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ORIGINAL ARTICLE

Serial Exhaled Nitric Oxide Measurements in the Assessment of Laboratory Animal Allergy

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Background. Laboratory animal allergy (LAA) may cause eosinophilic airway inflammation, for which exhaled nitric oxide (FE_{NO}) measurements are sensitive and specific. Our objective was to assess whether serial FE_{NO} measurements might detect exposure-related inflammation in laboratory animal workers. **Methods.** Fifty laboratory animal workers participated. Measurements of FE_{NO} and spirometry were obtained at baseline (Friday) and twice-daily following a weekend with no animal contact. **Results.** Eleven of 50 subjects had work-related symptoms, and 2 of 11 had positive serology for LAA. Baseline FE_{NO} was high (>150 ppb) in the two seropositive subjects and increased progressively during the working week in one subject, confirming exposure-driven airway inflammation. In seronegative subjects, mean FE_{NO} levels were 19.8 (standard deviation [SD], 20.1) and 21.7 (SD, 20.8) in the symptomatic and nonsymptomatic groups, respectively, with no significant changes in FE_{NO} over time. **Conclusion.** Serial FE_{NO} measurements may provide complementary information in the assessment of possible occupational sensitisation. The sensitivity and specificity of this approach to diagnosing occupational asthma requires further evaluation.

Keywords asthma, exhaled nitric oxide, laboratory animal allergy

INTRODUCTION

Laboratory animal allergy (LAA) is a common cause of occupational asthma (1, 2). The risk of developing LAA is determined by host-susceptibility factors and allergen exposure. LAA is more common among previously atopic individuals (3). The symptoms are nonspecific, ranging from conjunctivitis to cough and wheeze and occur in up to 44% of workers (4). Among symptomatic exposed individuals, a minority are sensitized to laboratory animal proteins and, conversely, only 30% of sensitized individuals develop symptoms consistent with a diagnosis of asthma (5, 6). Thus, lack of specificity makes the interpretation of respiratory symptoms in exposed individuals problematic, particularly in seropositive individuals.

Laboratory animal allergens are high-molecular-weight antigens, which elicit an IgE-mediated response (2). The late allergic reaction (LAR) is associated with an increase in airway inflammatory cells, particularly eosinophils (7). The allergen-induced LAR can be quantified indirectly by measuring the fraction of nitric oxide in exhaled air (FE_{NO}) (8).

In a cross-sectional study, FE_{NO} was increased in subjects with LAA (9). However, little is known about the potential diagnostic information, which might be available using serial measurements. Further, raised FE_{NO} levels are known to occur in asymptomatic airway inflammation (10), raising the possibility that, particularly if the sensitivity of serological testing is lower than for an increase in FE_{NO}, subclinical air-

way inflammation might be detected in some asymptomatic nonsensitized exposed individuals.

Our study was based on an index case in which a laboratory animal challenge resulted in an increase in FE_{NO} over 24 hours. Based on this experience, we designed a prospective investigation to explore whether serial FE_{NO} measurements might be used to detect occupationally induced airway inflammation in at-risk laboratory animal workers. We hypothesized that FE_{NO} would show variation over a typical working week in relation to allergen exposure. To the best of our knowledge, this is the first study using serial FE_{NO} measurements in an occupational setting.

CASE REPORT

The case was a 30-year-old nonsmoking male with a five-year history of exposure to rats in a university animal laboratory. He had no prior history of childhood asthma. After two years of regular contact with the rats, the patient developed occasional rhinitis and conjunctivitis in relation to attendance at work. After the third year, he noted occasional work-related cough. During the fifth year of exposure, intermittent mild wheeze and exercise-related dyspnea occurred. He sought medical advice and was referred for assessment.

Initial investigations included: spirometry, FEV₁ 4.93 L (120% predicted); FVC 5.65 L (109% predicted); FEV₁/FVC: 87%; change in FEV₁ following bronchodilator 1%; diurnal peak flow variation (amplitude % mean while at work): 2.3%. Challenge tests with methacholine and hypertonic saline were negative for airway hyper-responsiveness (PD₂₀ > 8 μmol and PD₁₅ > 12 mL, respectively). FE_{NO} was increased at 20.8 ppb at a flow rate of 250 mL/s using a Logan 2000 analyzer (Logan Research, Rochester, UK), equivalent

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to 45.2 ppb at a flow rate of 50 mL/s (11). The patient was atopic with a positive skin-prick test (>3 mm) to grass and horses. A RAST test for rat epithelial antigen was "weakly positive."

After a four-week absence from work, the patient underwent two animal challenges. These were characterized by the close handling of (1) a cat (control) and (2) a laboratory rat, each for 30 minutes on two separate days under medical supervision with full emergency equipment available. Serial spirometric and FE_{NO} measurements were obtained for 24 hours after each challenge. The results on the day of rat exposure are shown in Figure 1. They confirmed the presence of a late and early inflammatory reaction to rat antigen, with an increase in FE_{NO} , which was greatest 24 hours after exposure. There were no changes in any measured parameter following exposure to cat.

PROSPECTIVE STUDY: METHODS

Based on this case, and in consultation with the directors of four University animal laboratories, we designed a prospective study. The protocol was approved by the Lower South Island Ethics Committee, and each participant gave written, informed consent.

Subjects

We recruited subjects who worked with laboratory animals in the four research centers. Workplace protection was not uniform between the centers. Each subject was exposed on a minimum of two occasions per week for six months or longer. No subject was excluded on the basis of their clinical history, and subjects both with and without symptoms were enrolled. No subject had occupational asthma at study entry. Three subjects with doctor-diagnosed asthma, who were taking inhaled corticosteroid (ICS) treatment, had their medication withdrawn for six weeks prior to study procedures.

Study Design

This was a longitudinal study designed to follow changes in symptoms, peak flows, and FE_{NO} in relation to workplace

animal exposure during a typical working week and weekend. It was not logistically possible to withdraw any individual from their place of work.

Subjects were initially assessed on a Friday. Each completed an occupational questionnaire (from Bush et al.) (12), as well as the following procedures: skin testing for common allergens, venous blood sample for specific IgE, and FE_{NO} measurement, spirometry, and methacholine challenge in a fixed sequence. Subjects were instructed in the use of a peak flow meter and a symptom diary. Having avoided contact with laboratory animals over the weekend, they were assessed at the beginning and end of each working day from Monday to Friday of the following week. On each occasion, FE_{NO} and spirometric measurements were performed. Subjects recorded serial PEFs on waking each morning and at two-hour intervals throughout each study day until bedtime. Each evening, they also recorded: how much time (hours) was spent in close proximity to laboratory animals and the severity of seven common symptoms potentially related to LAA, using a visual analog scale (0–10).

Study Procedures

FE_{NO} was measured according to current guidelines (13) at an expiratory flow rate of 50 mL/s. Two measurements were obtained at each time point using a NiOX MINO (Aerocrine, Solna, Sweden) nitric oxide analyzer (14). $FE_{NO} > 35$ ppb was deemed to be outside the normal range, and an increase of >25 ppb was deemed to be clinically significant (15). In order to validate each FE_{NO} result, the sensors from the NiOX MINO device were tested at the end of the study against a calibrated standard and where appropriate a correction factor was applied to take account of signal drift.

Spirometry was performed according to ATS/ERS criteria (16) using either a rolling seal spirometer (Sensormedics Corporation, Yorba Linda, California, USA) or the Koko PFT system, version 4 (PDS Instrumentation, Louisville, Colorado, USA). The serial PEFs were obtained using the pocket electronic PIKO-1 meter (Ferraris Medical Ltd., Hertford, UK).

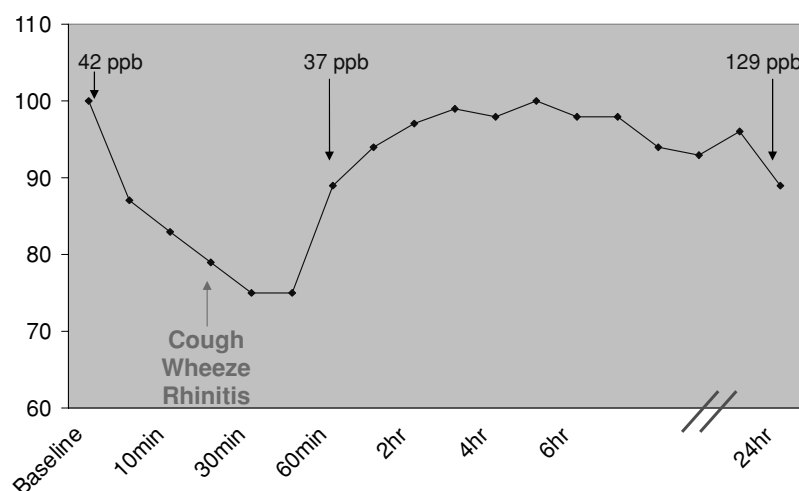


FIGURE 1.—Percentage changes in FEV_1 from baseline, where baseline is recorded as 100%, following exposure for 30 minutes to a laboratory rat in the index case. FE_{NO} measurements (ppb) were made at baseline and at 1 and 24 hours (arrows).

The methacholine challenges were performed using a modified version of the rapid challenge procedure (17). Skin allergen testing for atopy was performed using allergens to cat, grass pollen, and house dust mite (*D. pteronyssinus*) with negative and positive controls (Hollister-Stier Laboratories LLC, Spokane, Washington, USA).

Specific IgE was measured by means of radioallergosorbent testing (RAST) to relevant antigens: mouse, rat, guinea pig, rabbit, and cat. Serological evidence of LAA was defined by one or more positive specific IgE RAST tests (score 3 or greater). Environmental allergens were grass pollen, house dust mite, and latex for animal proteins.

Statistical Analysis

Subjects were stratified by the presence or absence of work-related LAA symptoms. Comparisons between these two groups were made using the Student's *t*-test. For serial FE_{NO} and PEFR measurements, diurnal variation and changes over time were compared using analysis of variance.

RESULTS

Fifty-one subjects were enrolled. One subject was withdrawn because of respiratory symptoms after discontinuing inhaled steroid therapy. She did not demonstrate any specific IgE to laboratory animal proteins. The demographic details of the remaining 50 subjects are contained in Table 1. Mean duration of exposure to laboratory animals was 10.5 years (range, six months to 39 years). During the study week, the mean cumulative exposure to laboratory animals was 16.0 hours (range, 1–40).

Prior History of Asthma

Eleven of 50 subjects reported a prior history of asthma (median duration, 16 years; range, 1–56 years). In 8 of 11, the diagnosis of asthma predated their exposure to laboratory animals. Of these, only four of eight had a PD₂₀ methacholine of <8 µmol and FE_{NO} >35 ppb, and two had an FEV₁/FVC ratio of <70%. The remaining four of eight subjects had normal spirometry, no evidence of AHR, and normal FE_{NO}. In the three cases where asthma was diagnosed *after* commencing exposure to laboratory animals, their respiratory symptoms were equally evident at and away from work, and occupational asthma was considered unlikely. None of these three had positive specific IgE tests for laboratory animal proteins.

Work-Related Symptoms

Eleven subjects (three with a prior diagnosis of asthma) reported work-related symptoms. Of these, two reported cough, wheeze, or chest tightness: one with a diagnosis of asthma that predated her laboratory animal exposure and the other with no previous asthma diagnosis. These two subjects had worked with laboratory animals for 13 and 30 months, respectively. At the end of the study, none of these 11 subjects fulfilled criteria for a diagnosis of occupational asthma (18).

Serological Evidence of LA Sensitization

Two of the 50 subjects had a positive specific IgE for one or more laboratory animal antigen. Details are contained in

TABLE 1.—Demographic details, reported symptoms, and animal exposure data for study participants.

N	50		
Male/female	18/32		
Age (years)	Mean = 34.5 (range, 19–59)		
Cigarette smoking	Current	Ex	Never
	9	10	31
Atopy ^a to common allergens	28 (56%)		
Diagnosed rhinitis	18 (36%)		
Regular antihistamine use	1 (2%)		
Previously diagnosed asthma	11 (22%)		
Corticosteroid use ^b	Nasal	Inhaled	
	0	2 (4%)	
Work-related symptoms at time of commencing study ^c			
Rhinoconjunctivitis	9 (18%)		
Lower respiratory tract	2 (4%)		
Skin rash	3 (6%)		
Duration of exposure to laboratory animals			
6–24 months	12 (24%)		
24–60 months	10 (20%)		
>60 months	28 (56%)		
Animal exposure			
Mice	40 (80%)		
Rats	36 (72%)		
Rabbits	14 (28%)		
Guinea pigs	14 (28%)		
Other	11 (22%)		
Exposure during study week			
<8 hours	18 (36%)		
8–24 hours	17 (34%)		
>24 hours	15 (30%)		

^aAtopy defined as one or more positive skin test to any of the following: house dust mite, cat, grass pollen.

^bInhaled corticosteroid therapy discontinued for six weeks prior to study entry.

^cSymptoms reported by subject that occurred only at work.

Table 2. One was a 46-year-old female, asymptomatic non-smoker with normal spirometry and a negative methacholine challenge. Six months after the conclusion of the study, she presented with symptoms typical of asthma and was diagnosed as having occupational asthma. The other was a 24-year-old female, symptomatic (rhinoconjunctivitis, cough, and wheeze), atopic nonsmoker with positive skin tests to cat, grass pollen, and house dust mite.

TABLE 2.—Details regarding the two cases who were seropositive for one or more laboratory animal antigens.

	Case 1	Case 2
Age	46	24
Sex	Female	Female
Smoking history	Never	Never
Prior history of asthma	No	Yes, since age 4
Inhaled steroids	No	Stopped prior to study
Atopy	No	Yes
Duration of exposure	24 years	one year
Ongoing work exposure	Intermittent, up to one hour/occasion	Daily up to one hour/day
Symptoms	Occasional rhinitis; cough and wheeze six months <i>after</i> completing the study	Intermittent conjunctivitis, rhinitis, and wheeze, not worse in relation to work exposure
Total IgE kIU/L	107	1110
Specific IgE	Mouse	Guinea-pig, rat, mouse; cat, grass pollen, house dust mite
Spirometry	Normal	Normal
Methacholine challenge	Negative	Positive

^aDefined as RAST score of 3 or greater.

TABLE 3.—Baseline measurements for study participants (*n* = 50).

Parameter	
FEV ₁ % predicted	106.6 (SD, 13.8) Number <80% = 3
FEV ₁ /FVC %	80.1 (SD, 6.7) Number <70% = 3 (6%)
FE _{NO} (ppb, geometric mean)	17.7 (SD, 36.7) Number >35 ppb = 9 (18%)
PD ₂₀ methacholine (μmol)	Number <8 μmol = 6 (12%)
Total IgE (mIU)	Mean = 108.5, Range, 16–1110 Number >100 IU = 13 (26%)
Specific IgE: number (%) of positives ^a	
House-dust mite	8 (16%)
Cat	1 (2%)
Grass pollen	3 (6%)
Mouse	2 (4%)
Rat	1 (2%)
Rabbit	0
Guinea pig	1 (2%)
Latex	0

^aVAS, Visual analog scale, 0–10 where 0 = none and 10 = very severe.

Baseline Measurements

Baseline measurements obtained on Day 1 are shown in Table 3. Three subjects had spirometric evidence of airflow obstruction at visit 1. One was diagnosed with chronic obstructive pulmonary disease (COPD). The two other subjects with airflow obstruction were male, atopic nonsmokers with a prior diagnosis of asthma, increased FE_{NO}, a PD₂₀ methacholine of <8 μmol, and an elevated total IgE with specific IgE to house dust mite. None of these three subjects demonstrated serological evidence, or had consistent work-related symptoms, to support a diagnosis of LAA.

FE_{NO} and PEFRs

Baseline FE_{NO} was highest in the two subjects who were seropositive for laboratory animal antigen(s) at 213.0 and 179.0 ppb, respectively. Mean FE_{NO} was 19.8 (standard deviation [SD], 20.1) and 21.7 (SD, 20.8) ppb in the remaining seronegative symptomatic and nonsymptomatic groups, respectively. Progressive increases in FE_{NO} occurred in one of the seropositive individuals during the working week (see Figure 2A), but not in the other (Figure 2B). There were no particular exposure characteristics that distinguished these two subjects.

No exposure-related changes occurred in any of the seronegative subjects (Figure 2C), irrespective of whether or not they reported respiratory symptoms. Four subjects demonstrated a change in FE_{NO} of >25 ppb during the working week (two seropositive plus two seronegative), but they did not differ as to symptom scores during the working week from those who showed no such FE_{NO} variation (Table 4).

There were no significant changes in symptoms or PEFRs in relation to time during the study in any group (data not shown).

DISCUSSION

In this study, our aim was to assess whether serial FE_{NO} measurements might identify exposure-related airway inflammation, possibly occurring subclinically, in individuals at risk of developing LAA. We confirmed this in one seroposi-

TABLE 4.—Comparison of daily reported symptom scores between subjects showing significant FE_{NO} change (>25 ppb) and those who did not show significant FE_{NO} change during the working week.

	Δ FE _{NO} greater than 25 ppb (<i>n</i> = 4)	Δ FE _{NO} less than 25 ppb (<i>n</i> = 46)	<i>p</i>
VAS ^a score, mean over five days (SD)			
Cough	2.2 (0.9)	5.4 (6.9)	0.422
Wheeze	5.9 (7.8)	4.7 (8.3)	0.812
Sneezing	13.2 (15.2)	5.5 (6.6)	0.080
Runny nose	12.8 (10.2)	5.7 (7.3)	0.118
Blocked nose	4.3 (3.4)	4.3 (6.4)	0.984
Itchy/watery eyes	6.6 (5.5)	5.1 (7.7)	0.737
Skin itch/rash	1.8 (0.8)	2.1 (2.9)	0.889

tive subject in whom baseline FE_{NO} levels were high and there was a progressive day-on-day increase in FE_{NO} in relation to allergen contact. The pattern in this subject indicated a time-dependent, exposure-driven increase in airway inflammation during the working week (see Figure 2A). With exposure the subject had only mild upper respiratory tract symptoms, no changes in PEFR, and no airway hyper-responsiveness (AHR), and so a diagnosis of occupational asthma was not confirmed. Interestingly, the patient subsequently developed cough and wheeze six months after the study, and based on the previous serological evidence, the FE_{NO} measurements and the development of AHR, a diagnosis of occupational asthma was made. This highlighted the prognostic significance of the earlier FE_{NO} measurements.

The second seropositive subject had high FE_{NO} levels at baseline and throughout the study week, without any incremental increase associated with exposure. This subject had life-long mild asthma, with a broad range of skin-prick reactions to common allergens. High FE_{NO} levels may occur even in mild asthma especially if untreated, and this needs to be borne in mind when interpreting FE_{NO} measurements (19). Exposure to other allergens such as house dust mite, driving continuous eosinophilic airway inflammation, may have masked potential work-related changes in FE_{NO}.

In another two (seronegative) subjects, changes in FE_{NO} of greater than 25 ppb, which were deemed to be clinically significant (15) occurred, but they had no particular identifying symptoms, abnormal lung function, or AHR. In the remaining 46 seronegative subjects, no important changes in FE_{NO} were seen, including the nine subjects with symptoms. Time-related changes in FE_{NO} did not distinguish symptomatic from asymptomatic seronegative subjects (Figure 2C). Thus, there was no evidence that serial FE_{NO} measurements identified otherwise “occult” inflammatory responses to a workplace antigen.

Our study was not designed to evaluate whether FE_{NO} measurements might be used to screen for occupational asthma as such. The pretest probabilities were low, and controls were not recruited. It was, therefore, not possible to calculate the sensitivities and specificities of FE_{NO} as a diagnostic test in this setting. Nevertheless, in the symptomatic subjects who were seronegative, we are confident that the absence of changes in FE_{NO} was a robust finding. Low, unchanging FE_{NO} levels provided reassurance that such symptomatic individuals did not have exposure driven airway inflammation (20), and that serological testing was not falsely negative. Conversely, 7 of 39 subjects with no symptoms

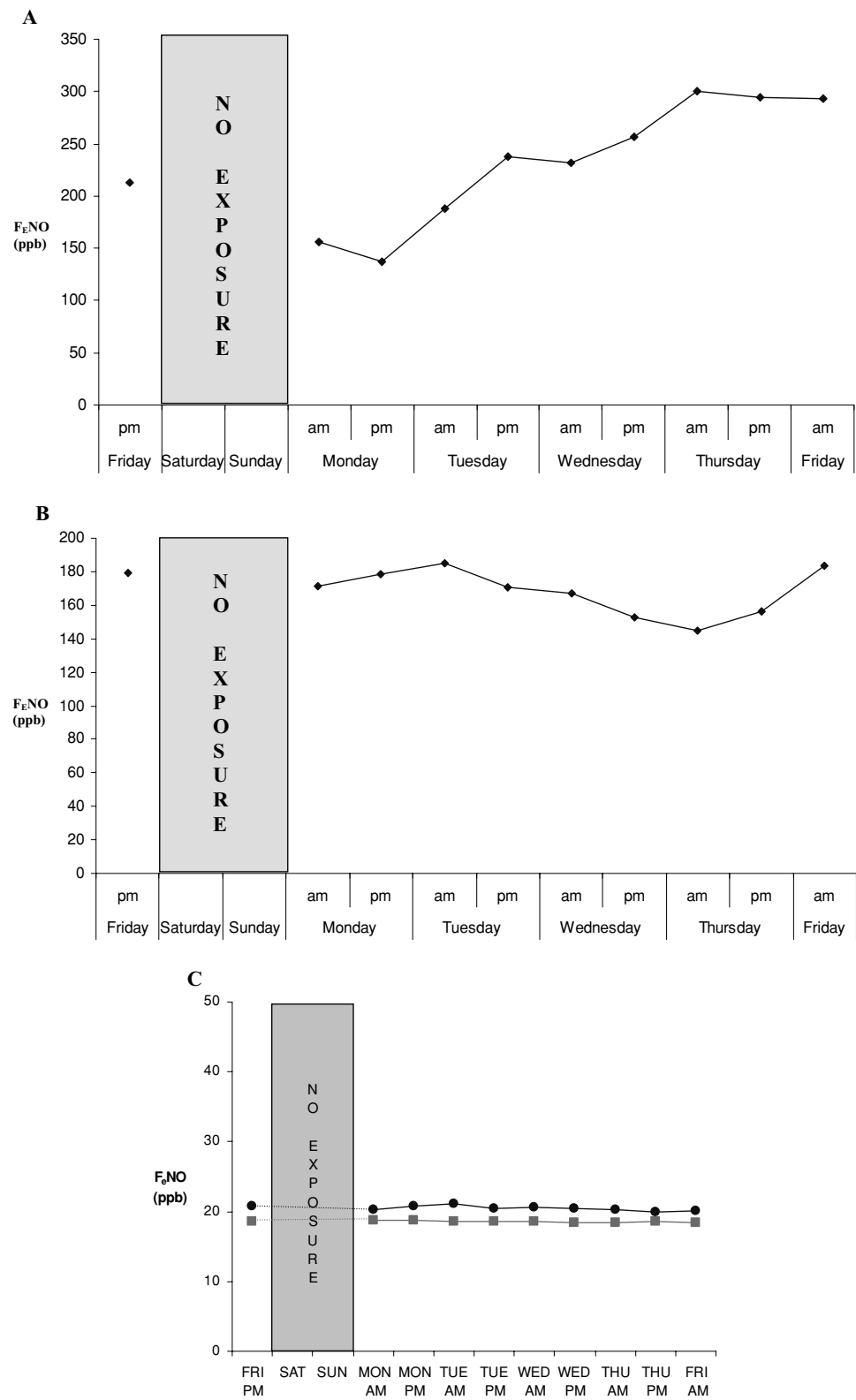


FIGURE 2.—(A and B). Serial FE_{NO} measurements in two sensitized subjects. After a decrease during the course of a weekend, there was a progressive increase over the period Monday to Friday in Case 1 (A). The grey bars represent time away from work with no animal exposure. In Case 2 (B), there were sustained high FE_{NO} levels: the subject had multiple other skin-prick test sensitivities. (C). Serial FE_{NO} measurements in non-sensitized symptomatic (■, $n = 9$) and asymptomatic (●, $n = 39$) subjects during a typical working week. The grey bar represent time away from work with no animal exposure.

to suggest laboratory animal asthma had baseline FE_{NO} levels of greater than 35 ppb ($n = 6$) and/or changes in FE_{NO} during the working week of greater than 25 ppb ($n = 2$). However, no consistent exposure-response pattern was observed. The raised FE_{NO} in these individuals was deemed to be due to other causes, notably non-occupational atopy (six of the seven were atopic).

In earlier studies, the limits of agreement for within-subject repeatability for FE_{NO} measurements were ± 4.4 ppb (21) and up to 26% in subjects with asthma (22). In deteriorating asthma, an increase of 25 ppb or 60% is associated with loss of asthma control and deemed to be clinically relevant (15). Thus, the magnitude (maximum minus minimum) of FE_{NO} variation over the working week in the two seropositive subjects (134 and 51 ppb) was clinically, as well as statistically, significant. In contrast, in the seronegative subjects with symptoms, FE_{NO} variation ranged from 3 to 31 ppb, with a mean of 11.7 ppb, which is not clinically significant.

Hitherto, confirming the diagnosis of allergic occupational asthma has been based on symptoms, positive skin-prick and IgE tests, nonspecific AHR, or typical PEFR variation at or away from the workplace (18). Recently, Girard et al. have reported that "inflammometry" improves the sensitivity and specificity of lung function tests in the diagnosis of occupational asthma (23). Perhaps, in a study with higher pretest probabilities for occupational asthma, serial FE_{NO} measurements would have provided supportive evidence that complements, or is superior to, these other investigations. Reliance is often placed on serial PEFR measurements, which are obtained over a prolonged interval at and away from work and whose sensitivity is generally low (24). Interestingly, the changes in FE_{NO} in Case 1 (Figure 2A) occurred within a short time frame, in the absence of changes in peak flows. Repeated low-dose allergen challenge may result in short-term increases in induced sputum eosinophilia and FE_{NO} (25, 26) in the absence of symptoms (10). Thus, FE_{NO} measurements may potentially offer evidence that an active response to antigen exposure is occurring, notably in sensitized subjects, in a shorter time period.

Our study design and results differ somewhat from those of Adisesh et al. (9). In that study, subjects with "constitutional asthma" were excluded *a priori*. Further, the proportion of sensitized subjects was higher (16/39, 41%). These authors reported raised one-off measurements of FE_{NO} in sensitized individuals, a similar finding to our own. However, serial FE_{NO} measurements were not measured. If they had done so, then exposure-related changes in airway inflammation might have been identified.

CONCLUSION

In conclusion, serial measurements of FE_{NO} over a typical working week provided confirmatory evidence of exposure-related airway inflammation in a laboratory animal worker who subsequently went on to develop asthma. In seronegative symptomatic subjects, no clear pattern was seen. Although the diagnostic yield of FE_{NO} measurements was low, these results provide evidence that serial FE_{NO} may be helpful in the assessment of occupational airway disease. Further studies in at-risk populations with higher pretest probabilities for

occupational asthma secondary to allergic, eosinophilic airway inflammation are needed in order to define the utility of FE_{NO} measurements in this setting.

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ABBREVIATIONS

AHR	Airway hyper-responsiveness
COPD	Chronic obstructive pulmonary disease
FE_{NO}	Fraction of nitric oxide in exhaled air
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
LAA	Laboratory animal allergy
LAR	Late asthmatic reaction
PD_{20}	Provocative dose causing a 20% fall in FEV_1
PEFR	Peak expiratory flow rate
RAST	Radio-allergosorbent test
SD	Standard deviation

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