 2000 group compared to the 2010 group

**Fig. 3:** Percentage distribution of the CAP classes for glucoamylase-, cellulase- and α-amylase-sensitized (CAP class ≥ 2) subjects in the 2010 group
was identified as a relevant occupational allergen in more than a quarter of bakers affected by disease. A retrospective study published in 1998 [6] showed a sensitization rate to glucoamylase at that time of 8% in affected bakers, suggesting a significant rise in the sensitization rate to date. At 16%, sensitization to cellulase is also more common than to α-amylase. In contrast to serological investigations in sensitized bakers conducted between 1992 and 2001 using baking enzymes coupled to cellulose slides – which showed a decrease in sensitization rates to α-amylase from 23% to 15%, as well as an increase in the prevalence of glucoamylase from 8% to 13% and of cellulase from 13% to 15% [14] – the present study, using ImmunoCAP, found 26% of subjects in the 2000 group to be sensitized to α-amylase at a comparable point in time. There are several possible explanations for these changes in the allergen spectrum. On the one hand, baking agents products and formulations have changed: paste-like, granular, or liquid products are now used more frequently than baking agents in powder form. On the other hand, the need for and use of particular enzymes currently required, e.g., for frozen dough pieces and pre-baked goods, has changed. These changes are reflected in the declining α-amylase exposure rates: in the years 2009–2011, significantly fewer bakers were exposed to α-amylase than in the preceding ten years, and this at a significantly lower enzyme concentration while total dust exposure remained almost unchanged, so that a marked reduction in enzyme exposure in the inhalable dust fraction can be assumed. No correlation between sensitization and individual exposure could be identified, likely due to the fact that these measurements represent mere snapshots. Changes in the allergen spectrum over the period of a decade make it clear that both industrial exposure and the methods of serological diagnostics used are subject to a development process. Thus, the high sensitization rate to glucoamylase gives cause to monitor baking agent enzyme exposure in bakeries and to regularly check for this sensitization in the context of expert assessments of occupational disease.

**Table 2**

### Statistical characteristics of personal α-amylase and dust exposure data

|                      | 2000 Group | 2010 Group | U/χ² Test (p) |
|----------------------|------------|------------|---------------|
| α-Amylase in ng/m³   | 5.7 ± 22.1 | 1.1 ± 2.2  | < 0.01        |
| PSS filter with exposure¹ | 177 (64%) | 193 (63.7%) | 0.03          |
| PSS filter without exposure² | 99 (36%) | 68 (33%) | 0.09          |
| Personal total dust level in mg/m³ | 3.8 ± 4.3 | 3.0 ± 2.7 | ³*          |

*Specified: mean±SD, median (min-max), Filter with α-amylase level greater/equivalent to or ¿α-amylase level below the LOQ

LOQ, limit of quantification; PSS, personal sampling system (for hazardous substances)

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