

ORIGINAL ARTICLE

Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers

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ABSTRACT

Objective Various studies of the usefulness of fractional exhaled nitric oxide (FeNO) in occupational settings remain inconclusive. The objective was to investigate the determinants of increased FeNO in bakery workers.

Methods A cross-sectional study of 424 supermarket bakery workers used a questionnaire and serum specific IgE to wheat, rye and α -amylase. FeNO during the work shift were assessed using a hand-held portable sampling device (NIOX MINO).

Results The median FeNO was 15 ppb, in atopics 21 ppb and current smokers 12 ppb. Increased FeNO was strongly associated with IgE to wheat independent of smoking and atopy status. In the multivariate model, IgE to wheat, current smoking, atopy and age were significantly associated with FeNO. Stratified analysis in a subgroup of atopic non-smokers demonstrated the strongest relationship between FeNO and various clinical endpoint such as wheat (OR=9.43) or rye (OR=11.76) sensitisation, work-related allergic rhinitis (OR=8.13) or asthma (OR=5.44), and probable baker's asthma (OR=6.72).

Conclusions Sensitisation to cereal flour allergens rather than asthma symptoms is a major determinant of elevated FeNO among bakers. This relationship is modified by atopy and current smoking status.

BACKGROUND

Various studies have demonstrated that the measurement of fractional exhaled nitric oxide (FeNO) is a useful non-invasive method for assessing eosinophilic inflammatory airway disease.¹ It is widely accepted that airway inflammation is associated with occupational asthma and rhinitis; however, the sensitivity and specificity of FeNO have not been sufficiently assessed in occupational settings.² While the predictive value of FeNO has been shown to be higher than spirometry or peak expiratory flow,³ more studies are required to examine the effectiveness of FeNO in the early detection of respiratory disease due to workplace exposures.⁴

There is currently conflicting evidence in the literature concerning the role of FeNO in occupational asthma, and hence the need to clarify the interpretation of changes in FeNO following exposure to occupational agents.⁴ FeNO has been demonstrated as a useful marker of exposure to gases and dusts in construction workers,⁵ organic solvents in leather workers⁶ and among aluminium potroom workers.⁷ Exhaled nitric oxide is also reported to be elevated

What this paper adds

- ▶ Studies of the relationship between FeNO and baker's allergy and asthma are scant.
- ▶ This is the first detailed analysis of factors associated with changes in FeNO in supermarket bakery workers known to be at an increased risk of developing occupational allergy and asthma.
- ▶ This study demonstrated that sensitisation to cereal flour allergens, rather than asthma symptoms, is a major determinant of elevated FeNO among bakers.
- ▶ The strong relationship between FeNO and sensitisation to cereal flour allergens is modified by atopy and current smoking status.

in workers exposed to organic dusts,⁸ laboratory animal workers⁹ and workers in swine confinement buildings.¹⁰ The FeNO increase in the latter group was demonstrated 5 h after exposure. In another study, Baur and Barbinova¹¹ demonstrated a greater than 50% increase in asthmatic healthcare workers 22 h after specific inhalation challenge to latex. However, other studies on the impact of swine dust exposure showed no apparent effect on FeNO¹² while other studies showed no clear relationship between FeNO in subjects exposed to latex or isocyanates.¹³ Recently, a strong association was reported in a subgroup of non-smoking, non-atopic farmers and agricultural processing workers exposed to endotoxin.¹⁴ Studies have also demonstrated an increase in FeNO in relation to immunoglobulin (Ig)E sensitisation^{15 16} and allergen exposure,¹⁷ and a decrease in FeNO with allergen avoidance.¹⁸ A recent review concluded that FeNO is a signal of allergen-triggered Th2-driven inflammatory mechanisms within the bronchial mucosa and can be considered as a marker of allergen exposure in sensitised subjects.¹⁹

Studies on the relationship between FeNO and asthma outcomes in bakers are scant. A study among bakers, farmers and healthcare workers showed a significant increase in FeNO only 24 h after a specific inhalation test²⁰ while a 15-month follow-up study among apprentice bakers demonstrated that an increase in FeNO correlated with the occurrence of bronchial hyperresponsiveness.²¹ Recently, a study by Pedrosa *et al*²² demonstrated an increase in

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Workplace

FeNO levels 24 h after allergen exposure in subjects with a positive bronchial allergen challenge with high-molecular weight agents, including cereal flour.

The aim of this study was to evaluate the determinants of FeNO for various clinically relevant endpoints in baker's allergy and asthma.

METHODS

Study design, population and sampling

A cross-sectional study was conducted on 424 workers in 31 supermarket bakeries of a supermarket chain store in the Western Cape province of South Africa. This is a follow-up investigation of a previous study of 517 workers originally assessed as part of a medical surveillance programme for baker's allergy and asthma. At follow-up 3 years later, 93 workers from the original cohort were no longer working in the bakery and were not traceable for further evaluation. Ethical clearance of the protocol was obtained from the University of Cape Town prior to the study being conducted.

Questionnaire

Each worker answered a questionnaire which included a history of recent chest infections, asthma symptoms, rhinitis and work-related respiratory symptoms. Information was also obtained on alcohol use, medication use, exercise and dietary history (nitrate rich foods). Smoking status was classified into two categories, that is, non-smoker as lifelong abstinence from smoking and current smoker (currently smoking for as long as a year). Symptoms were considered to be work-related if they were reported to have worsened during the work shift as reported previously.²³

Immunological tests

Quantification of serum specific IgE antibodies CAP-FEIA (fluorescence enzyme immuno assay) was performed using the ImmunoCAP 100 System (Phadia, Uppsala, Sweden) using wheat flour (f4), rye flour (f5) and fungal α -amylase (k87) according to the manufacturer's instructions. The presence of atopy in workers who did not undergo skin prick tests (SPTs) was defined by a positive Phadiatop test (ImmunoCAP 100 System; Phadia). An ImmunoCAP result of ≥ 0.35 kU_A/l was regarded as positive.²³

SPTs were performed using the following standard common local aeroallergens (ALK-Abello' A/S, Horsholm, Denmark): house dust mite (*Dermatophagoides pteronyssinus*), bermuda grass (*Cynodon dactylon*), rye grass (*Lolium perenne*), grass mix (Pollen III: Avena, Hordeum, Triticum, Secale), cockroach (*Blattella germanica*), cat (*Felis domesticus*), dog (*Canis familiaris*), mould mix (*Cladosporium herbarum*, *Alternaria alternata*, *Fusarium*) and *Aspergillus* (*Aspergillus fumigatus*) as previously reported.²³ For the purposes of this study, atopy was considered to be present if the SPT to one or more common aeroallergens was positive.

Exhaled FeNO tests

A hand-held portable sampling device (NIOX MINO) was used to determine FeNO during the work shift according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.^{24 25} The tests were conducted during the work shift at the various supermarket bakery stores in a distant room removed from the bakery area. Testing of workers occurred throughout the working week and work shift, with no particular variation with regard to time of testing for the different jobs. Three technically adequate FeNO were measured and

an average determined. Special instructions were given to workers to refrain from smoking, eating or drinking (at least 1 h) before the test. This was confirmed prior to testing, and those who did not follow the instructions were tested at a later stage after establishing their full compliance with these instructions.

Statistical analysis

Statistical analysis was performed using STATA V8.²⁶ Linear regression models were developed to describe the determinants of variability in FeNO. The natural logarithm (ln) of the measured FeNO average was used as the dependent variable since the data followed a log-normal distribution. Multiple linear regression was performed with variables that had an r^2 value of 0.01 or greater and a p value of 0.1 or less in the univariate analyses. Simultaneous quantile regression was also performed to determine the effect of the predictor variables for different levels (centiles) of FeNO. A p value of 0.10 was used as criterion to determine the best predictive model for FeNO, using stepwise forward and backward model selection procedure. This analysis included all predictor variables for FeNO that were significant in the univariate regression analysis, which resulted in some variables not remaining in the final model. The strength of the associations were summarised using multiple logistic regression stratified by atopy and smoking, with a cut-off of >25 ppb defined as abnormal.²⁵

RESULTS

Characteristics of subjects

A total of 424 currently employed workers participated in the study. The population comprised bakers (40%), counterhands (26%), confectioners (7%), supervisors/managers (14%) and ex-bakers (13%) who were removed from the bakery and transferred to other departments. Other characteristics of the study population are outlined in table 1. Work-related (WRS) ocular-nasal symptoms (44%) were more prevalent than chest symptoms (26%). A higher proportion of workers were sensitised to wheat (31%) or rye (28%) than to fungal α -amylase (2%). Sensitisation to wheat and rye were highly correlated ($r=0.83$, $p<0.001$). There were 6% of workers with probable baker's asthma (WRS, specific allergic sensitisation and previous history of a positive methacholine challenge test), based on the findings of our initial survey.²³ Stratified analysis across job categories revealed a higher prevalence of work-related chest symptoms among counterhands in comparison with bakers (table 2). However, the prevalence of sensitisation to occupational allergens was higher for bakers than for counterhands. No significant differences were observed for FeNO levels across job categories.

In a subgroup analysis of SPT data the prevalence of sensitisation was the highest to storage mite (19%), followed by rye grass (18%), grassmix (17%) and bermuda grass (11%). The overall prevalence of sensitisation to any grass pollen (rye grass, bermuda grass and grass mix) was 25%. Among those sensitised to wheat and/or rye, only 48% were also cosensitised to grass pollen.

Among subjects with cereal flour sensitisation based on allergen specific IgE ($n=138$), there were 31 (22%) and 79 (57%) subjects respectively who reported allergic asthma and rhinitis, while a greater proportion, 57 (41%) and 87 (63%), of subjects reported work-related allergic asthma and rhinitis.

The median FeNO was 15 ppb (range: 4–251); 74% had low FeNO (<25 ppb) levels, 15% intermediate (25–50 ppb) levels and 11% high (>50 ppb) levels.²⁵ Atopics had significantly

Table 1 Univariate analysis of factors associated with fractional exhaled nitric oxide (FeNO) (ln) levels among bakery workers

Factor	N=424	β	r^2	p Value
Demographic and personal factors				
Age (years)	32 (27–40)	−0.0132	0.0198	0.004
Gender (M:F)	43:57	0.2134	0.0170	0.007
Height (m)	1.65 (1.59–1.72)	0.7438	0.0064	0.186
Weight (kg)	74 (61–90)	−0.0039	0.0087	0.056
BMI (kg/m ²)	28 (23–33)	−0.0080	0.0051	0.237
Atopy	109 (39%)	0.5572	0.1088	<0.001
Blood pressure				
Systolic (mm Hg)	120 (110–127)	0.0022	0.0017	0.396
Diastolic (mm Hg)	76 (67–85)	0.0006	0.0001	0.823
Hypertension*	84 (20%)	−0.0234	0.0001	0.813
Smoking†	183 (43%)	−0.4496	0.0754	<0.001
Number of cigarettes smoked per day	6 (4–10)	−0.0237	0.0191	0.061
Cigarettes/day <5	53 (13%)	0		
Cigarettes/day 5–9	71 (17%)	−0.3261	0.0408	0.020
Cigarettes/day ≥10	59 (14%)	−0.3657	0.0408	0.012
Recent alcohol intake‡	27 (6%)	−0.1596	0.0023	0.324
Recent vegetable intake‡	89 (21%)	0.0096	0.0000	0.921
Recent exercise‡	29 (7%)	0.3203	0.0099	0.040
Steroid use	16 (4%)	0.2722	0.0041	0.189
Recent lung function test‡	3 (1%)	−0.3600	0.0014	0.445
Recent chest infection§	177 (42%)	0.1592	0.0094	0.046
Occupational history				
Counterhands	148 (31%)	0		
Confectioners	34 (7%)	−0.2641	0.0144	0.123
Supervisor/managers	68 (14%)	0.1257	0.0144	0.338
Bakers	234 (48%)	−0.0793	0.0144	0.422
High dust exposure job¶	310 (74%)	−0.0228	0.0002	0.801
Employment duration on bakery	7±5	−0.0132	0.0063	0.187
Employment duration in current job	4±4	−0.0116	0.0030	0.368
Symptom history in past 12 months				
Hayfever	153 (36%)	0.2477	0.0215	0.002
Dry cough	129 (30%)	−0.0319	0.0003	0.711
Shortness of breath	118 (28%)	−0.1776	0.0096	0.043
Chest tightness	110 (26%)	−0.0691	0.0014	0.443
Wheezing	87 (21%)	−0.1037	0.0027	0.289
Doctor diagnosed asthma	40 (9%)	0.1943	0.0049	0.150
Work-related symptoms				
Chest	111 (26%)	0.0716	0.0015	0.426
Ocular–nasal	187 (44%)	0.0946	0.0033	0.235
Serum specific IgE				
Wheat IgE (kU/l)	0.07 (0.04–0.70)	0.0196	0.0999	<0.001
Rye IgE (kU/l)	0.06 (0.03–0.65)	0.0142	0.1201	<0.001
Fungal α -amylase	0.001 (0.00–0.10)	0.0878	0.0104	0.036
Serum specific IgE				
Wheat IgE (>0.35 kU/l)	131 (31%)	0.5849	0.1110	<0.001
Class I: >0.35–<0.7	25 (6%)	0.2806	0.1546**	0.074
Class II: 0.7–<3.5	43 (10%)	0.4786	0.1546**	<0.001
Class III: 3.5–<17.5	40 (9%)	0.6272	0.1546**	<0.001
Class IV: 17.5–<50	14 (3%)	0.8988	0.1546**	<0.001
Class V: 50–<100	5 (1%)	1.0181	0.1546**	0.003
Class VI: ≥100	3 (1%)	2.0737	0.1546**	<0.001

Continued

Table 1 Continued

Factor	N=424	β	r^2	p Value
Rye IgE (>0.35 kU/l)	119 (28%)	0.5742	0.1012	<0.001
Class I: >0.35–<0.7	16 (4%)	0.4852	0.1533**	0.012
Class II: 0.7–<3.5	38 (9%)	0.3041	0.1533**	0.019
Class III: 3.5–<17.5	30 (7%)	0.3742	0.1533**	0.010
Class IV: 17.5–<50	16 (4%)	0.8567	0.1533**	<0.001
Class V: 50–<100	9 (2%)	1.2320	0.1533**	<0.001
Class VI: ≥100	10 (2%)	1.2992	0.1533**	<0.001
Fungal α -amylase IgE (>0.35 kU/l)	7 (2%)	0.6033	0.0090	0.051
Class I: >0.35–<0.7	2 (0.5%)	0.6912	0.0145	0.229
Class II: 0.7–<3.5	2 (0.5%)	−0.1044	0.0145	0.856
Class III: 3.5–<17.5	3 (0.5%)	1.0165	0.0145	0.031
Cereal flour sensitisation††	138 (33%)	0.5521	0.1017	<0.001
Allergic symptoms‡‡				
Work-related allergic rhinitis	87 (21%)	0.4727	0.0554	<0.001
Work-related allergic asthma	57 (13%)	0.3938	0.0274	0.001
Probable baker's asthma	27 (6%)	0.1790	0.0029	0.268

Median and inter-quartile range presented for continuous variables since data skewed.

*Systolic ≥140 and/or diastolic ≥90.

†Currently smoking for as long as a year.

‡Recent=1 day ago.

§Self-reported flu or sinusitis in the past 3 weeks.

¶High dust exposure jobs=bakers, confectioners, managers and supervisors versus low dust exposed job category of counterhands.

** r^2 represents one model.††Cereal flour sensitisation=sensitised to wheat, rye or α amylase on IgE (>0.35 kU/l).

‡‡Allergic symptoms=self-reported work-related symptoms in the presence of cereal flour sensitisation.

BMI, body mass index.

higher FeNO than non-atopics (median 21 vs 13 ppb; $p<0.001$), while current smokers had significantly lower FeNO (median 12 vs 19 ppb; $p<0.001$).

Correlation between FeNO and IgE

A modest positive correlation was observed between FeNO and log(n) wheat IgE ($r=0.36$; $p<0.001$). Stratification by atopic status revealed similar correlations for atopics ($r=0.30$; $p=0.001$) and non-atopics ($r=0.31$; $p<0.001$). Similar significant correlations were demonstrated between FeNO and rye specific IgE, while the relationship for fungal α amylase was weaker overall ($r=0.19$; $p=0.004$), and not significant when stratified by atopic status.

Factors associated with variability in FeNO

Host factors such as current smoking ($\beta:-0.4496$) and age ($\beta:-0.0132$) were associated with lower FeNO, while atopy ($\beta:0.5489$), recent exercise ($\beta:0.3203$), male gender ($\beta:0.2134$) and recent chest infections ($\beta:0.1592$) were associated with increased FeNO (table 1). A self-reported history of hayfever ($\beta:0.2477$, $p=0.002$), but not work-related upper ($\beta:0.0716$; $p=0.235$) or lower respiratory symptoms ($\beta:0.0946$; $p=0.426$) were associated with higher FeNO. However, allergic work-related upper ($\beta:0.4727$, $p\leq 0.001$) as well as lower respiratory symptoms ($\beta:0.3938$, $p=0.001$) were significantly associated

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Table 2 Prevalence of sensitisation, work-related symptoms and fractional exhaled nitric oxide (FeNO) levels by job category among bakery workers

Outcome	Job category			
	Counterhand (n=109)	Supervisor/managers (n=58)	Confectioners (n=28)	Bakers (n=172)
Work-related symptoms				
Chest	27 (25%)	15 (26%)	4 (14%)	32 (18%)
Ocular–nasal	42 (39%)	22 (38%)	6 (21%)	72 (42%)
FeNO				
Low <25 ppb	79 (72%)	38 (65%)	24 (86%)	135 (78%)
Medium 25–50 ppb	20 (18%)	8 (14%)	3 (11%)	20 (12%)
High >50 ppb	10 (10%)	12 (21%)	1 (3%)	17 (10%)
Serum specific IgE				
Wheat (>0.35 kU/l)	20 (18%)	17 (29%)	9 (32%)	53 (31%)
Rye (>0.35 kU/l)	21 (19%)	16 (28%)	6 (21%)	45 (26%)
Fungal α -amylase (>0.35 kU/l)	0 (0%)	2 (3%)	0 (0%)	3 (2%)
Allergic respiratory symptoms				
Work-related allergic rhinitis	14 (13%)	11 (19%)	2 (7%)	29 (17%)
Work-related allergic asthma	7 (6%)	8 (14%)	3 (11%)	12 (7%)

with elevated FeNO. A greater increase in the β estimate was demonstrated for IgE to fungal α -amylase (β :0.0878) than to wheat (β :0.0196), or rye (β :0.0142).

A subgroup analysis of the relationship between sensitisation to common aeroallergens and FeNO demonstrated strong positive associations for rye grass (β :0.3672), grass mix (β :0.3915), storage mite (β :0.3354) and grass pollen (β :0.3551, $p=0.002$).

After adjusting to control for sensitisation to mites and grass pollen (ryegrass, grassmix, bermuda grass), there was an even stronger association between FeNO and sensitisation and to wheat (adjusted β :0.6897, $p<0.001$; unadjusted β :0.5849, $p<0.001$) and rye (adjusted β :0.6993, $p<0.001$; unadjusted β :0.5742, $p<0.001$).

There was no association demonstrated between FeNO and either occupational category or employment duration.

In the simultaneous quantile regression analysis atopy, smoking, recent chest infections, a history of hayfever and serum specific IgE to flour allergens (wheat, rye) were consistently associated with FeNO at each quantile (0.25, 0.50, 0.75) (data not shown). Although no association was demonstrated in the overall simple linear regression model at the upper quantile (0.75), a strong positive relationship was found for both doctor diagnosed asthma (β :0.6074; $p=0.004$) and probable baker's asthma (β :0.6329; $p=0.001$) with FeNO.

In the final multiple linear regression model including all variables significantly associated with FeNO in the univariate analysis, only IgE to wheat, current smoking, atopy and age remained significantly associated with FeNO (table 3) using a

stepwise model building approach. Due to the high correlation between sensitisation to wheat and rye ($r=0.83$), sensitisation to the latter allergen did also not appear in the final model. In this final model, IgE to wheat accounted for most of the variability in FeNO (15%) followed by smoking (6%) and atopy (5%). The β estimate increased by 0.2 with each class increase in wheat IgE level up to class 5 and was even more pronounced for class 6.

Predictors of elevated FeNO in logistic regression models

In the stratified logistic models, for atopy and smoking, a strong relationship was found for workers with elevated FeNO (>25 ppb) and sensitisation to wheat or rye, but not with fungal α -amylase, due to lack of power (table 4). A strong association was observed between FeNO and work-related allergic rhinitis irrespective of smoking and atopic status. However, the association with probable baker's asthma was only evident in non-atopics. Although the relationship between elevated FeNO and sensitisation to wheat was similar in both atopics and non-atopics, a stronger association was observed for non-atopics (OR=6.4) than atopics (OR=3.3) sensitised to rye.

Analysis for a possible interaction between atopy and smoking demonstrated strong positive associations with FeNO among atopic non-smokers for various clinical endpoints such as wheat (OR=9.43) or rye (OR=11.76) sensitisation, work-related allergic rhinitis (OR=8.1) or asthma (OR=5.4) and probable baker's asthma (OR=6.7) (table 5). The analysis further showed that the effect of smoking on FeNO appears to override atopy, demonstrating a clear dose-dependent effect with increasing number of cigarettes smoked per day.

DISCUSSION

This study, to our knowledge, represents the first detailed analysis of factors associated with FeNO among workers exposed to flour dust in supermarket bakeries, and one of few reported for bakery workers overall.^{20 21}

Normative values for FeNO have been reported in a number of different populations ranging between 27 and 57 ppb for non-asthmatics.²⁵ In comparison with these studies, FeNO in our study (90% upper limit: 57 ppb) was much higher than the levels reported by Travers *et al*¹⁵ in a random community survey (90% upper limit: 41 ppb) but similar to levels reported by

Table 3 Multiple linear regression model of fractional exhaled nitric oxide (ln) predictors among bakery workers

Factor	Estimate (β)	95% CI	p Value
Wheat	0.4746	0.28 to 0.67	<0.001
Current smoker	-0.4550	-0.63 to -0.28	<0.001
Atopy	0.3442	0.16 to 0.53	<0.001
Age	-0.0121	-0.02 to -0.01	0.023

Overall multiple regression model, $\beta_0=3.2$, $p<0.001$, cumulative $r^2=0.2573$.

Table 4 Predictors of elevated fractional exhaled nitric oxide (FeNO) (>25 ppb) stratified by atopy and smoking among bakery workers in separate multiple logistic regression models

Predictor	Smoking (OR, CI)*		Atopy (OR, CI)*	
	Smoker	Non-smoker	Atopic	Non-Atopic
General symptoms				
Wheeze	1.5 (0.6 to 3.5)	1.5 (0.7 to 3.1)	1.4 (0.6 to 3.5)	1.2 (0.5 to 3.3)
Hayfever	2.6 (1.1 to 6.3)	1.9 (1.1 to 3.5)	1.9 (0.8 to 4.9)	1.8 (0.7 to 4.4)
Doctor diagnosed asthma	2.1 (0.7 to 6.9)	3.2 (1.2 to 8.4)	2.4 (0.8 to 6.8)	3.1 (0.9 to 9.6)
Work-related symptoms				
Chest	1.0 (0.4 to 2.5)	1.5 (0.8 to 2.9)	0.8 (0.4 to 1.8)	1.7 (0.7 to 4.4)
Ocular–nasal	1.2 (0.5 to 2.7)	1.0 (0.6 to 1.8)	1.3 (0.6 to 2.9)	1.0 (0.4 to 2.4)
Allergic symptoms				
Work-related allergic rhinitis	3.2 (1.4 to 7.7)	2.9 (1.5 to 5.6)	2.8 (1.2 to 6.6)	4.1 (1.5 to 11.4)
Work-related allergic asthma	2.5 (0.9 to 6.9)	2.3 (1.1 to 5.0)	1.8 (0.7 to 4.4)	2.4 (0.7 to 8.0)
Serum specific IgE				
Wheat (>0.35 kU/l)	3.5 (1.5 to 7.9)	3.8 (2.1 to 6.9)	3.9 (1.7 to 9.5)	4.1 (1.6 to 10.3)
Rye (>0.35 kU/l)	3.7 (1.6 to 8.7)	4.4 (2.4 to 7.9)	3.2 (1.4 to 7.3)	6.5 (2.5 to 16.4)
Fungal α amylase (>0.35 kU/l)	2.6 (0.2 to 35.7)	†	2.9 (0.4 to 19.4)	†
Cereal flour sensitisation	3.1 (1.4 to 7.1)	4.0 (2.3 to 7.2)	3.8 (1.6 to 9.2)	5.0 (2.0 to 12.5)
Probable baker's asthma	1.5 (0.4 to 6.2)	2.4 (0.8 to 7.1)	0.8 (0.3 to 2.5)	4.3 (1.1 to 16.3)

*Adjusted for age and recent chest infection.

†Undefined since none of the subjects had elevated FeNO (>25 ppb).

Olin *et al.*¹⁶ With particular reference to studies in occupational populations, the median and/or mean FeNO in the current study of bakery workers were also higher than levels reported in other studies of workers exposed to endotoxins,¹⁴ organic dust,¹⁰ laboratory animals⁹ and latex,¹¹ irrespective of smoking and atopic status. This could be attributed to differences in timing of measurements, exposure duration of subjects, and inclusion and exclusion criteria based on symptom history use in different studies, unlike the current study that included all workers.

In this study, the major determinants for FeNO aside from serum specific IgE to wheat or rye were gender, age, smoking, atopy, recent chest infections, hayfever and allergic upper and lower respiratory symptoms. The higher FeNO in male subjects is consistent with several studies reporting similar findings.^{15–25} The association between FeNO levels and height as with other variables has been inconsistent in studies such as Sandrini *et al.*²⁷ FeNO also correlates strongly with atopy as had been observed in large cohort studies^{15–28} while higher FeNO has been observed in asymptomatic subjects with atopy, other studies only demonstrating this association in subjects with asthma or rhinitis.²⁹ In the current study, no significant association was observed between elevated FeNO (>25 ppb) and hayfever or asthma irrespective of atopic status, although in the unadjusted models hayfever, but not asthma, showed a positive relationship. However, in the current study, work-related allergic rhinitis was consistently positively associated with an elevated FeNO in the unadjusted models irrespective of atopic status. The findings are consistent with the study of Travers *et al.*¹⁵ in which allergic rhinitis was associated with higher FeNO.

While age appears to be an important determinant in children, there is less agreement in studies among adults. The finding of the current study, among a relatively young adult workforce, showing an inverse relationship with FeNO is at variance with other studies showing a positive association between age and FeNO. The possibility of selection bias cannot be excluded due to the cross-sectional nature of the study. Olin

*et al.*¹⁶ found an independent positive relationship with age, with the oldest group (>64 years) having 40% higher FeNO compared with the youngest group, while Franklin *et al.*³⁰ also found that FeNO increased with age. However, a study from Hong Kong found no correlation ($r=0.12$; $p=0.17$) between age and FeNO.³¹

The current study showed a significant decrease in FeNO among smokers even after adjusting for other potential confounders. It is well described that smoking leads to a decrease in FeNO³² and that smoking cessation may result in an increase in FeNO.³³ In multivariate linear models, the overall estimate of FeNO corresponding to smoking varied between $\beta=-0.7$ and $\beta=-0.8$ for smokers compared with non-smokers.^{15–16} However, smoking effect was not as strong in our study ($\beta=-0.45$). Furthermore, a strong association between elevated FeNO and doctor-diagnosed asthma was only observed among non-smokers as reported previously,¹⁶ despite asthmatic smokers having a higher FeNO compared with asthmatic non-smokers in this and other studies.³⁴

In the current study, no significant association was demonstrated between occupation and FeNO. This lack of association with occupation (high dust exposure jobs vs low dust exposure) could be explained by the possible delayed reaction of increase in FeNO following allergen exposure relating to timing of measurements. In this current study, due to logistical considerations only one cross-sectional measurement was done, although two consecutive measurements would have been more appropriate to determine an increase in FeNO following allergen exposure. This increase in FeNO has been documented by Pedrosa *et al.*²² 24 h after allergen exposure and Swierczyńska-machura *et al.*²⁰ after specific inhalation challenge. Another possible explanation for this finding could be possible exposure misclassification since job titles, instead of actual environmental measurements, were used as a proxy for exposure in this study. Although individuals had different job titles, the open plan design of the bakery resulted in possible increased exposures in individuals not directly involved in the baking process. Future studies should

Workplace

Table 5 Predictors of elevated fractional exhaled nitric oxide (>25 ppb) among bakery workers in multiple logistic regression models exploring interaction between smoking and atopy

Predictor	Atopic Smoker (OR, CI)*	Atopic Non-smoker (OR, CI)*	Non-atopic Smoker (OR, CI)*	Non-atopic Non-smoker (OR, CI)*
Work-related chest symptoms	1.55 (0.62 to 3.86)	4.26 (2.03 to 8.93)	0.39 (0.17 to 0.92)	1.08 (0.59 to 1.99)
Cigarettes/day <5	1.42 (0.33 to 6.14)	†	0.49 (0.14 to 1.76)	†
Cigarettes/day 5–9	0.82 (0.13 to 5.15)	†	0.28 (0.05 to 1.62)	†
Cigarettes/day ≥10	0.47 (0.05 to 4.84)	†	0.16 (0.12 to 1.61)	†
Work-related ocular–nasal symptoms	1.47 (0.58 to 3.72)	4.04 (1.94 to 8.40)	0.37 (0.16 to 0.86)	1.01 (0.57 to 1.81)
Cigarettes/day <5	3.29 (0.72 to 15.11)	†	1.13 (0.35 to 3.66)	†
Cigarettes/day 5–9	1.92 (0.31 to 11.82)	†	0.66 (0.14 to 3.19)	†
Cigarettes/day ≥10	1.12 (0.12 to 10.60)	†	0.38 (0.05 to 3.10)	†
Wheat (>0.35 kU/l)	3.66 (1.41 to 9.48)	9.43 (4.44 to 20.04)	1.62 (0.66 to 3.95)	4.16 (2.19 to 7.92)
Cigarettes/day <5	4.74 (1.13 to 19.87)	†	2.45 (0.69 to 8.78)	†
Cigarettes/day 5–9	2.50 (0.41 to 15.26)	†	1.29 (0.23 to 7.28)	†
Cigarettes/day ≥10	1.32 (0.13 to 13.54)	†	0.68 (0.07 to 6.79)	†
Rye (>0.35 kU/l)	4.19 (1.59 to 11.07)	11.76 (5.26 to 26.27)	1.60 (0.68 to 3.78)	4.50 (2.40 to 8.43)
Cigarettes/day <5	4.96 (1.14 to 21.23)	†	2.06 (0.61 to 6.96)	†
Cigarettes/day 5–9	2.52 (0.41 to 15.57)	†	1.05 (0.20 to 5.61)	†
Cigarettes/day ≥10	1.29 (0.13 to 13.14)	†	0.53 (0.06 to 5.08)	†
Cereal flour sensitisation	3.89 (1.49 to 10.17)	10.33 (4.78 to 22.35)	1.68 (0.69 to 4.08)	4.46 (2.35 to 8.48)
Cigarettes/day <5	4.36 (1.01 to 18.82)	†	2.06 (0.56 to 7.55)	†
Cigarettes/day 5–9	2.41 (3.38 to 15.33)	†	1.14 (0.19 to 6.68)	†
Cigarettes/day ≥10	1.33 (0.12 to 14.56)	†	0.63 (0.06 to 6.54)	†
Work-related allergic rhinitis	3.11 (1.17 to 8.28)	8.13 (3.72 to 17.76)	1.13 (0.46 to 2.79)	2.97 (1.53 to 5.70)
Cigarettes/day <5	5.87 (1.11 to 30.99)	†	2.23 (0.54 to 9.20)	†
Cigarettes/day 5–9	3.48 (0.44 to 27.41)	†	1.33 (0.20 to 8.98)	†
Cigarettes/day ≥10	2.07 (0.16 to 27.35)	†	0.79 (0.07 to 9.57)	†
Work-related allergic asthma	2.01 (0.74 to 5.44)	5.44 (2.36 to 12.54)	0.61 (0.24 to 1.55)	1.65 (0.80 to 3.41)
Cigarettes/day <5	1.88 (0.37 to 9.55)	†	0.63 (0.14 to 2.78)	†
Cigarettes/day 5–9	0.97 (0.13 to 7.32)	†	0.33 (0.05 to 2.32)	†
Cigarettes/day ≥10	0.50 (0.04 to 6.34)	†	0.17 (0.01 to 2.12)	†
Probable baker's asthma	2.54 (0.85 to 7.64)	6.72 (2.50 to 18.06)	0.67 (0.24 to 1.87)	1.78 (0.75 to 4.23)
Cigarettes/day <5	1.55 (0.30 to 8.17)	†	0.61 (0.12 to 2.99)	†
Cigarettes/day 5–9	1.00 (0.15 to 6.87)	†	0.39 (0.06 to 2.61)	†
Cigarettes/day ≥10	0.65 (0.06 to 5.56)	†	0.25 (0.03 to 2.55)	†

*Adjusted for age and recent chest infection.

†Analysis only applicable for current smokers.

rather assign exposure categories based on personal exposure measurements to better evaluate the relationship between occupational exposure (job title) and FeNO. There is also the added possibility that movement of workers within the bakery from high to lower exposed jobs, due to symptoms, may have further contributed to this misclassification.

The final multivariate model explained just over a quarter ($r^2=26$) of the total variability in FeNO, suggesting that other unknown factors may contribute substantially to the variability in FeNO. Levesque *et al*³⁵ also reported an overall variability of 26% that was slightly higher than Travers *et al* (22%).¹⁵ The latter study, however, did not account for serum specific IgE in their model. It has therefore been suggested that the variation in FeNO may be caused by a complex interaction of genetic, biologic and environmental factors, not all of which are easily measurable.³⁵

Among those factors, serum specific IgE to wheat or rye was the strongest determinant of the variability in FeNO (15%) in this study. Furthermore, we found slightly higher correlations for specific IgE to wheat or rye and FeNO ($r=0.36$) in comparison with other studies that used total serum IgE ($r=0.11$), which is not as specific.¹⁵ This association has been ascribed to

asthma being predominantly an IgE-mediated allergic airways disease. However, the association appears quite modest suggesting that the association between atopy and FeNO may be due to shared genetic determinants.³⁶

Several studies indicate that smoking and atopy are major determinants of FeNO. However, few studies have investigated the effect of a possible interaction between smoking and atopy on FeNO. In a study of farmers and agricultural processing workers, Smit *et al* found a strong association between increasing endotoxin exposure and FeNO only in non-smoking, non-atopic workers.¹⁴ However, although our study also demonstrated strong associations among non-smoking, non-atopic workers, a stronger association was observed between sensitisation to wheat or rye and elevated FeNO (FeNO>25 ppb) in atopic non-smokers. Furthermore, a strong association was also observed between elevated FeNO and cereal flour-related allergic symptoms as well as probable baker's asthma in atopic non-smoking workers. It can be concluded from this study that the overall effect of smoking ($\beta=-0.4550$) on FeNO overrides atopy ($\beta=0.3442$) in a dose-dependent relationship when these characteristics coexist in bakery workers sensitised to flour allergens.

In conclusion, the present study demonstrated that sensitisation to wheat or rye, but not symptoms, is a major determinant of FeNO among bakery workers. Furthermore, a strong association was observed between FeNO and allergic upper and lower respiratory disease associated with sensitisation to cereal flour allergens. The usefulness of FeNO is increasingly being acknowledged in occupational settings when compared with sputum eosinophil count for identifying workers with asthma, as it is well tolerated, less time consuming, inexpensive, and could be performed in various workplace settings using hand-held portable sampling devices. It may therefore provide a valid alternative to sputum eosinophil counts to evaluate bronchial inflammation when sputum collection is unavailable, unfeasible or unsuccessful. However, further studies are needed to assess the usefulness of FeNO to address various diagnostic challenges in occupational contexts. These include their use in medical surveillance programmes for the early detection of work-related allergic respiratory disease as has been reported in isolated studies evaluating the effectiveness of control measures³⁷ or before and after a specific bronchial challenge when evaluating difficult cases of occupational asthma.³⁸

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Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers

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