

Increase in Exhaled Nitric Oxide Is Associated with Bronchial Hyperresponsiveness among Apprentices

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Rationale: Airway inflammation is a hallmark of asthma. Several studies have validated the use of the fractional concentration of exhaled nitric oxide (F_{ENO}) as a surrogate marker of airway inflammation in asthma.

Objectives: We examined how the change in F_{ENO} levels, since the beginning of occupational exposure, could be associated with the incidence of bronchial hyperresponsiveness (BHR) among baker, pastry maker, and hairdresser apprentices during their 2-year training.

Methods: A standardized questionnaire was administered; skin prick tests for common and specific occupational allergens were done; methacholine challenge and measurement of F_{ENO} were performed 6, 12, and 15 months after the first examination.

Measurements and Main Results: Of 441 apprentices initially included, 351 completed the study. The increase in F_{ENO} , since the beginning of exposure, was associated with the incidence of BHR (odds ratio, 2.00 [95% confidence interval, 1.21–3.32] per unit increase in log parts per billion) both in atopic and nonatopic subjects. The average increase in F_{ENO} was similar in atopic and nonatopic subjects and was unrelated to past or current smoking habits, sex, or training track. Atopy in bakers/pastry makers and sensitization to alkaline persulfates in hairdressers were also independently associated with the incidence of BHR. BHR occurred sooner among bakers/pastry makers than among hairdressers, but its incidence leveled off later.

Conclusions: Our results suggest that measurement of F_{ENO} , a simple and reproducible test, could be useful in the screening of BHR in workers newly exposed to agents known to cause occupational asthma.

Keywords: occupational asthma; airway inflammation; exhaled nitric oxide; bronchial hyperresponsiveness

The understanding of the inflammatory nature of asthma has encouraged the development of noninvasive, surrogate markers of airway inflammation (1). Among these markers, the fractional concentration of exhaled nitric oxide (F_{ENO}) has been validated and standardized procedures of measurement have been proposed to minimize false results (2). F_{ENO} is increased in subjects with established asthma (3) or bronchial hyperresponsiveness (BHR) to methacholine (4); it is a more accurate

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Increased fractional concentration of exhaled nitric oxide (F_{ENO}) has been reported in subjects exposed to occupational agents known to be risk factors of occupational asthma. However, there is still conflicting evidence regarding the usefulness of F_{ENO} in detecting bronchial hyperresponsiveness (BHR) in an occupational context.

What This Study Adds to the Field

This study shows that in a population of bakery and hairdressing apprentices, an increase in F_{ENO} over a relatively short period of time (15 mo) is correlated with the occurrence of BHR regardless of atopy. Our data suggest that serial F_{ENO} measurement in workers exposed to agents that can cause occupational asthma could detect those at risk of asthma.

detector of asthma than conventional tests, such as peak flow and FEV_1 (5), and it has proved useful to monitor asthma treatment (3). Further, the test is simple and quick to perform, and it has good reproducibility (6) despite modifying factors such as atopy, smoking, or respiratory infections (7). Predicted values have been proposed that take into account several factors including sex, height, smoking habits, and atopic status (8).

BHR is also strongly associated with occupational asthma (OA), in particular after specific challenge tests, and measuring BHR is a validated step for diagnosing OA, even though BHR may occasionally be absent in confirmed OA (9). Increased F_{ENO} values have been reported in symptomatic subjects exposed to occupational agents known to be risk factors for occupational asthma (OA) (10), and also after specific challenge tests for various occupational allergens (11). There is still conflicting evidence regarding the usefulness of F_{ENO} in detecting BHR (1).

In this study, we investigated, in apprentices enrolled in bakery, pastry-making, or hairdressing training programs, whether F_{ENO} levels or their evolution over a 2-year follow-up since inception of exposure to airborne allergens or irritants would be predictive of changes in BHR to methacholine. Some of the results of this study have been previously reported in the form of an abstract (12).

METHODS

For details, see the online supplement. Apprentice bakers, pastry makers, and hairdressers starting career programs in six vocational schools were invited to participate. Subjects were included provided they had neither been previously exposed to substances known to induce OA, nor had physician-diagnosed asthma. The study was

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approved by the local ethics committee (the Comité de Protection des Personnes) and both apprentices and parents (for students less than 18 yr old) gave written consent. Examinations were performed, on average, within 3 months of the beginning of apprenticeship and 6, 12, and 15 months thereafter.

Clinical Examination and Skin Prick Tests

A standardized questionnaire was used for personal and demographic information and smoking habits (13). Atopy, assessed at entry and end of study, was defined as the presence of a positive skin reaction to common allergens. Sensitization to baking- and hairdressing-related antigens was also tested.

Functional Respiratory Measurements and Definition of BHR

Spirometry was performed according to the recommended criteria (14). BHR was assessed by methacholine challenge test (MCT), using three cumulative successive doses (0.5, 3.0, and 8.0 μ mol) as previously described (15). The methacholine test was considered positive (MCT⁺) if FEV₁ decreased by at least 20% below the baseline value and the corresponding provocative concentration (PC₂₀) was recorded. According to previous publications (16) a linear two-point dose-response slope (DRS) was then calculated. To avoid zero or negative values, a constant of +2.5 was added to all DRS values and the values were normalized as NDRS = 1/(slope + 2.5). "BHR incidence," taken as the main outcome, was defined according to the following criteria of occurrence or aggravation:

- Occurrence of a positive MCT (MCT⁺) at any visit in subjects with a negative MCT (MCT⁻) at inclusion
- Decrease in the PC₂₀, by at least one dose in subjects with MCT⁺ at the first visit
- Decrease in the NDRS by 0.100 or more at any visit compared with the NDRS measured at the first visit, in subjects exhibiting at least a decrease of 15% in FEV₁, whatever the visit; the 0.100 cutoff point corresponds to the mean decrease in all MCT⁺ subjects

F_{ENO} was measured in accordance with the recommended criteria (17). In particular, subjects with a recent respiratory infection were excluded from the F_{ENO} tests. Results were expressed as parts per billion (ppb) and as a percentage of the predicted values presented by Travers and colleagues (8), which take into account sex, smoking habits, and atopy.

Statistical Analysis

The Stata 10 package (18) was used. Comparisons of continuous variables were performed by mixed-models analysis with random

intercepts on log-transformed F_{ENO} measurements (expressed as parts per billion or as a percentage of predicted values). Evolution of F_{ENO} was calculated as the difference between log F_{ENO} values at the visit during which BHR appeared the first time (last visit for subjects without BHR) and log F_{ENO} values at first visit. For BHR, incidence rates over all subjects and according to the training track were calculated at each visit (19). Logistic regression was performed to assess the relationship between BHR incidence and F_{ENO} evolution while taking into account the training track, smoking, and atopy; effect modification was tested for each of these factors.

RESULTS

The mean (SD) age of the apprentices at the beginning of their training program was 17.5 $\pm$ 1.4 years (Table 1). Mean duration of exposure was 15 months since beginning of the training program. The number of subjects seen at enrollment and still present at the last visit was 351 (80% of inclusions). Of 90 apprentices who did not attend the last visit, 77 (86%) were interviewed by telephone and justified their absence by lack of time and departure from the vocational school; only 1 said he had a respiratory condition. The remaining 13 apprentices were not found, probably because of change of name after marriage (for women) or because they had moved away (*see* Table E1 in the online supplement for details).

F_{ENO} was missing, either because of technical problems or because it had not been measured because of a recent infection, for 69 subjects (16.5%) at either the first or the last visit. F_{ENO} values were higher among men, atopic subjects, nonsmokers, and ex-smokers (Table 2); this remains true for values expressed as a percentage of the predicted value, even among atopic subjects although atopy is taken into account in calculating the predicted values. Overall, the median values of F_{ENO} showed little variability over the study and were less than 100% of predicted. However, BHR⁺ subjects exhibited higher F_{ENO} values than those whose methacholine test was (at first visit) or remained negative, with values higher than 100% of predicted at the last visit.

Among the 76 BHR⁺ subjects at the end of follow-up (17% cumulative incidence), 51 subjects (67%) did not have BHR at enrollment ("new-onset BHR") and 25 subjects (33%) had experienced a worsening of their initial BHR. Figure 1 shows the BHR incidence rates by training track. Figures for bakers

TABLE 1. CHARACTERISTICS OF STUDY SUBJECTS AT INCLUSION AND DURING FOLLOW-UP

	Bakers	Pastry Makers	Hairdressers	Total
Number of subjects, n	161	111	169	441
Men, n (%)	152 (94.4%)	87 (78.4%)	12 (7.1%)	251 (56.9%)
Women, n (%)	9 (5.6%)	24 (21.6%)	157 (92.9%)	190 (43.1%)
Atopy at inclusion, n (%)	56 (34.8%)	42 (37.8%)	43 (25.4%)	141 (31.9%)
Age at beginning of training program (yr), mean (SD)	17.5 (1.4)	17.4 (1.2)	17.6 (1.5)	17.5 (1.4)
Smoking status at inclusion				
Nonsmoker, n (%)	77 (47.8%)	58 (52.25%)	86 (50.89%)	221 (50.1%)
Current smoker, n (%)	79 (49.1%)	52 (46.85%)	75 (44.38%)	206 (46.7%)
Ex-smoker, n (%)	5 (3.1%)	1 (0.90%)	8 (4.73%)	14 (3.2%)
Pack-years, mean (SD) (in smokers and former smokers only)	1.5 (1.3)	1.9 (1.9)	1.6 (1.6)	1.7 (1.6)
Lost to follow-up, n (%)	39 (24.2%)	24 (21.6%)	27 (15.9%)	90 (20.4%)
Number of visits per subject, mean (SD)	3.2 (0.9)	3.3 (0.8)	3.5 (0.7)	3.3 (0.8)
Number of lung function tests per subject, mean (SD)	3.0 (0.9)	3.2 (0.8)	3.3 (0.7)	3.2 (0.8)
Number of F _{ENO} measurements per subject, mean (SD)	3.0 (1.0)	3.1 (0.9)	3.2 (0.8)	3.1 (0.9)
Number of methacholine challenge tests per subject, mean (SD)	3.0 (1.0)	3.2 (0.8)	3.3 (0.8)	3.2 (0.9)
Number of skin prick test sessions per subject, mean (SD)	1.6 (0.6)	1.6 (0.6)	1.6 (0.6)	1.6 (0.6)
Smoking status at last examination				
Nonsmoker, n (%)	65 (40.4%)	49 (44.1%)	76 (45.0%)	190 (43.1%)
Current smoker, n (%)	93 (57.8%)	61 (54.9%)	85 (50.3%)	239 (54.2%)
Ex-smoker, n (%)	3 (1.8%)	1 (0.90%)	8 (4.7%)	12 (2.7%)
Incidence of sensitization to occupational agents at the end of the follow-up, n (%)	19 (11.8%)	9 (8.1%)	7 (4.1%)	35 (7.9%)

TABLE 2. FRACTIONAL EXHALED NITRIC OXIDE CONCENTRATIONS STRATIFIED BY SEX, ATOPY, SMOKING STATUS, AND METHACHOLINE AIRWAY CHALLENGE AT EACH VISIT

Parameter	V1 (n = 441)	V2 (n = 315)	V3 (n = 381)	V4 (n = 351)	P Value*
Sex					
Female, n (%)	190 (43.1%)	137 (43.5%)	171 (44.9%)	157 (44.7%)	Sex
FE _{NO} , ppb	11.8 [8.1–17.1]	10.6 [7.8–15.5]	12.2 [8.8–18.6]	10.8 [8.1–16.5]	P value (ppb) < 0.0001
FE _{NO} , % predicted	77.8 [54.1–103.4]	68.0 [51.1–99.3]	79.5 [57.6–117.5]	71.8 [50.1–109.5]	P value (% predicted) = 0.551
Male, n (%)	251 (56.9%)	178 (56.5%)	210 (55.1%)	194 (55.3%)	
FE _{NO} , ppb	13.9 [9.5–20.7]	13.6 [9.0–20.0]	14.8 [10.6–25.5]	14.4 [9.7–23.7]	
FE _{NO} , % predicted	68.0 [48.1–94.0]	65.2 [47.7–101.8]	79.7 [54.7–119.7]	74.8 [49.9–119.7]	
Atopy					
Missing data	19 (4.3%)	5 (1.6%)	9 (2.4%)	2 (0.57%)	Atopy
Nonatopic, n (%)	281 (63.7%)	209 (66.4%)	246 (64.6%)	232 (66.1%)	P value (ppb) < 0.0001
FE _{NO} , ppb	11.7 [8.1–15.4]	10.7 [7.9–15.3]	12.7 [9.5–18.2]	12.3 [8.6–16.4]	P value (% predicted) < 0.0001
FE _{NO} , % predicted	66.1 [48.6–89.5]	63.6 [48.1–87.4]	75.6 [57.0–104.5]	70.0 [51.1–96.8]	
Atopic, n (%)	141 (32.0%)	101 (32.1%)	126 (33.1%)	117 (33.3%)	
FE _{NO} , ppb	18.5 [11.0–34.5]	16.3 [11.1–36.5]	19.7 [10.7–42.6]	17.4 [9.6–34.5]	
FE _{NO} , % predicted	85.2 [55.2–169.5]	80.5 [55.4–186.8]	96.1 [53.1–200.9]	86.9 [48.3–156.4]	
Smoking status					
Nonsmokers and ex-smokers, n (%)	235 (53.3%)	163 (51.6%)	185 (48.6%)	170 (48.4%)	Smoking status
FE _{NO} , ppb	15.1 [11.0–22.0]	14.6 [9.5–22.5]	16.0 [10.5–23.0]	14.7 [10.3–22.1]	P value (ppb) < 0.0001
FE _{NO} , % predicted	79.1 [56.1–107.6]	73.6 [53.7–110.7]	80.2 [54.7–120.5]	75.0 [52.6–115.6]	P value (% predicted) = 0.223
Smokers, n (%)	206 (46.7%)	152 (48.3%)	196 (51.4%)	181 (51.6%)	
FE _{NO} , ppb	10.2 [7.6–15.2]	9.8 [7.2–14.6]	12.4 [9.0–19.7]	11.2 [7.1–17.9]	
FE _{NO} , % predicted	62.1 [45.3–91.9]	59.2 [47.0–89.3]	78.8 [56.4–119.4]	72.1 [44.6–117.4]	
Methacholine airway challenge (MCT)					
Missing data	11 (2.5%)	26 (8.3%)	13 (3.4%)	19 (5.4%)	Methacholine airway challenge
MCT ⁺ , n (%)	404 (91.6%)	271 (86.0%)	333 (87.4%)	308 (87.8%)	P value (ppb) = 0.036
FE _{NO} , ppb	12.5 [8.8–18.1]	12.4 [8.6–19.1]	13.3 [9.6–20.3]	13.1 [8.9–20.1]	P value (% predicted) = 0.060
FE _{NO} , % predicted	72.5 [50.6–98.5]	67.8 [49.7–101.8]	79.3 [55.2–119.4]	72.4 [50.8–113.8]	
MCT ⁺ , n (%)	26 (5.9%)	18 (5.7%)	35 (9.2%)	24 (6.8%)	
FE _{NO} , ppb	16.4 [10.3–35.0]	13.0 [8.2–48.1]	17.1 [11.0–26.6]	23.0 [10.4–41.0]	
FE _{NO} , % predicted	79.2 [48.6–174.5]	71.0 [48.4–208.7]	78.3 [61.7–150.7]	115.9 [57.6–230.3]	

Definition of abbreviations: FE_{NO} = fractional exhaled nitric oxide concentration; V1–V4 = first to fourth visit.

Data for FE_{NO}, expressed as parts per billion or as a percentage of the predicted value (8), are presented as medians with the 25th–75th percentiles in square brackets.

* P values for a common main effect in all four visits in a mixed models analysis with random intercepts on log-transformed FE_{NO} measurements.

and pastry cooks are similar (see Figure E1), and were merged. In the latter group, incidence rates were high soon after inception of exposure (31.9 cases per 100 person-years [95% confidence interval, 18.3–51.9]) and then decreased with time. Thus, at the first visit, the incidence of BHR among bakers and pastry cooks was significantly higher than among hairdressers (20.6 case per 100 person-years [95% confidence interval, 1–40]), but later the figures were similar for the two groups.

Table 3 presents the FE_{NO} levels at the first and last visits and their relative evolution according to BHR and atopic status. Median FE_{NO} did not vary among subjects who remained BHR[−]; in contrast, FE_{NO} increased in subjects who experienced occurrence or aggravation of BHR during the follow-up. Among these “reactive” individuals, the relative increase in FE_{NO}, calculated as the difference in FE_{NO} between the first and last visits divided by the first visit value, amounted to 20 and 16% for nonatopic and atopic subjects, respectively. Table 4 presents the results of a logistic regression model to study factors that influence the incidence of BHR during the follow-up. Two different models are presented. Model 1 presents the association with increase in FE_{NO}, atopy, and sensitization, adjusting for smoking, sex, and training tracks. The second model introduces interaction terms between training tracks and atopy or sensitization to occupational allergens, respectively. Table 4 shows that BHR is associated with the increase in FE_{NO} values, with no effect modification by atopy ($P = 0.95$); likewise, the smoking status did not influence the association between FE_{NO} concentrations and BHR (P for interaction = 0.35). Sensitization to occupational allergens is associated with BHR incidence among hairdressers, but not among bakers and pastry cooks. Conversely, BHR is more frequent among atopic

than among nonatopic bakers and pastry cooks, but not among atopic hairdressers.

DISCUSSION

The main objective of this article was to study in a population of bakery, pastry cooking, and hairdressing apprentices whether the change in FE_{NO} levels was associated with the incidence of BHR to methacholine, during 15 months of occupational exposure. Our results show that the increase in FE_{NO} levels is highly correlated with occurrence of BHR during this short follow-up period, regardless of atopy. Atopy at enrollment in the training program was associated with the incidence of BHR among bakers and pastry makers, but not among hairdressers.

Older studies in bakers and hairdressers focused on subjects with prevalent OA, using cross-sectional designs. In 1997, De Zotti and colleagues (20) published the first study of bakery apprentices reporting that, after 6 months of follow-up, work-related respiratory symptoms were significantly higher in subjects with atopy or sensitization to occupational allergens. The same year, Gautrin and colleagues reported an association between specific sensitization and atopy and BHR among 769 apprentices in animal health or veterinary medicine, pastry making, and dental hygiene (21). Thereafter, several longitudinal studies were published dealing with apprentices exposed to either low molecular weight (LMW) or high molecular weight (HMW) agents, to better understand the determinants of the occurrence of occupational sensitization and OA (4, 22–32). In general, these studies had similar methodology except for the follow-up duration and measured parameters. To the best of our

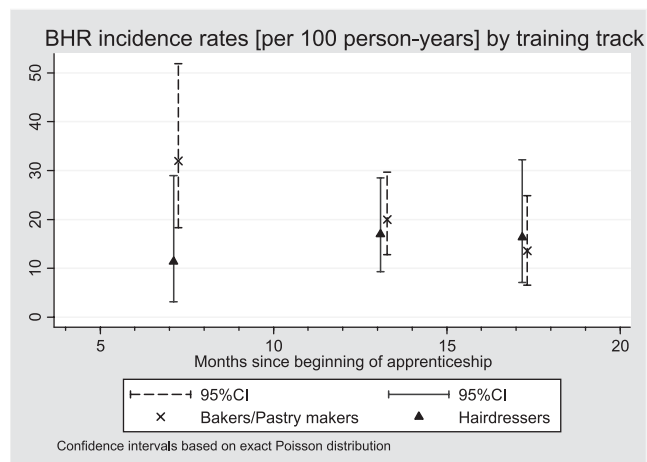


Figure 1. Incidence rate of bronchial hyperresponsiveness (BHR) during apprenticeship according to the training track ($n = 418$; see METHODS for the definition of BHR).

knowledge, our study is the first to use F_{ENO} to assess airway inflammation in a cohort of apprentice bakers and hairdressers.

F_{ENO} is known to be associated with BHR in adults (33). Several factors influence this association, including atopy (34), the degree of sensitization to common allergens (35), and the existence of respiratory symptoms (4). In a cross-sectional study of 7- to 12-year-old school children, Steerenberg and colleagues found that, in comparison with children without symptoms, blood eosinophilia, or BHR, a relative increase of 1.55 ppb of F_{ENO} was positively associated with BHR in atopic but not in nonatopic children (34). Olin and colleagues reported that the increase in F_{ENO} in a sample of 1,506 healthy subjects was associated with an increased risk of developing wheeze over a follow-up of 4 years (36). No clear results were observed after specific inhalation challenges to occupational allergens, although some authors suggested an association with a positive late response, depending on allergens (10, 11, 37) and on basal F_{ENO} levels (38). Piipari and colleagues measured F_{ENO} during specific challenge tests to assess occupational asthma in 40 patients and found a significant increase among patients having

a normal or slightly increased (<14.5 ppb) basal F_{ENO} level and a late (i.e., >1 h) bronchoconstriction, but not among patients having a high basal F_{ENO} level (>14.5 ppb) and bronchoconstriction, either immediate or late (38).

In our study, which concerns nonasthmatic subjects, we found an association between F_{ENO} evolution and incidence of BHR in bakery, pastry cooking, and hairdressing apprentices, suggesting that the observed correlation is independent of the high or low molecular weight nature of the agent. Obviously, these results need to be confirmed by further studies involving different occupational allergens. The correlation between F_{ENO} and BHR was equally observed in nonatopic and atopic subjects, the former having a higher basal level of F_{ENO} , however. This finding is in contrast to the aforementioned studies, a discrepancy that might be explained by differences in the study populations (34), study designs (39), or sample sizes (38). In the present work, the average F_{ENO} values were lower than 100% predicted according to Travers and colleagues (8). However, these predicted values are based on subjects aged 25 to 75 years taken from the general population, whereas our study concerns younger subjects in an occupational setting. No other study, to our knowledge, accounts for age. We are not aware of F_{ENO} predictive values adapted to the population we studied. We also reanalyzed our data with the adoption of a more sensitive definition of BHR, that is, using a threshold of 15% for the decrease in FEV_1 (PC_{15}), as has been sometimes proposed for screening purposes (40). However, this did not modify our main findings. Thus, our results support the view that measuring F_{ENO} may be a useful strategy to screen airway inflammation in occupational epidemiology.

Atopy is a well-known risk factor of work-related sensitization, BHR or OA. Our observed prevalence of atopy, 29%, is similar to the figures (29–34%) reported in European studies (30, 41). Some studies of apprentice bakers (23, 30, 32) showed atopy to be a risk factor for work-respiratory symptoms or OA, whereas others did not (42). Similar associations were also reported for subjects exposed to other HMW allergens such as laboratory animals (43), although less consistent results were also reported (25, 39, 44). On the other hand, the role of atopy in the response to exposure to LMW agents (e.g., isocyanates) (45) is thought to be weak. Our results show a strong and significant association between atopic status, defined as a positive response to skin prick testing for common allergens at

TABLE 3. FRACTIONAL EXHALED NITRIC OXIDE VALUES AT FIRST AND LAST VISITS AND THEIR RELATIVE INCREASE ACCORDING TO ATOPY AND RESPONSE TO METHACHOLINE CHALLENGE TEST

	First Visit	Last Visit*	Relative Increase
Negative BHR			
Atopic ($n = 81$)			
F_{ENO} (ppb)	20.4 [11.2–37.6]	18.8 [12.0–34.5]	–2% [–28%, 20%]
F_{ENO} (% predicted)	91.5 [55.3–171.0]	94.3 [54.8–157.9]	–2% [–27%, 22%]
Nonatopic ($n = 196$)			
F_{ENO} (ppb)	12.1 [8.4–16.0]	12.5 [8.3–17.0]	5.6% [–21%, 40%]
F_{ENO} (% predicted)	71.5 [51.3–90.1]	69.3 [51.8–97.6]	6.6% [–20%, 45%]
Incident BHR			
Atopic ($n = 25$)			
F_{ENO} (ppb)	17.8 [13.8–29.2]	18.5 [12.3–48.0]	16% [–12%, 41%]
F_{ENO} (% predicted)	83.5 [57.4–126.8]	93.2 [57.5–208.7]	17% [–12%, 45%]
Nonatopic ($n = 37$)			
F_{ENO} (ppb)	9.8 [7.5–14.8]	12.5 [8.6–18.1]	20% [–12%, 100%]
F_{ENO} (% predicted)	57.6 [37.5–89.7]	69.6 [55.2–101.9]	20% [–12%, 100%]

Definition of abbreviations: BHR = bronchial hyperresponsiveness; F_{ENO} = fractional exhaled nitric oxide concentration.

Data for F_{ENO} , expressed as parts per billion or as a percentage of the predicted value (8), are presented as medians with the 25th–75th percentiles in square brackets.

* Last visit among negative BHR subjects = true last visit during the follow-up; last visit among incident BHR subjects = visit at which BHR first appeared.

TABLE 4. FACTORS ASSOCIATED WITH INCIDENCE* OF BRONCHIAL HYPERRESPONSIVENESS AMONG APPRENTICES

Variable	Model 1		Model 2	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Increase in F _{ENO} between first and last visit, per unit expressed in log ppb	1.94 (1.18–3.20)	<0.01	1.99 (1.20–3.29)	<0.01
Atopy	1.55 (0.82–2.92)	0.17		
Among bakers/pastry makers			2.37 (1.19–4.70) [†]	0.01
Among hairdressers			0.51 (0.13–2.04) [†]	0.34
Sensitization to occupational agents	1.48 (0.63–3.46)	0.35		
Among bakers/pastry makers			0.95 (0.35–2.55) [‡]	0.92
Among hairdressers			4.29 (0.86–21.40) [‡]	0.08
Active smoking at last visit	0.75 (0.42–1.33)	0.32	0.73 (0.41–1.30)	0.28
Bakers/pastry makers vs. hairdressers	1.62 (0.63–4.20)	0.32		
Female vs. male	1.38 (0.55–3.44)	0.48		

Definition of abbreviations: CI = confidence interval; F_{ENO} = fractional exhaled nitric oxide concentration; OR = odds ratio.

* Incidence of BHR as defined in METHODS.

[†] Test for interaction between atopy and training track significant ($P = 0.04$).

[‡] Test for interaction between sensitization to occupational agents and training track ($P = 0.13$).

baseline, and the incidence of BHR within 15 months of follow-up in bakery and pastry-making, but not in hairdressing, apprentices. This observation is in line with the current opinion, according to which atopy is a risk factor for OA or occupational sensitization induced by HMW but not LMW agents (46). Evidence of effects of exposure on sensitization have been described for HMW agents including flour dust, and LMW agents, such as platinum salts and acid anhydrides (47). Moreover, some experimental findings have demonstrated that dermal sensitization in rodents before specific challenges to toluene diisocyanate (48) enhances methacholine responsiveness. Some authors have also shown that sensitization to specific allergens is a risk factor for BHR during specific inhalation challenge (49). This was observed in occupational settings, including in apprentice cohorts (21, 26, 50). Surprisingly, our results confirm this association for hairdressers, with occupational sensitization to persulfate (an LMW agent) showing an almost significant association with BHR, but not for bakery allergens. We offer no explanation for this finding, which deserves further investigation.

The incidence rate was highest in the first months and decreased up to the end of follow-up in subjects exposed to HMW agents and was stable for subjects exposed to LMW agents. This could relate to differences in the mechanism of sensitization between LMW and HMW agents (46). However, differences in incidence rates may also be partly explained by exposure levels in the two vocational groups as measured in our population (51, 52). For bakery and pastry makers, mean particulate concentrations ranged between 0.50 and 0.71 mg·m⁻³ for PM_{2.5} and between 0.63 and 1.10 mg·m⁻³ for PM₁₀, that is, levels exceeding the value of 0.5 mg·m⁻³ recommended by the American Conference of Governmental Industrial Hygienists for the prevention of sensitization. By contrast, personal exposures in hairdressers were lower than existing occupational threshold limit values for three agents, namely H₂O₂, NH₃, and persulfates, with concentrations of 0.05 (SD, 0.04), 0.90 (SD, 0.76), and 0.016 (SD, 0.021) mg·m⁻³, respectively.

One limitation of this study is the time at which the first visit took place. Because of the procedures required to obtain informed and signed consents from parents and volunteer apprentices, 85% of subjects attending the first examinations, could not be first examined before 3 months after starting the training program. This might explain why 22 subjects (13 bakers, 5 pastry cooks, and 4 hairdressers, representing 6.4% of our cohort) were sensitized to occupational allergens at the first visit. Early sensitization rates ranging from 0.5 to 1.7%

were reported previously in bakery and other apprentices (21, 30, 32, 41). However, the main consequence of this delay is likely to be an increase in the percentage of subjects with BHR at inclusion, and a reduction of the statistical power of the study. So we assume that this limitation had no substantial effect on our main results.

Our 12.9% cumulative incidence of sensitization at end-study was greater than the 6.1% (over 20 mo) described by Skjold and colleagues (30), who explained their low incidence by a possible poor sensitivity of the allergenic extracts used for the skin prick tests (30). In a cohort study of flour-exposed workers, Cullinan and colleagues found a sensitization rate to flour of 2.2 cases per 100 person-years (42). On the basis of cross-sectional studies, Jacobs and colleagues (53) and Houba and colleagues (54) reported prevalence rates of 12% (among bakers aged 40 yr on average) and 10% (in the baking industry) for sensitization to flour allergen.

Cigarette smoking could have influenced our results, and previous studies have examined the influence of smoking on BHR, yielding contradictory results (27, 55). The association between F_{ENO} and BHR persisted after adjustment on chronic and current smoking status, as well as on CO measurements (data not shown), which included the changes in smoking status that occurred in some apprentices. One should note that our subjects were young and that their smoking consumption, on average 1.7 pack-years, was probably too small to have an impact on BHR.

The number of subjects lost to follow-up was reasonably low (about 20%) for this kind of longitudinal study. Higher dropout numbers were reported by Skjold and colleagues (54.3%) (30), De Zotti and colleagues (62.4%) (41), and Walusiak and colleagues (37.7%) (32). Our low figures illustrate the effectiveness of our enrollment procedure (56) and the acceptability of the examinations. The proportion of smokers was the only feature that differed between subjects lost to follow-up and those remaining in the study, thus suggesting little selection bias in our prospective study.

Last, we chose to study BHR instead of asthma, and this obviously limits our conclusions. Even if BHR and OA are strongly linked, discrepancies exist in the literature as asthma may be present in the absence of BHR and vice versa. Moreover, BHR may disappear after cessation of exposure to occupational allergens, especially if cessation occurs soon after the beginning of exposure and start of symptoms (9). A longer survey is required to evaluate whether or not the BHR changes observed in our study predict the later occurrence of OA in these subjects. Moreover, further analyses are also warranted to assess the

predictive value of F_{ENO} changes (alone or in combination with clinical or functional features) relative to BHR incidence.

Conclusions

This study shows that, in a population of bakery and hairdressing apprentices, an increase in F_{ENO} over a relatively short period of time (15 mo) is highly correlated with the occurrence of BHR regardless of atopy. Our data give support to the idea that serial F_{ENO} measurements in workers starting exposure to agents known to cause OA could help detect those who develop BHR and, as a consequence, are at risk of asthma. Further studies, including consideration of ethical aspects of such screening, in other occupational settings are warranted to confirm this observation.

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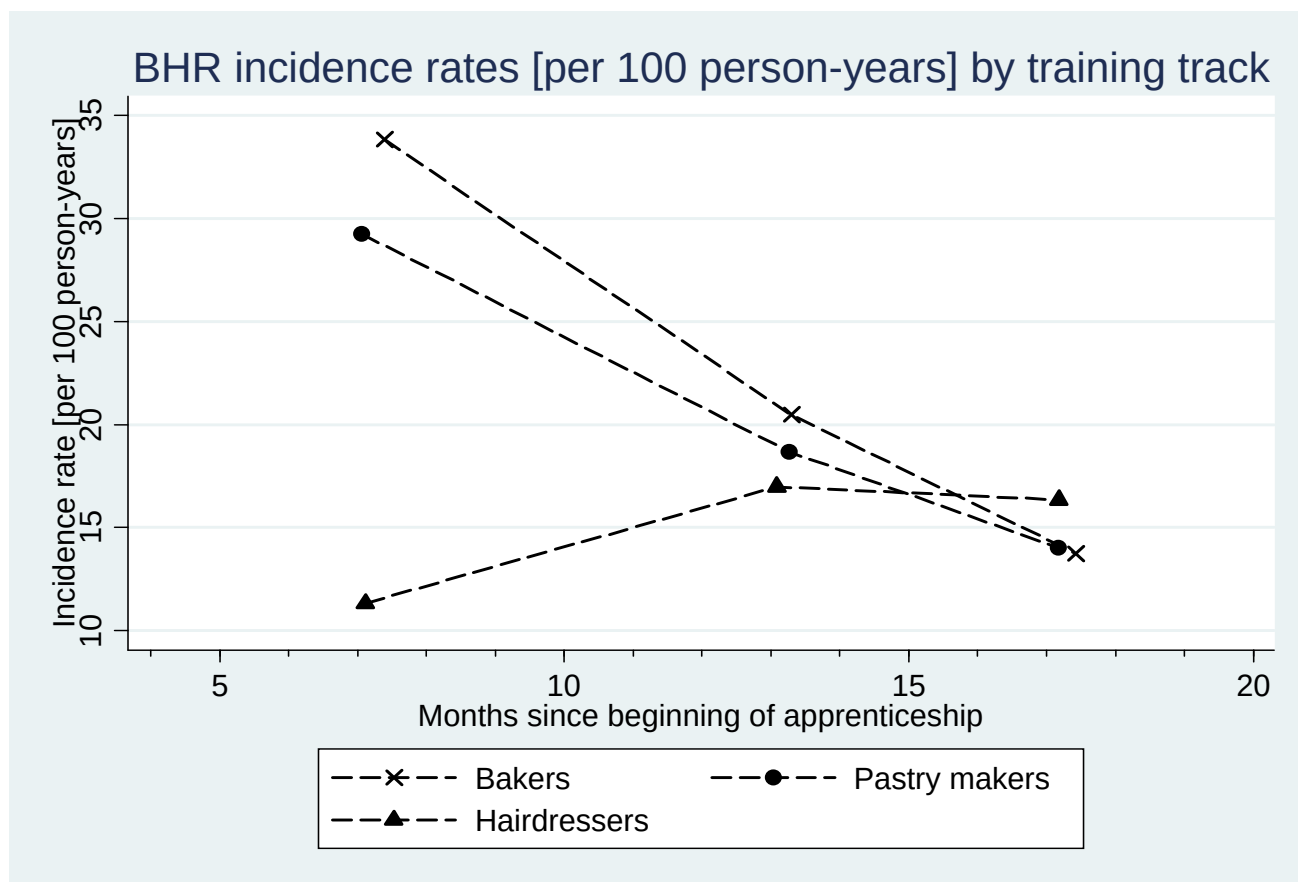
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Increase of Exhaled Nitric Oxide is Associated with Bronchial Hyperresponsiveness among Apprentices

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Online Data Supplement

Figure E1: Incidence rate (per 100 person-years) of BHR* along the training program according to the training track (bakers and pastry makers showed separately) (n=418)



* Incidence of BHR as defined in "Material and Methods"