Allergy risk in an enzyme producing plant: a retrospective follow up study

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Abstract

***Objective*—To investigate the risk of enzyme sensitisation and clinical allergy in workers exposed to enzymes at Novo Nordisk A/S.**

***Methods*—The study was a retrospective follow up study based on medical history and test data originally collected at routine screenings for enzyme allergy by the Occupational Health Service (OHS) of Novo Nordisk A/S during the period 1970-92. Workers were exposed to proteases, lipases, cellulases, and carboxyhydrases. Medical records of 3815 subjects were registered in the OHS database. According to criteria including possible enzyme exposure, allergy tests at the time of engagement, and participation in the allergy screening programme 1064 were selected for the present study. Outcomes were allergy symptoms, specific IgE test (radioallergosorbent test (RAST)) to enzymes, skin test reactions to common allergens and enzymes, forced expiratory volume in one second (FEV_1), and forced vital capacity (FVC). Potential risk factors were smoking habits, workplace, type of job, age, and sex.**

***Results*—Sensitisation occurred to all types of enzymes handled in the plant, most often in production areas and laboratories; 8.8% developed clinical enzyme allergy during the first three years of employment. The risk declined during the period. The frequency of enzyme sensitisation, expressed as RAST values > 0.5 SU, was 36%, and the frequency of significant RAST values ≥2 SU was 8%. Ranking diagnoses of enzyme allergy by severity, the frequency of asthma was 5.3%, rhinitis 3.0%, and urticaria 0.6%. Half of the cases occurred within the first 15 months of exposure. Smoking was an independent risk factor for clinical enzyme allergy (odds ratio (OR)=2.3 (95% exact confidence interval (95% CI) 1.4 to 3.9), measurable RAST ≥0.5 SU (OR=1.5 (95% CI 1.1 to 2.1)), and RAST ≥2 SU (OR=4.5 (95% CI 2.2 to 8.4)). Atopic predisposition at the time of engagement was not a significant risk factor for enzyme allergy. This could be due to various selection mechanisms.**

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Novo Nordisk A/S has produced detergent enzymes since 1962, and later on also other types of industrial enzymes. Clinical enzyme allergy and enzyme sensitisation in employees exposed occupationally to enzymes is well known.[1–6] Previous publications gave results on allergy frequencies to selected proteases (alcalase and esperase).[6 7] In this study we evaluated the risk at Novo Nordisk A/S to a wider variety of enzymes.

Since 1974, all applicants to jobs in departments handling enzymes were counselled about the allergy risk, offered the opportunity to participate in the health surveillance programme, and tested for atopy[6 7] with a skin test panel of common northern European allergens. Pepys' definition of atopy as a positive skin test reaction was used.[8 9] Up to 1980, a positive skin test excluded applicants from employment in jobs with exposure to enzymes. Since 1980 jobs have been classified by assumed risk and atopic subjects were from then on engaged in departments with lower exposure (for example, laboratories). From February 1987, the pre-employment tests have served solely as a background for counselling the applicants. Departments were included in the allergy screening programme when enzyme sensitisation had occurred "recently", or if evaluations of exposure and working conditions indicated a high risk of sensitisation. The health surveillance programme included a test for specific IgE antibodies (radioallergosorbent test; RAST), information about exposure to enzymes, medical history, and clinical tests, including lung function and, in some cases, skin tests. The screening intervals varied, but were typically once or twice yearly.

The study comprised: (1) evaluation of medical records and registration of the data in a database; (2) registration of exposure related to information about job and working area and; (3) estimation of enzyme allergy risk in relation to smoking and atopy before employment.

A distinction was made between sensitised employees with and without symptoms. Sensitisation represents a measurable RAST value, or a positive skin test, or both.

Material and methods

Data from personnel files, medical records, and a previous database holding information since 1970 were evaluated and recorded in a relation database (ORACLE). For the period 1970-92, 3815 employees were registered in the database, and from these a cohort of 1064 persons were identified according to the following

criteria: (1) employment in the company for the first time; (2) employment in a department which was included in the allergy screening programme; (3) a pre-employment test had been performed between 90 days before and not later than 14 days after date of employment; (4) at least one medical examination had been carried out after engagement.

The aim of this design was to build up a cohort in which employees' exposure to enzymes was certain and in which the quality of follow up data was optimal. Thus the risk of underestimating risk of enzyme allergy should be minimised. From the population of 3815 these inclusion criteria resulted in exclusion of 862 lacking medical examination at employment, 496 with employment examination only, and 1393 employed outside a screening department or examined outside the interval for a pre-employment test. Enzyme sensitisation at the time of engagement was a criterion for exclusion from the cohorte.

Evaluation of the medical records included information of previous and present allergy symptoms, lung function tests (Vitalograf spirometry, forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), peak flow monitoring (Vitalograf peak flowmeters)), measurement of specific IgE to enzymes (RAST),[10-12] and a description of the job and exposure to enzymes.

For a definition of clinical enzyme allergy, all the following criteria needed to be met:

(1) A formal diagnosis or information in the record of one or several of the following: (*a*) asthma, given as coughing, shortness of breath, wheezing, or tightness of the chest; (*b*) allergic rhinitis and conjunctivitis given as sneezing, rhinorrhoea, itching and congestion of the nose, eye itching, redness, and oedema of mucous membranes and eyelids; (*c*) urticaria by itching, redness, and wheals.

(2) Symptoms related to exposure to enzymes.

(3) Positive skin scratch test, or measurable RAST, or a positive workplace challenge to enzymes.

A positive skin test—prick or scratch—was defined as a wheal with a cross diameter mean of 3 mm and a reaction of at least 2 × the negative control or half of the positive control with histamine.[13]

Values for RAST (in Sorbent Units (SU)) are given at three levels: <0.5 (below the detection limit), 0.5-1.9, and ≥2.0 SU. Traditionally RAST ≥2.0 SU has been regarded as clinically relevant.[10-12]

Results were calculated according to the following: (1) most important allergy diagnosis (ranked for asthma, rhinoconjunctivitis, and urticaria with or without enzyme sensitisation); (2) RAST values at intervals of 0.5-1.9 and ≥2 SU.

The follow up refers to periods after engagement and calculation of risk was performed according to these at intervals of 0-15, 16-27, and 28-39 months.

EMPLOYEES

The cohort of 1064 employees (age range 17-60, mean 32 years) comprised 150 women (14.1%, mean age 25.2) and 914 men (85.9%, mean age 33); 82% of the cohort were employed in production departments and 18% in laboratories.

ETHICS

The study was approved by the Danish Regional (Copenhagen and Frederiksberg) scientific ethics committee.

Results

PRE-EMPLOYMENT EXAMINATION

For the period 1981-92 the OHS recorded the mean number of atopic applicants irrespective of later employment as 17.2% (period range 12.4%-24.1%). Among the subjects employed the outcome of the pre-employment examination showed that 103 (9.7%) subjects had a positive skin test and by this were defined as atopic subjects in accordance with the criteria given by Pepys.[9] Only 24 skin test positive subjects had symptoms of respiratory allergy judged from the medical history, three with asthma and 21 with rhinoconjunctivitis. Also, four of the non-atopic subjects (skin test negative) had symptoms of rhinoconjunctivitis. The relative distribution of positive reactions to common inhalable allergens were as expected.[14 15] At the time of the employment 57% were smokers and 41% non-smokers, and for 2% no information on smoking habits could be found.

FOLLOW UP

The population

During the three screening periods 69, 78, and 46 subjects respectively left the company leaving 871 persons followed up for at least 39 months.

DIAGNOSES

During the period 1970-92 a total of 94 (8.8%) people were diagnosed as having definite enzyme allergy and 27 (2.5%) were classified as suspected of having enzyme allergy (table 1). These cases presented within the first 39 months after employment and the annual number ranged from 1 to 13. The incidence of enzyme allergy declined with time during the first 39 months of employment (0-15 months 52 (4.9%); 16-27 months 30 (2.8%); 28-39 months 12 (1.1%).

After 39 months of employment no systematic screening has been carried out. However, 23 (2.2%) additional cases of enzyme allergy

Table 1 Outcome of follow up examination

	Enzyme allergy	
Follow up diagnosis	*Yes*	*No*
Asthma	56	8
Rhinoconjunctivitis	32	28
Urticaria	6	3
Other or none		931*
Sum	94 (8.8%)	970

* Including 27 with suspected enzyme allergy, not confirmed.

from an exposed group of 871 were identified after more than 39 months of employment among workers consulting the OHS or in random surveys in certain departments.

During 1970-92 the frequency of symptomatic allergy tended to decline. In 1970-79 the frequency was 10.3%, in 1980-86 9.5%, and in 1987-92 6.1% (P=0.07; Mantel-Haenszel test for trend).

RAST

Quantities and types of enzymes produced and the degree of exposure have varied with time, which is reflected in the number of RAST tests for each enzyme. Table 2 shows the results of RAST analyses of the most important enzymes.

Most RAST analyses were made in connection with scheduled screenings. Three hundred and fifty five (36%) subjects of 995 tested at follow up had a RAST value above the detection limit of 0.5 SU and 76 (8%) ≥2 SU for one or more enzymes (table 3).

Occurrence of enzyme allergy and RAST correlated well: 52 (68%) with RAST ≥ 2.0 SU had allergy symptoms; 11 (1.7%) with negative RAST were diagnosed as having allergy to enzyme (table 3).

However, of those with RAST ≥2.0 SU, eight (10.5%) had no diagnosis of symptomatic allergy. Of subjects with RAST between 0.5 and 2.0 SU 240 (86%) had no enzyme allergy.

LUNG FUNCTION

Outcome of lung function tests during the screening periods were compared with corresponding values at pre-employment examination for each person. Statistically, individual changes were compared by *t* test. For non-smoking workers who remained non-allergic, no decline was detected during the 39 months of screening. By contrast, smoking independently of allergy status led to a significant fall in FEV_1 during the three screening periods (0.08 *v* 0.01 l, P=0.011)). Particularly, a remarkable fall in lung function was found among smokers who developed enzyme allergy (0.21 *v* 0.08 l, P=0.009). Decline in lung function was not associated with allergy status in non-smokers.

RISK FACTORS

There was no tendency for an association between age or sex and enzyme allergy. This is remarkable, most male workers being employed in production areas in which the exposure levels were highest but also with the greatest selection.

The risk of symptomatic allergy was significantly increased in smokers compared with non-smokers (11% *v* 5%, odds ratio (OR) 2.3 (95% exact confidence interval (95% CI) 1.4 to 3.9)).

Sensitisation to enzymes, expressed as increasing RAST value in SU, was significantly increased in smokers compared with non-smokers (RAST value 0.5-<2 SU: 29% *v* 23%, OR 1.5 (95% CI 1.1 to 2.1) and RAST value ≥2 SU: 10% *v* 3%, OR 4.5 (95% CI 2.2 to 8.4)).

A positive skin test at the pre-employment examination did not predispose to clinical enzyme allergy (OR 1.0 (95% CI 0.45 to 2.0)).

Likewise, clinical allergy at the pre-employment examination did not predispose to enzyme allergy or sensitisation (tables 4 and 5). Combining smoking habits, atopy, and sex in a multivariate analysis did not give any clear correlation between outcome at pre-employment examination and later enzyme allergy.

Time to sensitisation may be a more sensitive indicator of the impact of atopy and smoking—of those workers becoming allergic to enzymes and being smokers and atopic before employment 93% showed enzyme allergy in the first or second screening period compared with 62% of sensitised non-smoking non-atopic subjects.

Subanalysis of data for different exposure groups—for example, laboratory technicians *v* production workers—disclosed no significant difference in risk between groups.

Table 2 Results of RAST analyses of the most important enzymes after employment, stating those who have had the RAST value (SU) measured in the interval at the specific test

	Percentage distribution of RAST values (SU)				
Enzyme	*< 0.5*	*0.5-< 2*	*≥ 2*	*People (n)*	*Tests (n)*
Alcalase	84	13	3	799	2445
AMG	77	16	7	510	1455
BAN	77	17	6	587	1682
Celluzyme	96	3	1	284	489
Esperase	78	19	3	653	1989
Fungamyl	77	17	6	410	1147
Lipolase	97	2	1	287	507
Neutrase	81	13	6	357	928
Rennilase	84	9	8	367	1103
Savinase	94	4	1	540	1164
Sweetzyme(T)	100	0	0	68	101
Termamyl	82	14	4	715	2028
Trypsin(PTN)	89	10	1	288	754

For repeated tests for a specific enzyme, the highest value has been chosen.

Table 3 RAST value (SU) in relation to diagnoses during follow up period until 39 months (number of people and percentage of RAST value stated)

	RAST value		
Diagnosis	*< 0.5 (%)*	*0.5-< 2 (%)*	*≥ 2 (%)*
Asthma with enzyme allergy	5 (0.8)	15 (5.4)	33 (43.4)
Rhinoconjunctivitis with enzyme allergy	6 (0.9)	10 (3.6)	16 (21.1)
Urticaria with enzyme allergy	0 (0.0)	3 (1.1)	3 (3.9)
Suspected enzyme allergy	0 (0.0)	11 (3.9)	16 (21.1)
Asthma without enzyme allergy	7 (1.1)	1 (0.4)	0 (0.0)
Rhinoconjunctivitis without enzyme allergy	19 (3.0)	5 (1.8)	2 (2.6)
Urticaria without enzyme allergy	2 (0.3)	1 (0.4)	0 (0.0)
Other or none	601 (93.9)	233 (83.5)	6 (7.9)
Sum*	640 (100)	279 (100)	76 (100)

* 69 (6.5%) people were not tested by RAST, three of whom were diagnosed allergic by skin test and anamnesis.

Discussion

The three year cumulated incidence proportion of clinical enzyme allergy was 8.8%; in addition 23 cases were identified later than 39 months. In order not to underestimate the risk of enzyme allergy in exposed workers the inclusion in the cohort was restricted to workers in departments with probable exposure to enzymes and to those for whom start and follow up data were optimal. We expect that the vast majority of clinical enzyme allergies were identified, as symptoms are characteristic, well known among workers, and often clearly work related, and there is a systematic surveillance

Table 4 Outcome of pre-employment examination v later symptomatic enzyme allergy

	Enzyme allergy		
Pre-employment diagnoses	*No (%)*	*Yes (%)*	*OR (95% CI)*
Asthma*	2 (67)	1 (33)	0.8 (0.1 to 3.3)
Allergic rhinitis†	24 (96)	1 (4)	
Positive skin test without symptoms	72 (91)	7 (9)	1.0 (0.4 to 2.3)
Other or none	872 (91)	85 (9)	
Sum	970 (91)	94 (9)	

Values in parentheses are % accross rows.
* All had positive skin tests at pre-employment test.
† 21 of 25 had positive skin test before employment.

Table 5 Outcome of pre-employment examination v RAST for enzymes in the follow up period

	RAST value			
Pre-employment diagnosis	*< 0.5*	*0.5-< 2*	*≥ 2*	*Not done*
Asthma*	1 (33)	0 (0)	1 (33)	1 (33)
Allergic rhinitis†	11 (44)	10 (40)	1 (4)	3 (12)
Positive skin test without symptoms	43 (54)	26 (33)	7 (9)	3 (4)
Other	585 (61)	243 (25)	67 (7)	62 (7)
Sum	640 (60)	279 (26)	76 (7)	69 (7)

Values in parentheses are % across rows.
* All had positive skin test at pre-employment test.
† 21 of 25 had positive skin test before employment.
OR (95% CI) for: positive skin test and RAST value 0.5-<2 SU 1.6 (0.8-2.5); inhalation allergy and RAST value 0.5-<2 SU 2.0 (0.8-5.1); positive skin test and RAST value ≥ 2 SU 1.4 (0.5-3.4); inhalation allergy and RAST value ≥ 2 SU 1.5 (0.2-6.8).

and an open access to health check in the OHS. Likewise, replacement to non-exposure workplaces is offered to enzyme allergic employees and salary compensation from insurance given. The surveillance programme does not include an exit examination and therefore some employees may have left with an unidentified enzyme allergy. The recruitment practices and the occasional intervention by replacement of persons with raised RAST values irrespective of symptoms may both add to an underestimation of the risk.

In previous studies[6 7] lower frequencies of enzyme allergies were found. These studies, however, only included two selected *Bacillus subtilis* enzymes. For those enzymes (Alcalase and Esperase) frequencies of RAST ≥2.0 SU were similar to the 3% found in our study.

Scratch tests were usually carried out for rapid diagnosis of enzyme allergy or as a supplement to low RAST values. The subjects were usually tested with a full enzyme panel, disregarding actual exposure history. A higher proportion of positive skin tests than RAST was found with up to 25% positive reactions for some allergens. The rationale for testing with RAST or scratch tests means that the tests are incomparable in respect of sensitivity or for assessment of the relative allergenicity of enzymes. Cross reactions between enzymes occur, and must be taken into consideration.

Some studies report the risk of enzyme allergy in industries using enzymes,[2 3 16 19] especially in production of detergents with enzymes added. From these studies prevalences of 17%-21% for positive skin tests were found and Flood *et al*[2] estimate the incidence proportion of symptoms as 4.5%. These figures cover a period in which the formulation changed from powders to granulates. Since then coating of the granulates further reduced enzyme dust concentrations .[20] However, corresponding data on allergy risk are not published.

Thus, risk of enzyme allergy is comparable with allergy risk in other protein processing industries (10-30%)—for example, baking,[21-23] coffee,[24] fish,[25] and egg processing industries.[21]

Our population was preselected on the basis of allergy tests as previously described. The rationale for the selection was the assumed risk for people predisposed to allergy.[6 7 22 26] The prevalence of atopy among applicants from 1981-92 was 17.2% mean (range 12.4-24.1%). This prevalence includes subjects not employed and therefore excluded from the database. It is higher than that found in the study group and somewhat lower than expected in a population of Danish adults (prevalences 21%-28%).[14 15] The differences may be a result of selection in the recruitment procedure before the pre-employment test and after the examination. A selection may also have taken place before application for a job, as the allergy risk associated with the company may be known in the community.

Among the atopic subjects we did not see the expected excess risk for enzyme sensitisation. However, only the "mildest" cases were engaged (for example, low asthma frequency among applicants) and they were probably employed in departments with the lowest exposure and followed up by more rigorous medical surveillance. Further, as a secondary preventive measure, atopic subjects with increased RAST may have been moved more readily and earlier to non-exposed jobs, than non-atopic subjects. These factors will mask a correlation between atopy and subsequent enzyme allergy. However, we cannot confirm that atopy is a definite factor for enzyme allergy. Future studies of populations less selected will, therefore, be more valid regarding the importance of allergic predisposition. The results do not justify rigorous selection on the basis of pre-employement examination.

Smoking was an unequivocal risk factor of allergy. Smoking was not a criteria for selection, but the risk from smoking should be included in future counselling. The explanation of the negative correlation between smoking and atopy at engagement may be that atopic smokers were selected out on account of several symptoms. The impact of smoking on production of specific IgE antibody and as a risk factor in occupational disease still needs further study.[2 5] As mentioned, decline in lung function tests overall correlated well with smoking habits and to development of allergy among smokers. However, this does not imply that this test can be considered to be suitable for screening for allergy. Rather, single lung function tests reflect the gravity and nature of lung disease.

No published studies consider the risk of enzyme allergy in relation to threshold limit values (TLVs) (TLV for proteases= 0.06 μg/m^3).[27] The TLV was originally set as a recommendation in the late 1960s and early 1970s.[16 19 28 29] The obtainable exposure concentration in the production of detergents with granulated subtilisin, rather than scientific

results, seems to have been the guiding factor for the TLV. The general dust concentrations have decreased over the period of 20 years covered by this study. In laboratory areas dust concentrations, characterised by 50 and 90% percentiles, have ranged between 0.05 and 0.1 $\mu g/m^3$ (maximum 0.8 $\mu g/m^3$ enzyme protein). In production areas handling enzyme powder, dust concentrations of about 0.1-1.0 $\mu g/m^3$ have been measured (maximum 2 $\mu g/m^3$). Face masks were prescribed at enzyme concentrations above 0.6 $\mu g/m^3$.

The National Research Council's Ad Hoc Committee stated in 1971 that despite a great number of consumers, only six cases of definite IgE induced allergy to enzyme detergents were found in consumers tested.[26 30] Since the introduction of granulated enzymes no cases of sensitisation or allergic respiratory disease in consumers have been reported.

Despite increased quantities produced, the number of cases of enzyme allergy at Novo Nordisk A/S seems to have decreased over the period of investigation, probably due to improved industrial hygiene and training. However, 8.8% still developed allergy, half of them with lung symptoms.

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