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Bronchial responsiveness to adenosine-5′-monophosphate and methacholine as predictors for nasal symptoms due to newly introduced allergens. A follow-up study among laboratory animal workers and bakery apprentices

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Summary

Background In asthma patients, bronchial hyper-responsiveness (BHR) to adenosine-5′-monophosphate (AMP) reflects bronchial inflammation more closely than BHR to methacholine. In this follow-up study we studied bronchial responsiveness to both stimuli as predictors of new-onset airway symptoms.

Methods We included 118 laboratory animal workers and bakery apprentices with a work experience of maximally 1 year. The baseline survey comprised a questionnaire, skin prick tests (SPTs) to common and work allergens, blood eosinophil counting, and bronchial challenge with methacholine and AMP. At follow-up, questionnaire and SPTs to work allergens were repeated. Airway symptoms to common allergens and work allergens were defined as nasal symptoms, chest tightness or asthma attack during or after contact with either common or work allergen. Bronchial challenge tests were analysed by BHR at a 15% fall in forced expiratory volume of 1 s (FEV_1), and by dose–response-slope (DRS).

Results Fourteen subjects (12%) developed airway symptoms to work allergens, of whom 12 had nasal symptoms. A positive SPT to work allergens occurred in 64%, and was the strongest predictor of airway symptoms [relative risk (RR) 7.5, 95% confidence interval (CI) 2.0–28.6]. Other predictors were airway symptoms to common allergens (RR 4.3, 95% CI 1.4–12.8), blood hypereosinophilia (RR 4.4, 95% CI 1.2–15.4) and BHR, with a slightly higher risk estimate for AMP than for methacholine (RR_{AMP} 3.7, 95% CI 1.1–12.5 and RR_{meth} 2.8, 95% CI 1.0–8.5). The difference was more distinct analysing airway responsiveness by DRS, for which AMP predicted symptoms better than methacholine ($P < 0.05$).

Conclusions Pre-existent bronchial inflammation or a preinflammatory state marked by AMP (hyper)responsiveness increases the vulnerability to develop nasal symptoms.

Keywords adenosine, bronchial hyper-responsiveness, follow-up study, methacholine, occupational allergy

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Introduction

Although bronchial hyper-responsiveness (BHR) is considered a hallmark of asthma, it may also exist in subjects without respiratory symptoms or rhinitis. In children, asymptomatic BHR has shown to be a predictor of asthma later in life [1, 2]. In adults, asymptomatic BHR predicts the development of asthmatic symptoms as well, especially if peripheral blood eosinophilia is present [3, 4]. A recent study by Ulrik et al. showed that BHR also predicts the development of rhinitis [5].

The role of BHR as a predictor may depend on the stimulus used. Methacholine and histamine cause bronchoconstriction by a direct effect on airway smooth muscle cells, whereas adenosine-5′-monophosphate (AMP) exerts its bronchoconstrictive effect mainly by the release of mast cell mediators [6, 7]. Clinical studies in patients with allergic asthma have shown that BHR to AMP reflects the underlying bronchial inflammation more accurately than BHR to methacholine [8–10]. AMP responsiveness is known to be increased in atopic non-asthmatics, particularly if they have rhinitis symptoms [11, 12]. We have recently confirmed the results of clinical studies in a cross-sectional study among apprentices before exposure to occupational allergens and found bronchial responsiveness to AMP to be more strongly related to allergic nasal as well as bronchial symptoms than bronchial responsiveness to methacholine [13].

The aim of the present study was to assess whether bronchial responsiveness to methacholine and AMP comprise risk factors for new-onset symptoms of upper and lower airways to allergens of the work environment. To this aim we have studied

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newly employed laboratory animal (LA) workers and bakery apprentices prior to their practise-year. Both occupational groups are at risk of airway allergy due to aero-allergens in the work environment [14].

Materials and methods

Study design

From 1995 to 1999, newly employed LA-workers were recruited by occupational health services at four universities. Within 4 months after employment, LA-workers were invited for the baseline survey. Bakery apprentices were recruited at school prior to their practise year. Both LA-workers and bakery apprentices were excluded if aged ≥ 45 years, had been working with LA or bakery products for more than 1 year prior to the study, or reported work-related respiratory symptoms at baseline. The baseline survey comprised a questionnaire, skin prick tests (SPTs) to common allergens and allergens of the work environment, peripheral blood eosinophil counting, and bronchial challenge tests with AMP and methacholine. During follow-up a questionnaire was completed, and SPTs on work allergens were repeated though only in the LA-workers. Figure 1 shows the stepwise selection protocol with numbers of participants. We ended up with 105 LA-workers and 13 bakery apprentices who completed both bronchial challenge tests at baseline, and at least one follow-up questionnaire.

The Medical Ethics Committee of the University of Wageningen approved the protocol, and written informed consent was obtained from participating subjects.

Questionnaire

Similar questionnaires were used for the LA-workers and bakery apprentices, with slight differences regarding the work environment. Both baseline and follow-up questionnaires comprised detailed questions on respiratory symptoms to allergens of the work environment, work experience, exposure to LA or bakery products, and smoking habits. At baseline additional data were collected on airway symptoms to common allergens. We defined symptoms as:

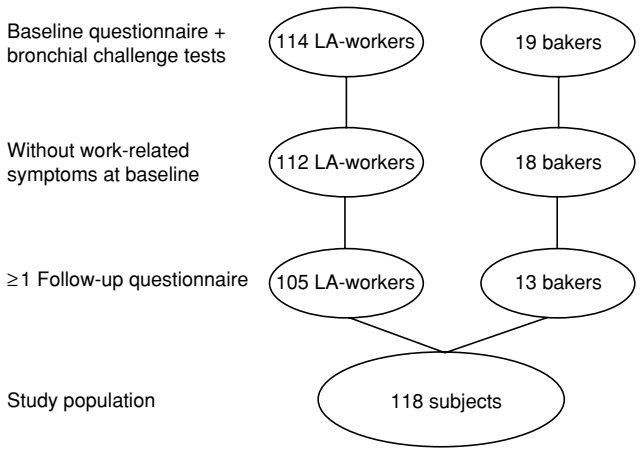


Fig. 1. Stepwise selection procedure of study population for data analyses.

- Airway symptoms to *common* allergens if subjects reported at baseline: a history of sneezing, blocked or runny nose, chest tightness or asthma attacks during or after contact with any agent except occupational allergens mentioned below.
- New-onset airway symptoms to *work* allergens if subjects reported at baseline or follow-up: sneezing, blocked or runny nose, chest tightness or asthma attacks during or after contact with rat, mouse, guinea pig or rabbit for the LA-workers, and wheat, rye or α-amylase for the bakery apprentices.

The questions distinguished between symptoms occurring during work hours and after work shift. Subjects were also asked for the month and year they first noticed symptoms.

Skin prick tests

SPTs were performed with common allergens and allergens of the work environment (ALK Benelux, Houten, the Netherlands). Common allergens comprised a mixture of grass pollen (SQ293), tree pollen (SQ197), cat fur (SQ555), dog fur (SQ553) and a mixture of the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* (SQ510). LA-workers were also tested for rat fur (15.09) and urine (15.79), mouse fur (15.08) and urine (18.78), guinea pig fur (15.05) and rabbit fur (15.07). Occupational allergens for the bakery apprentices comprised wheat (52.06) and rye flour (52.05), fungal α-amylase (52.52) and baker's yeast (52.01). Positive and negative control solutions consisted of histamine (10 mg/mL) and phosphate-buffered-saline (PBS). All tests were done on the forearm and read after 15 min. A SPT was considered positive if the mean weal diameter exceeded that of the negative control with 3 mm or more.

Eosinophil counting

Venous blood was taken in an EDTA-containing tube before bronchial challenge. The number of leucocytes and eosinophils was counted in 1 : 11 dilution using a Bürk counting chamber. The same technician performed all countings in duplicate and the mean was used in the analyses. Based upon the frequency distribution, we defined hypereosinophilia as the upper decile of the distribution (= 90th percentile: 200/mL).

Lung function and bronchial challenges

Lung function was determined using a pneumotachometer (Jaeger Toennies, Hoechberg, Germany). Prior to the challenge tests, full forced spirometry was assessed according to the guidelines of the ERS. Administration of methacholine and AMP (both Sigma, St Louis, MO, USA) occurred by a breath-actuated dosimeter (Jaeger) driven by compressed air at 20 psi. The challenge tests were performed using a modification of the protocol used in the European Community Respiratory Health Survey [15, 16]. Briefly, inhaled doses were quadrupled from 0.02 to 38.4 mg (90.5 μM) for AMP, and 0.01–2.4 mg (10.0 μM) for methacholine. Two minutes after each dose step, two reproducible measurements of forced expiratory volume of 1 s (FEV_1) were made and the higher of two acceptable measurements was selected. Both challenges were performed at the same time of day with an interval of 2–14 days. Salbutamol was stopped 8 h, and salmeterol and antihistaminics 48 h before the test. Subjects with a baseline $FEV_1 < 65\%$ of the predicted value were excluded.

The dose needed to cause a 15% fall in FEV_1 (PD_{15}) was assessed by linear interpolation between the last two points of the dose–response curve. BHR was present if a 15% fall in FEV_1 occurred within maximum cumulative dose inhaled, i.e., $PD_{15} \leq 10\,\mu M$ for methacholine and $PD_{15} \leq 90.5\,\mu M$ for AMP, respectively. For each subject, dose–response slopes (DRS) were calculated as the maximum percentage fall in FEV_1 during the test per the inhaled cumulative dose of agent in μMs [17, 18]. Based upon the frequency distribution of DRS-values we classified the values as: 'low' ($\leq$ 33rd percentile), 'intermediate' (33rd−67th percentile) and 'high' ($>$ 67th percentile).

Statistical analysis

To prevent bias by selective dropout of subjects with BHR in the first test, we only included subjects who completed both challenge tests. Relative risks (RR) and 95% confidence intervals (95% CI) were calculated for new-onset airway symptoms to work allergens using PROC PHREG in the statistical package SAS 6.11. If no information was obtained on the date of symptom onset, this was set at the midpoint between the follow-up in which symptoms were reported for the first time, and the previous survey. All risk estimates were adjusted for potential confounding by age, gender and smoking status.

Results

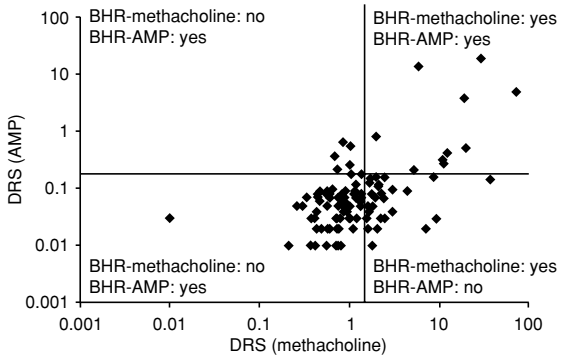
Baseline and follow-up data were available for 105 out of 112 LA-workers and 13 out of 18 bakery apprentices; baseline characteristics are described in Table 1. Bakery apprentices were younger and more often male than LA-workers; they reported airway symptoms to common allergens less frequently, and were more often hyper-responsive to methacholine. The mean follow-up period was 2.1 years (0.9–4.0 years) for both occupational groups together; due to the design of the study the bakery apprentices had a shorter follow-up.

Eleven subjects were hyper-responsive to both methacholine and AMP, for whom the geometric mean value for PD_{15} methacholine was 10 times smaller than that for PD_{15} AMP (1.2 and 12.3 μM, respectively). Figure 2 shows the correlation between methacholine and AMP responsiveness, expressed as DRS-values. The correlation coefficients were similar for the whole population and for the 11 subjects with BHR to both agents: 0.46 and 0.48, respectively.

New-onset airway symptoms to work allergens occurred in 14 subjects (12%), of whom 12 were LA-workers and two bakery apprentices. All symptoms occurred during work and no exclusive late reactions were reported. In 12 cases, symptoms involved a blocked or runny nose, and in two subjects chest tightness. Table 2 shows characteristics of these 14 cases. At baseline, SPTs were performed in 13 subjects of whom three were sensitized to work allergens. The case with missing data on baseline SPTs was excluded because of pregnancy, but at follow-up she had a positive SPT to work allergens. New sensitization to work allergens occurred in three of the 10 new-onset cases. All were LA-workers, and neither of them had a positive SPT to common allergens. At follow-up, SPTs were not repeated in three non-sensitized subjects that had developed symptoms. One of them was a bakery student for who SPTs were not included in the follow-up survey. In the LA-workers,

Table 1. Baseline characteristics of the study population ($n = 118$)

	Laboratory animal workers ($n = 105$) *n* (%)	Bakery apprentices ($n = 13$) *n* (%)
Mean age, years (range)	25 (14–43)	19 (18–22)
Female	61 (58.1)	5 (38.5)
Smoker	20 (19.0)	2 (15.4)
Airway symptoms to common allergens	33 (31.4)	1 (7.7)
$\geq$ 1 SPT common allergen, $n = 115$	36 (35.3)	5 (38.5)
$\geq$ 1 SPT work allergen, $n = 115$	10 (9.8)	2 (15.4)
PD_{15} methacholine $\leq$ 10 μmol	29 (27.6)	6 (46.2)
PD_{15} AMP $\leq$ 90.5 μmol	14 (13.3)	2 (15.4)
Nasal or inhaled corticosteroid use	4 (3.8)	0 (0)
Mean period of follow-up, years (range)	2.3 (1.0–4.0)	1.1 (0.9–1.2)



Pearson correlation coefficient $R = 0.46$ ($P < 0.001$) for DRS values ($N = 118$); $R = 0.48$ ($P < 0.14$) for subjects with BHR to both methacholine and AMP ($N = 11$)

Fig. 2. Correlation of dose-response slopes (DRS) for methacholine and AMP challenge. Lines indicating cut-off values for bronchial hyper-responsiveness (BHR).

Table 2. Baseline characteristics of 14 new-onset cases with respiratory symptoms due to allergens of the work environment

Patient no.	Work allergen		Common allergen		PD_{15} methacholine $\leq$ 10 μmol	PD_{15} AMP $\leq$ 90.5 μmol
	Symptoms	$\geq$ 1 SPT	$\geq$ 1 SPT	Symptoms		
1	Ast	Y	Y	Y	N	N
2	Ast	Y	ND	Y	Y	Y
3	Rhi	Y	Y	Y	Y	Y
4	Rhi	Y	Y	N	Y	N
5	Rhi	Y	Y	N	Y	Y
6	Rhi	Y	N	N	Y	N
7	Rhi	Y	N	N	N	N
8	Rhi	ND	Y	Y	N	Y
9	Rhi	ND	N	N	Y	Y
10	Rhi	ND	N	N	N	N
11	Rhi	N	Y	N	N	N
12	Rhi	N	N	Y	Y	N
13	Rhi	N	N	Y	N	N
14	Rhi	N	N	Y	N	N

Ast, asthma symptoms; Rhi, nasal or eye symptoms. Y, yes; N, no; ND, not determined.

participation rates were similar for those who developed symptoms and those who did not (83% and 87%, respectively).

BHR to methacholine was present in seven, BHR to AMP in five and BHR to both stimuli in four of the 14 new-onset cases of symptoms to work allergens. In five of 11 (45%) new-onset cases we observed BHR to methacholine and a positive SPT to work allergens. The combination of BHR to AMP and a positive SPT to work allergens was present in three out of 11 cases (27%) that were all hyper-responsive to methacholine as well.

Table 3 shows the results of regression analyses. The strongest predictor for new-onset airway symptoms to work allergens was a positive SPT to allergens of the work environment at baseline or follow-up. A history of airway symptoms to common allergens and blood hypereosinophilia were also risk factors for new-onset symptoms to work allergens, whereas a positive SPT to common allergens was not. Furthermore, BHR to methacholine and AMP defined by a $PD_{15} \leq 10.0$ and $PD_{15} \leq 90.5$ μM, respectively, were both predictors of new-onset airway symptoms to work allergens. The risk estimate for BHR to AMP was slightly greater than that for methacholine.

The difference between AMP and methacholine responsiveness became more distinct using the DRS classified as 'low', 'intermediate' and 'high'. Comparison of the fit of the models showed that the one including AMP responsiveness explained development of new-onset symptoms better than the model with methacholine responsiveness (likelihood ratios: AMP 19.5, methacholine 13.8; test for difference $P < 0.05$).

To test whether bronchial responsiveness to either stimulus was an independent predictor, both were included in the regression model. The results did not change markedly and again the risk estimate for AMP was greater than for methacholine. Including atopy and blood eosinophilia in the regression models did not yield sensible results due to the few number of cases that were hyper-responsive as well.

Restricting the analyses to the 12 subjects that had developed only nasal symptoms did not change the results essentially, and neither did exclusion of subjects using corticosteroid treatment. Because of the difference in baseline characteristics between the LA-workers and bakery apprentices, we repeated the analyses including a dummy variable for this in the regression models, but the results did not change.

Discussion

In this follow-up study among newly employed LA-workers and bakery apprentices, new-onset airway symptoms (predominantly nasal in origin) to allergens of the work environment were most strongly predicted by allergic sensitization to allergens of the work environment. Increased bronchial responsiveness to either methacholine or AMP were predictors as well, AMP responsiveness being a slightly stronger predictor than methacholine responsiveness.

LA-workers and bakers are at increased risk for developing airway allergy due to allergen exposure at work. In our study, 12% of the population developed new-onset airway symptoms to work allergens, which is in agreement with other follow-up studies in these occupational groups [19–23]. Like others, we found that new-onset rhinitis occurred more frequently than asthma, which may be due to a greater sensitivity of the upper airways [24] or a too short follow-up period to develop asthmatic symptoms. Nevertheless our findings may have important bearings for the future, as it is known that allergic rhinitis may precede asthma [25], particularly if BHR is present as well [26]. Allergic sensitization to allergens of the work environment could be demonstrated in 64% of the new-onset cases in our study, and this pointed out to be the most important risk factor. Because sensitization is presumed to precede symptoms, we defined sensitization to work allergens as a positive SPT at

Table 3. Prevalence, relative risk (RR) and 95% confidence intervals (95% CI) for new-onset airway symptoms to work allergen, crude results and after adjustment for confounding by age, sex and smoking

Baseline characteristic	New-oneset cases, *n* (%)		Crude results	Adjusted for age, sex, smoking
	Yes	No	RR (95% CI)	RR (95% CI)
≥ 1 SPT to work allergen, baseline or follow-up	7 (64)	14 (18)	6.4 (1.9–22.0)‡	7.5 (2.0–28.6)‡
≥ 1 SPT to common allergen	7 (46)	35 (34)	1.6 (0.5–4.6)	1.6 (0.5–4.7)
Airway symptoms to common allergens	7 (50)	27 (26)	2.5 (0.9–7.2)*	4.3 (1.4–12.8)‡
Blood eosinophils ≥ 200/mL	4 (31)	8 (8)	4.5 (1.4–15.0)†	4.4 (1.2–15.4)†
Methacholine responsiveness				
DRS				
Low (≤ 0.72)	4 (29)	35 (34)	1.0 (–)	1.0 (–)
Intermediate (0.72–1.35)	2 (14)	39 (38)	0.4 (0.1–2.2)	0.3 (0.1–1.8)
High (> 1.35)	8 (57)	30 (29)	1.8 (0.6–6.1)	1.8 (0.5–6.4)
$PD_{15} \leq 10$ μMs (DRS ≥ 1.50)	7 (50)	28 (27)	2.4 (0.8–6.9)	2.8 (1.0–8.5)*
AMP responsiveness				
DRS				
Low (≤ 0.03)	1 (7)	41 (39)	1.0	1.0
Intermediate (0.03–0.08)	3 (21)	38 (37)	3.5 (0.4–34.1)	3.2 (0.3–30.8)
High (> 0.08)	10 (71)	25 (24)	11.2 (1.4–87.3)†	12.9 (1.6–102.4)†
$PD_{15} \leq 90.5$ μM (DRS ≥ 0.17)	5 (36)	11 (11)	3.3 (1.1–10.0)†	3.7 (1.1–12.5)*

*$P < 0.10$; †$P < 0.05$; ‡$P < 0.01$.

baseline or developed during follow-up. In our study, the sensitization rate to work allergens among asymptomatic subjects was higher than in other studies [20–22]. This could not be ascribed to a difference in participation to SPTs between subjects with and without new-onset symptoms (83 and 88%, respectively). A likely explanation is the shorter follow-up period in our study compared to those mentioned above. One other study with a similar follow-up period also found a relatively high proportion of work-related sensitization in asymptomatic subjects [27]. It might well be that a number of our subjects will develop symptoms after the study has ended.

We expressed bronchial responsiveness as: (i) BHR if the PD_{15} was smaller than the maximum dose inhaled, and (ii) DRS that was classified as 'low', 'intermediate', or 'high' by its frequency distribution. Using the DRS allows one to investigate all participants for their level of bronchial responsiveness irrespective of the presence of BHR as defined by a fall in FEV_1 of 15% or more. Furthermore, it allows analyses of increased responsiveness at multiple levels compared to a reference group with none or minimal responsiveness. Using the DRS in AMP responsiveness, it may suggest bronchial inflammation yet at a subclinical level [28].

Irrespective whether bronchial responsiveness was expressed as PD_{15} or DRS, AMP (hyper)responsiveness yielded a higher risk estimate than methacholine (hyper)responsiveness for the development of work-related airway symptoms. Interestingly, particularly a joint presence of BHR to AMP and methacholine seemed a predictor of developing symptoms to newly introduced allergens.

The observation that BHR is a risk factor for nasal symptoms may be explained by the anatomical and physiological link between the upper and lower airway tract. A number of studies have shown increased BHR to AMP, and higher levels of exhaled nitric oxide (NO) or sputum inflammatory markers in patients with allergic rhinitis, suggesting bronchial inflammation to be present in upper airway allergy [11, 12]. Because BHR to AMP more closely reflects bronchial eosinophilic inflammation, our results suggest that pre-existent bronchial eosinophilic inflammation increases the vulnerability to develop nasal symptoms to newly introduced allergens. This is in agreement with our observation that blood hypereosinophilia was a risk factor as well. In the baseline survey of this study we observed a positive relationship between AMP responsiveness and blood hypereosinophilia, whereas this was not the case for methacholine responsiveness [13]. However, we did not assess any inflammatory mediator in the bronchi. Although blood eosinophilia is related to BHR and airway symptoms, it does not reflect airway inflammation *per se*. Nevertheless, our results are in agreement with others who have shown that particularly a joint presence of blood hypereosinophilia and BHR comprises a risk factor for new-onset airway symptoms [4].

In our study, we aimed to collect specific data on allergic airway symptoms, by collecting data on symptom character and location, as well as the causal exposure. However, for symptoms due to allergens of the work environment only 64% had positive SPT. False negative reactions cannot be excluded, but more likely the concurrent use of other, non-allergic agents have caused symptoms. Unfortunately, we could not perform separate analyses for subjects with both a positive SPT and new-onset symptoms to work allergens as this occurred in only seven subjects.

We do not think that denial symptoms will have played a relevant role. Reporting symptoms would not have any consequence for a subject's work. Moreover, the informed consent included that personal data were not available for anyone else, including occupational health professionals, without the participant's permission.

Interestingly, allergic sensitization to common allergens did not constitute risk factor for new-onset symptoms. Furthermore, none of the newly sensitized subjects were atopic to common allergens. In the literature, results on the role of atopy to common allergens depend on the study design, length of follow-up, test methods used, and definitions of atopy and work-related symptoms [20–23, 27]. Because only a few follow-up studies have been conducted with standardized methods and design, the role of atopy to common allergens as a risk factor for the development of work-related allergy is still under discussion.

In contrast to allergic sensitization, symptoms to common allergens was a predictor for new-onset symptoms to work allergens. This discrepancy may be explained by the fact that the question regarding this issue was related to a lifetime history of symptoms, whereas SPTs are only related to a point in time. We observed a positive SPT to common allergens in 78% of the subjects reporting to have ever had allergic symptoms.

To increase statistical power, we used combined data of LA-workers and bakery apprentices. Both are at increased risk of developing occupational airway allergy, although exposure to relevant allergens may differ in potency to develop allergic sensitization and concentration or pattern during work. In our study, LA-workers and bakery apprentices differed considerably in baseline characteristics. Nevertheless, it is unlikely that these differences have influenced our results, as adjustment for occupational group did not change the results.

We made an attempt to select subjects with a minimal prior exposure by excluding subjects with more than a 1-year work experience with LAs or bakery products. Because most subjects had been exposed during their studies, we cannot completely exclude selection bias towards 'healthy workers'. As a result of existing privacy legislation baseline measurements in LA-workers had to be conducted after employment. Although the delay did not exceed 4 months, symptoms may have developed during this period leading to an underestimation of the incidence of new-onset symptoms to work allergens. In our study population, three subjects (2%) reported airway symptoms to work allergens at baseline and these were subsequently excluded from the analyses.

In summary, we have demonstrated that within 2 years 12% of bakery apprentices and newly applied laboratory animal workers develop respiratory symptoms related to allergens of the work environment. Specific allergic sensitization was the strongest predictor, followed by blood hypereosinophilia, and a history of airway symptoms to common allergens. BHR was a risk factor as well, and AMP responsiveness seemed a stronger predictor than methacholine responsiveness. Presumably, pre-existent bronchial eosinophilic inflammation predisposes for the development of nasal symptoms to newly introduced allergens. Our results support the hypothesis that allergic rhinitis and allergic asthma may represent similar pathology at least in some patients [25, 26]. Follow-up studies that include bronchial challenge tests with both methacholine and AMP, and inflammatory mediators derived from the bronchi, like exhaled NO,

or markers in induced sputum may unravel more of the association between bronchial (hyper)responsiveness, bronchial inflammation and the development of nasal symptoms.

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