Repeated exposure to an ambient level of NO₂ enhances asthmatic response to a nonsymptomatic allergen dose

V. Strand^{*,**}, M. Svartengren^{*,†}, S. Rak⁺⁺, C. Barck^{*}, G. Bylin^{*}

Repeated exposure to an ambient level of NO₂ enhances asthmatic response to a nonsymptomatic allergen dose. V. Strand, M. Svartengren, S. Rak, C. Barck, G. Bylin. ©ERS Journals Ltd 1998.

ABSTRACT: We investigated the effects of NO₂ and allergen on lung function in a repeated exposure model.

For 4 subsequent days, 16 subjects with mild asthma and allergy to birch or grass pollen were exposed at rest to either purified air or 500 µg·m⁻³ NO₂ for 30 min in an exposure chamber. Four hours later, an individually determined nonsymptomatic allergen dose was inhaled. Lung function (forced expiratory volume in one second (FEV₁)) was measured by a portable spirometer at early phase (EP) 15 min after allergen and at late phase (LP) 3–10 h after allergen. Subjective symptoms and medication were followed by diary cards.

Asthmatic response was significantly increased after repeated exposure to NO₂ and allergen compared to air and allergen. The 4-day mean fall in FEV₁ after NO₂ was at EP -2.5% versus -0.4% for air (p=0.02) and at LP -4.4% versus -1.9% for air (p=0.01, ANOVA). An increase in EP response was seen already after a single NO₂ exposure (p=0.03). There was a tendency (p=0.07) towards increased night-time symptoms of asthma after NO₂ plus allergen.

Although the effects were small, the results indicate that a repeated short exposure to an ambient level of NO₂ enhances the airway response to a nonsymptomatic allergen dose.

Eur Respir J 1998; 12: 6–12.

^{*}Dept of Respiratory and Allergic Diseases, and The Institute of Medicine at the Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden. ^{**}The Institute of Environmental Medicine at the Karolinska Institute, Stockholm, Sweden. [†]Dept of Occupational Medicine, Huddinge University Hospital, Stockholm, Sweden. ⁺⁺Allergy Center, Sahlgrenska University Hospital, Gothenburg, Sweden.

Correspondence: V. Strand, Dept of Respiratory and Allergic Diseases, Huddinge University Hospital, S-14186 Huddinge, Sweden. Fax: 46 87117306

Keywords: Air pollution, allergy, asthma, lung function, nitrogen dioxide, repeated exposure

Received: November 5 1997

Accepted after revision March 20 1998

This study was supported by grants from The Swedish Association against Asthma and Allergy, The Swedish Building Research Council, The Swedish Environmental Protection Agency, Swedish Heart Lung Foundation and King Oscar II:s Jubilee Foundation

Nitrogen dioxide (NO₂) is a major air pollutant produced by combustion, the main sources being traffic exhaust outdoors and gas appliances indoors. Evidence from several epidemiological studies suggests that ambient levels of nitrogen dioxide may increase the risk for exacerbations of asthma [1, 2]. Controlled human exposure studies on the other hand have not shown that lung function is affected by ambient levels of NO₂ [3, 4]. However, an increase in airway responsiveness to nonspecific stimuli like histamine and methacholine has been reported in asthmatics at NO₂ concentrations <1,000 µg·m⁻³ [5–7]. It is possible that NO₂ may exert its effects by an interaction with other air pollutants, such as particles, SO₂ and O₃. In a study by Jörres *et al.* [8], the lung function response was increased after NO₂ followed by SO₂ in asthmatics, and in healthy females when NO₂ was followed by O₃ exposure 3 h later [9], but not in asthmatics exposed to NO₂ and O₃ simultaneously [10]. Furthermore, in a recent study, bronchial response to birch and timothy pollen was enhanced by prior exposure to 500 µg·m⁻³ NO₂ for 30 min [11]. Similar results were shown for house dust mite after exposure to 800 µg·m⁻³ (0.4 ppm) NO₂ for 1 h [12]. These latter reports show that ambient levels of NO₂ can also enhance the airway responsiveness to allergens in humans. However, the allergen doses given in the experiments men-

tioned above considerably exceed those encountered in normal life. This makes it difficult to draw any firm conclusions on the clinical implications of the findings. As an increase in nonspecific bronchial responsiveness and the presence of late asthmatic reactions have also been reported after low doses of pollen allergen [13, 14], the use of a nonsymptomatic allergen dose would imitate seasonal exposure better.

In order to mimic real-life exposure, the aim of the present study was to investigate the effect of daily repeated exposure to a nonsymptomatic allergen dose preceded by a short exposure to 500 µg·m⁻³ NO₂. We wanted to determine whether the NO₂ effect on asthmatic response was reproducible with this significantly lower allergen dose and whether the magnitude of the response to NO₂ changed during repeated exposure.

Methods

Subjects

Subjects were recruited through the hospital outpatient clinic and by local advertising. All subjects gave informed

consent to participate in the study. The study was approved by the Ethics Committee at Huddinge University Hospital.

Sixteen subjects, 6 females and 10 males, age 21–52 yrs with mild-to-moderate seasonal asthma and allergy to pollen (12 to birch, 4 to timothy) participated in the study. Anthropometric and clinical data are given in table 1.

The subjects were included if they had a diagnosis of asthma based on reversible attacks of dyspnoea during the pollen season and airway hyperresponsiveness to histamine. Their allergy to either birch or timothy pollen was confirmed at an inclusion test by a positive skin-prick test and a positive bronchial challenge with the relevant allergen. The study was performed out of the pollen season. Lung function at inclusion expressed as forced expiratory volume in one second (FEV₁) was 93±16% predicted (mean ±SD).

They all used inhaled β_2 -agonist as needed, and nine used inhaled steroids during the pollen season. None received inhaled steroids or antihistamines during the study period, and the time span between the end of inhaled corticosteroid treatment and first visit in the study was at least 4 months.

All subjects were nonsmokers (13 had never smoked, three were exsmokers for at least 2 yrs). The subjects sensitive to animal dander did not have pets in their homes, and no subject had any domestic gas appliances.

Study design

The subjects were exposed for 30 min at rest in an exposure chamber to filtered air or to a concentration of 500 $\mu\text{g}\cdot\text{m}^{-3}$ (260 ppb) NO₂ on 4 consecutive days, at least 4 weeks apart. The exposures were performed single-blind

between 08:00 and 09:00 h and the order of exposure was randomized (9 subjects first to NO₂, 7 first to air).

The procedure on days 1–4 was as follows: after arrival at the laboratory, the subject rested for 15 min, and the specific airway resistance (*sRaw*) and thoracic gas volume (TGV) were measured with a whole-body plethysmograph and FEV₁ with a portable spirometer (Diary Card Spirometer®; Micromedical Ltd., Chatham, Kent, UK). The subject then rested for 10 min more before entering the exposure chamber. During the 30 min of exposure to air/NO₂, the lung function (*sRaw*, TGV) was measured at 4, 15, and 30 min, and an interview concerning symptoms after 3 and 26 min was conducted with the help of a questionnaire.

After exposure, FEV₁ was measured hourly with the portable spirometer. Four hours later, before the allergen inhalation, lung function was again measured by means of spirometry and plethysmography. After the allergen inhalation, the subject went home and continued to measure FEV₁ hourly. The subjects were instructed to use an inhaled bronchodilator if necessary and to keep a daily record on symptoms and medication.

On day 5, a histamine challenge was performed in the laboratory between 08:00 and 09:00 h preceded by *sRaw*, TGV and FEV₁ measurements.

The exposures were coded to the investigators analysing the results from the portable computerized spirometer.

Whole-body plethysmography

Airway resistance (*Raw*) and TGV were measured in a constant-volume body plethysmograph (Model 2000 TB; Cardio-Pulmonary Instruments, Houston, TX, USA) according to DuBois *et al.* [15, 16].

Table 1. – Anthropometric and clinical data

Subject	Age yrs	Sex	Asthma duration yrs	Allergen inhaled	Positive SPT for allergens*	Smoking	FEV ₁ % pred	Allergen dose** SQ units	Allergen PDs <i>Raw</i> 100% SQ units	Histamine PDs <i>Raw</i> 100% μg
1	31	F	15	B	T,C	N	93	28	239	1305
2	33	M	17	T	D,C,B	N	73	28	243	350
3	52	F	33	B	T,D,C	N	67	14	79	72
4	22	F	5	T	D,C	N	86	165	1474	172
5	38	M	36	T	B,D,C	N	130	55	568	133
6	25	M	22	B	T,D,H,C	N	96	14	111	102
7	40	M	39	B	D,H,C	E	85	49	446	163
8	21	F	3	B	T,D,C	N	89	21	181	145
9	28	M	19	B	D,H,C	N	99	21	147	461
10	29	M	3	T	-	E	104	110	1097	530
11	32	F	12	B	H,C	N	77	21	180	137
12	38	M	35	B	D,C	E	93	660	6654	124
13	41	F	35	B	D,C	N	86	42	411	187
14	38	M	23	B	T,D,H,C	N	124	28	279	395
15	32	M	22	B	T,C	N	94	35	326	128
16	31	M	15	B	T,D,C	N	97	7	52	74
Mean	33		21				93			
±SD	8		12				16			
Median								28	261	154
25th percentile								21	172	127
75th percentile								51	477	361

F: female; M: male; N: nonsmoker; E: exsmoker; SPT: skin-prick test; T: timothy grass pollen; B: birch pollen; D: dog; C: cat; H: horse; FEV₁: forced expiratory volume in one second; PDs*Raw*100%: provocative dose causing a 100% increase in specific airway resistance; SQ: standard quality. *: positive SPT defined as allergen induced weal of at least the size of the histamine skin prick (positive control); **: inhaled allergen dose study days 1–4;

The gas flow-/box pressure slopes were measured between gas flow $+0.5$ and -0.5 L·s⁻¹ (expiration - inspiration) as a mean of two to three slopes. The mouth-/box pressure slopes were measured between the end-points, again as the mean of two to three curves. All panting manoeuvres were made at about 1 Hz, the subject being guided by a metronome.

Spirometry

We used a portable combined spirometer and electronic diary card (EDC) symptom registration developed by Micromedical Ltd. The equipment has been used by several groups, and it is well accepted by the patients and seems to give reliable data [11, 17]. The best FEV₁ out of two blows was used in the analysis. The average FEV₁ value 3–10 h after allergen inhalation was used as a quantitative estimate of the asthmatic reaction during the late phase of the bronchial allergic response, and the minimum single FEV₁ value was used as a qualitative assessment of a late asthmatic reaction (LAR) defined as a $\geq 15\%$ decrease in FEV₁ from prechallenge values.

Histamine bronchial challenge

Histamine provocation tests were performed by using an automatic, inhalation synchronized dosimeter jet nebulizer (Spira Elektro 2; Respiratory Care Center, Hameenlinna, Finland) with an adjustable aerosol delivery time according to a method previously described [11, 18]. Briefly, doubling doses of histamine diphosphate at three concentrations, 1, 8, and 64 mg·mL⁻¹ were administered from an initial dose of 14 µg until a 100% increase in *sRaw* was reached. *sRaw* and TGV were measured 3 min after each dose. The provocative dose (PD_{*sRaw*100%}) was calculated by linear interpolation from a logarithmic scale.

Allergen challenge and allergen inhalation

The allergen challenge at study inclusion and the allergen inhalation during the study was performed with the same dosimeter jet nebulizer (Spira Elektro 2) as for the histamine inhalation. Standardized and freeze-dried birch or timothy allergen extracts (Aquagen, ALK, Copenhagen, Denmark) were used at a maximum of four concentrations, 1,000, 4,000, 16,000 and 64,000 standardized quality (SQ) allergen units·mL⁻¹. At each concentration, two and four breaths could be taken and, if needed, followed by eight and 16 breaths at the highest concentration. Fifteen minutes after each dose *sRaw* and TGV were measured. After measuring baseline *sRaw*, the subject inhaled doubling doses of allergen, starting from an initial dose of 14 SQ until a 100% increase in *sRaw* was reached. PD_{*sRaw*100%} for the allergen was calculated by linear interpolation from a logarithmic scale.

The daily inhaled allergen dose during the study period was set to 10% of the PD_{*sRaw*100%} at study inclusion or, when this was impossible to administer for practical reasons, the dose above (table 1). FEV₁ and *sRaw* were recorded immediately before and 15 min after the single dose of allergen was inhaled.

Questionnaire

After 3 and 26 min of exposure in the chamber, the subject was asked 16 questions concerning respiratory symptoms and perceptions of discomfort (*i.e.* tight chest, cough, headache, odour), estimated on a scale of 1–7. Throughout the exposure week, the subjects kept a self-administered daily record of airways symptoms, nose and eye symptoms, and bronchodilator medication. Medication was counted as the number of inhalations during the night and day, respectively. Nightly asthmatic bronchial symptoms were registered on a five-category scale: 1) no symptoms; 2) awoken by symptoms once or earlier than usual; 3) awoken more than once; 4) awake for greater part of the night; and 5) severe symptoms such that no sleep at all had been possible. Daytime symptoms were expressed on a six-category scale: 1) no symptoms; 2) symptoms once; 3) symptoms more than once; 4) symptoms during all day; 5) symptoms affecting normal activities; and 6) symptoms hindering normal duties. Irritability symptoms from bronchi, nose and eyes were noted for the last 24 h on a four-category scale: 1) no symptoms; 2) tolerable symptoms; 3) symptoms affecting normal activity; 4) no normal activity possible.

Gas dilution and exposure system

NO₂, kept in a gas bottle (Alfax, approximately 8,000 mg·m⁻³ NO₂) was diluted in two steps to a final concentration of about 500 µg·m⁻³ NO₂ and fed into the exposure chamber (volume 7 m³). The gas dilution and exposure system is presented in more detail elsewhere [19].

Chemical analyses

NO₂ concentrations in the exposure chamber were measured with a chemiluminescence instrument (Model 8440 Nitrogen Oxides Analyzer; Monitor Laboratories, Englewood, CO, USA). For calibration, a NO₂ permeation tube and NO calibration gas (Model 8500 Calibrator; Monitor Laboratories, Englewood, CO, USA; AGA Special Gas, 100 ppm NO) was used. A calibration procedure was run daily.

The subject's individual exposure to NO₂ was measured with a personal, passive (filter badge) sampler (Toyo Roshi Kaisha Ltd, Tokyo, Japan). Sampling was made from the morning of day 1 to the morning of day 5 with the exception of the 30 min of NO₂/air exposure in the chamber. The analytical technique as well as the accuracy and reproducibility of the measurements with the sampler are presented in detail elsewhere [20].

Exposure data

NO₂ concentrations in the exposure chamber were measured in the breathing zone of the subject. The concentration was 499 ± 8 ; 493 – 519 µg·m⁻³ (average of the mean concentrations during 4 days $\pm$ SD, range). During exposure to filtered air, the NO₂ concentration was <10 µg·m⁻³. The temperature in the exposure chamber was $25.1 \pm 1.1^\circ\text{C}$ (mean $\pm$ SD) during air and $25.0 \pm 1.0^\circ\text{C}$ during NO₂ exposure. The corresponding values for relative humidity was 35.9 ± 7.6 and $39.5 \pm 8\%$.

The exposure to NO₂ in ambient air measured with the personal sampler was 18±7, 9–33 µg·m⁻³ (4 days mean±SD, range) during NO₂ and 16±7, 4–32 µg·m⁻³ during air exposure.

The concentrations of outdoor air pollutants in the Stockholm area were low or moderate during the study period (NO₂ 40–60 µg·m⁻³, SO₂ 3–6 µg·m⁻³, O₃ 25–62 µg·m⁻³; monthly means, Stockholm Air Quality and Noise Analysis, The Environmental Administration of the City of Stockholm).

Data analysis

The early-phase (EP) reaction after allergen was defined as the percentage change in FEV₁ from immediately before to 15 min after the allergen. The late-phase (LP) reaction was defined as the percentage change in FEV₁ from immediately before to the average FEV₁ 3–10 h after the allergen inhalation. The minimum FEV₁ value 3–10 h after the allergen was used as a qualitative assessment of a late asthmatic reaction (LAR) defined as ≥15% decrease in FEV₁ from preallergen values.

The effects on FEV₁ and sRaw after a single exposure and also histamine responsiveness and subjective symptoms were tested using Wilcoxon's nonparametric sign rank test for two related samples for data not normally distributed. Differences between NO₂ and air in FEV₁ and sRaw after repeated exposure were tested by analysis of variance (ANOVA) for repeated measures. The statistical programme used was SPSS for Power Macintosh (SPSS Inc. Chicago, IL, USA). Probability values <5% were considered significant.

Results

All 16 subjects completed both exposure series.

Effects of NO₂ exposure on lung function

Data are shown in table 2. After a single exposure, there was no immediate reaction either during (mean sRaw, data not shown) or after NO₂ when comparing baseline (morning) FEV₁ to FEV₁ 15 min after the end of NO₂ exposure on day 1. In addition, there was no delayed reaction to NO₂ after a single exposure when comparing FEV₁ baseline (morning) values to the values 4 h after exposure, before the allergen.

After repeated exposure, the mean sRaw during the exposure sessions to NO₂ did not, on any day, differ significantly from the baseline (morning) values before exposure or from the air exposure week (data not shown). There was a trend, albeit not statistically significant (p=0.07), towards lower FEV₁ values 4 h after exposure during the NO₂ week compared to the control.

Effects of NO₂ on asthmatic reaction to allergen after single exposure

Spirometric results are shown in table 3. FEV₁ decreased significantly after allergen preceded by NO₂ at the

Table 2. – Baseline forced expiratory volume in one second before air or NO₂ and before allergen inhalation

		Before exposure		Before allergen	
		Mean	±SD	Mean	±SD
Day 1	Air	3.63	1.06	3.61	1.09
	NO ₂	3.67	1.11	3.70	1.07
Day 2	Air	3.52	1.18	3.57	1.17
	NO ₂	3.60	1.11	3.66	1.10
Day 3	Air	3.57	1.09	3.64	1.11
	NO ₂	3.63	1.15	3.65	1.13
Day 4	Air	3.59	1.13	3.61	1.12
	NO ₂	3.60	1.12	3.62	1.11

Table 3. – Individual changes in forced expiratory volume in one second (FEV₁) (%) after allergen preceded by air or 500 µg·m⁻³ NO₂

Subject	ΔFEV ₁ % EP								ΔFEV ₁ % LP							
	Day 1		Day 2		Day 3		Day 4		Day 1		Day 2		Day 3		Day 4	
	Air	NO ₂	Air	NO ₂	Air	NO ₂	Air	NO ₂	Air	NO ₂	Air	NO ₂	Air	NO ₂	Air	NO ₂
1	0.0	-0.9	0.6	2.5	-4.1	2.4	5.1	0.0	-3.0	-10.7	0.3	-2.2	-5.8	-4.8	-2.1	0.0
2	-3.1	-1.8	3.7	-13.8	-5.5	-7.5	-1.1	9.7	-4.9	-4.7	-3.7	0.8	-7.7	-5.7	-4.2	-0.4
3	0.0	-13.2	-1.3	-18.4	-15.9	-1.8	0.0	-11.2	-1.2	-17.5	6.4	-13.4	-9.3	-6.6	6.5	-13.4
4	6.4	2.9	-7.6	-6.2	-0.6	-3.6	-11.6	-2.8	0.3	-8.0	-3.7	-6.5	-14.8	-8.2	0.3	-12.0
5	0.0	-2.7	-1.5	-2.3	-4.8	-5.7	-7.3	-3.1	-3.1	0.6	1.9	-2.5	-2.9	-2.1	0.8	-2.5
6	0.0	-0.7	-0.7	2.9	0.9	3.0	0.7	1.4	-1.4	-0.5	3.2	1.8	-2.9	-0.5	1.1	0.9
7	5.1	0.0	3.5	-0.8	1.3	-4.6	-1.6	-3.3	9.8	-4.0	0.8	-2.8	-3.6	-3.3	-5.2	-7.2
8	-2.8	-2.8	1.1	-1.8	-0.7	-1.1	-1.8	0.8	-2.4	-1.5	3.0	-18.9	-5.0	-8.2	-2.2	-5.6
9	0.6	-1.5	1.1	4.0	-1.9	1.6	-3.0	4.7	2.5	-1.5	1.9	1.7	-0.6	-3.1	0.7	0.8
10	-4.8	-1.5	2.7	2.4	-0.8	-3.5	-0.7	-1.0	-5.3	4.5	1.0	1.0	-0.3	-9.1	-4.1	-7.6
11	-4.7	-8.8	5.9	-5.9	12.7	-11.9	-1.0	-7.2	-3.1	-8.8	10.1	0.9	-4.8	-4.3	-3.6	-8.6
12	1.4	-1.9	3.9	-6.1	0.8	-1.8	0.0	0.0	5.3	-2.7	9.3	-5.8	1.5	6.8	2.3	5.2
13	-1.9	0.0	0.8	-1.1	1.8	2.3	-1.1	0.0	-1.9	-0.8	2.3	-2.3	-4.4	0.4	-3.8	-0.8
14	1.5	-1.1	0.5	0.0	2.1	0.5	0.9	1.1	2.8	-2.9	-5.3	-0.2	-4.4	-6.6	-1.5	-1.5
15	-3.5	-2.2	1.7	-1.3	3.5	-6.8	10.9	-7.1	-17.4	-2.5	-9.0	-12.4	-10.3	-20.2	-3.8	-10.7
16	4.0	-3.9	-9.3	-6.2	-4.9	-5.0	-1.6	-6.4	-4.0	-7.1	-3.0	-1.4	-10.0	0.7	-9.2	-13.1
Mean	-0.1	-2.5	0.3	-3.3	-1.0	-2.7	-0.8	-1.5	-1.7	-4.2	1.0	-3.9	-5.3	-4.7	-1.7	-4.8
±SD	3.3	3.9	4.0	6.1	5.9	4.2	4.8	5.0	5.8	5.2	5.1	6.1	4.2	5.8	3.7	5.8

ΔFEV₁ EP: difference during early phase (EP) in FEV₁ 15 min after allergen inhalation as a percentage change from immediately before inhalation. ΔFEV₁ LP: difference during late phase (LP) in FEV₁ 3–10 h after allergen inhalation as a mean percentage change from immediately before allergen inhalation. Difference between air and NO₂ significant for EP p=0.02 and LP p=0.01, analysis of variance.

early phase with an average decrement of -2.5% ($p=0.01$) versus -0.1% after air ($p=0.94$) and with the difference between air-allergen and NO_2 -allergen responses reaching statistical significance ($p=0.03$). The corresponding change in FEV₁ after NO_2 at the LP response was -4.2% ($p=0.005$) versus -1.7% after air ($p=0.29$), although the difference between air and NO_2 was not statistically significant ($p=0.21$). Baseline (morning) FEV₁ and s_{Raw} did not change from day 1–2 either for air or for NO_2 , which indicates that no effect of NO_2 or allergen remained from day 1.

Effects of NO_2 on asthmatic reaction to allergen after repeated exposure

Spirometric results are shown in table 3, and figures 1 and 2. The EP response was significantly increased by NO_2 , and the allergen induced fall in FEV₁ for the 4 days was, on average, -2.5 versus -0.4% after air ($p=0.018$,

Fig. 1. – Repeated exposure, early phase response. Change in forced expiratory volume in one second (FEV₁) 15 min after inhalation of a nonsymptomatic allergen dose preceded by exposure to $500 \mu\text{g}\cdot\text{m}^{-3}$ NO_2 (□) or air (■). Values are shown as percentage mean and 95% confidence interval. Air versus NO_2 days 1–4, $p=0.02$, analysis of variance.

Fig. 2. – Repeated exposure, late phase response. Change in forced expiratory volume in one second (FEV₁) 3–10 h after inhalation of a nonsymptomatic allergen dose preceded by exposure to $500 \mu\text{g}\cdot\text{m}^{-3}$ NO_2 (□) or air (■). Values are shown as percentage mean and 95% confidence interval. Air versus NO_2 days 1–4, $p=0.01$, analysis of variance.

analysis of variance). The LP response was also significantly greater after NO_2 after repeated exposure for 4 days, with an average decrement in FEV₁ of -4.4 versus -1.9% after air ($p=0.009$, analysis of variance).

The number of late asthmatic reactions 3–10 h after allergen with a fall in FEV₁ $\geq 15\%$ was seven after air (five subjects) and 12 after NO_2 (6 subjects, ns). There was no difference in baseline (morning) FEV₁ or s_{Raw} values between the air and NO_2 weeks.

Histamine responsiveness

Histamine PD_{sRaw100%} was at inclusion 154 (126,372) μg and decreased after allergen preceded by air to 137 (58,261) μg and after allergen preceded by NO_2 to 100 (58,239) μg (median value (25th percentile, 75th percentile)). The decrease from inclusion in histamine PD_{sRaw100%} was significant after air plus allergen (-17% , $p=0.01$) as well as after NO_2 plus allergen (-35% , $p=0.002$). There was, however, no statistically significant difference between the decrease after air plus allergen and NO_2 plus allergen.

Subjective complaints and medication

Subjective symptoms and medication did not differ significantly between NO_2 -allergen and air plus allergen, although there was a trend towards increased night-time symptoms of asthma after NO_2 plus allergen (score 18) versus after air plus allergen (score 9), $p=0.07$. During the 4 days, four doses of inhaled β_2 -agonist were used after NO_2 plus allergen (one subject, days 2, 3 and 4) versus 6 after air plus allergen (3 subjects, days 2, 3 and 4).

Discussion

The major aim of the present study was to investigate the effects of $500 \mu\text{g}\cdot\text{m}^{-3}$ NO_2 and a nonsymptomatic dose of pollen allergen on both EP and LP asthmatic response after 1) single and 2) repeated exposure to allergen. An attempt to imitate real life exposure was made in three ways, using: 1) a NO_2 concentration that may occur in areas with heavy traffic or in homes with unvented gas appliances; 2) a repeated and short exposure to NO_2 for several days resembling the exposure pattern during morning rush hours; and 3) a repeated inhalation of a small dose of an outdoor allergen. In this group of subjects with mild asthma, we found a small, but statistically significant, enhancement by NO_2 on both the EP and the LP asthmatic response to the nonsymptomatic allergen dose. These effects remained after repeated exposure.

Exposure to $500 \mu\text{g}\cdot\text{m}^{-3}$ NO_2 alone did not induce any acute lung function changes either during or immediately after the exposure session, which is in accordance with earlier reports [3, 8, 19]. After repeated exposure, there was a trend to a delayed effect 4 h after NO_2 exposure, although the decrease in FEV₁ did not reach statistical significance.

We found an increase in EP response to allergen when preceded by a single exposure to NO_2 . It should be noted that the allergen dose was so low that it did not *per se* cause any change in FEV₁ at the control exposure. Our

present results not only confirm that the EP asthmatic response can be potentiated by NO₂, as shown by TUNNICLIFFE *et al.* [12] but also indicate that this effect can arise at a significantly lower dose of allergen and also a lower dose of NO₂. In our former study [11], a statistically significant effect was seen only on LP, but our method for assessing EP in that study (change in allergen provocative dose) might not have been sensitive enough.

In contrast to these short-exposure studies (30 min and 1 h, respectively), the response to allergen after a six-hour exposure to NO₂ alone was not altered [21]. The reason for this inconsistency is unclear but could be caused by differences in the subjects' sensitivity to allergen or the fact that the allergen was inhaled immediately after the end of NO₂ exposure in the six-hour study.

It was shown in this study for the first time that an enhancing effect of NO₂ on the asthmatic response is seen over 4 days of repeated exposure. This contrasts with the adaptation of lung function response known from repeated O₃ exposure [22, 23] and also with the inflammatory response to NO₂ in bronchoalveolar lavage (BAL) studies in healthy subjects where the response changes from an increase after single to a decrease after repeated exposure to NO₂ [24].

There is no clear time pattern of the EP and the LP response. The LP response after NO₂ does not show any obvious signs of adaptation, and the difference in FEV₁ between air and NO₂ on day 4 (-3.1%) is not less than the difference on day 1 (-2.5%). Nevertheless, the duration of the effect had vanished after 18 h (on the basis of morning FEV₁) also at the end of the exposure week. The LP response to allergen after air day 3 was equivalent to that after NO₂ but had returned on day 4 to the same level as on day 1 and cannot possibly be explained as a cumulative effect of the allergen.

Nitrogen dioxide alone may cause an increase in non-specific airway responsiveness in asthmatics [6], and repeated exposure to low doses of pollen allergen as well as natural pollen exposure is also known to increase non-specific bronchial responsiveness [13, 14]. In this study, there was an increase in histamine responsiveness after both the air and the NO₂ week, presumably caused by the allergen exposure. Although the air and the NO₂ week did not differ significantly, it should be noted that the relative shift in histamine responsiveness was twice as large after NO₂ as compared to air exposure.

A wide range of mechanisms may be involved in the NO₂-induced potentiation of the allergen response. Although the allergic reaction is relatively well known and there are data on cell and mediator changes after exposure to NO₂, there are few reports on the combined effect of NO₂ and allergen in the airways. Eosinophils and eosinophil cationic protein (ECP) in peripheral blood did not increase after airway exposure to 500 µg·m⁻³ NO₂ and allergen [11], indicating either that the enhancing effect of NO₂ on the late asthmatic reaction was not mediated by eosinophils or that a recruitment of eosinophils from the blood to the lung had already taken place. In a nasal lavage study, ECP was found to be increased after NO₂ and allergen, supporting the hypothesis that activated eosinophils are involved in the adjuvant effect of NO₂ [25]. Studies of the local allergic reaction in the lung after NO₂ by means of bronchial lavage and other techniques are needed.

This study shows that an ambient concentration of NO₂ enhances the asthmatic response to allergen at an allergen dose that does not cause any asthmatic reaction by itself, and that the effect is reproducible after repeated exposure. The results indicate that NO₂ may affect the health of individuals with allergic asthma in real life. However, it is difficult to assess how substantial this NO₂ effect could be based on data from this laboratory experiment. On the one hand, the mean FEV₁ change is small and close to the individual normal variability in asthmatics, and the subjects did not report any statistically significant increase in symptoms of asthma or use of bronchodilators. On the other hand, our group of subjects all had mild asthmatic disease, they were all nonsmokers with a normal lung function, with few exceptions. They did not require any inhaled steroids outside of the pollen season and had few subjective symptoms of asthma according to the diary card 24 h before the start of the study. In this group of mild asthmatics, a LAR was, nevertheless, experienced on 12 occasions after NO₂ compared to seven after air, and the tendency to develop asthmatic symptoms at night during the NO₂ week could harmonize with the spirometric results. Therefore, the small effects found in this study could be more emphasized and clinically manifested in allergic asthmatics with a more severe disease or when exposed to a combination of pollutants. Further studies are necessary in order to assess the clinical implications of NO₂ exposure on the population of people with allergic asthma.

In our exposure model, we intended to mimic natural exposure to NO₂ and allergen, but there are obvious limitations related to a study in a laboratory setting. One is the brief exposure period compared to the natural allergen exposure persisting for a whole pollen season or even throughout the whole year as for indoor allergens. Another limitation is the very low level of other pollutants and a favourable climate with a temperature of 25°C and relative humidity around 37% in the laboratory.

Epidemiological studies have suggested an association between ambient levels of NO₂ and increased risk of exacerbations of asthma [1, 2], first-time asthma in children [26] and a decrease in lung function [27]. However, in these studies, data on the exposure to allergens are rarely reported. During the outbreaks of asthma caused by soybean dust in Barcelona 1985–1987, an association with ambient NO₂ levels was discussed, although confounding meteorological factors could not be excluded [28].

We conclude that repeated exposure for 4 days to 500 µg·m⁻³ NO₂ for 30 min prior to a nonsymptomatic allergen dose enhances the early as well as late phase airway response. The enhancing effect of NO₂ on the late phase response remains unchanged during the 4 days.

Acknowledgements: The authors thank K. Örne-falk, M. Claesson, A-S. Lantz, S. Jonsson, L-G. Persson for skilful technical assistance.

References

1. Castellsague J, Sunyer J, Sáez M, Antó JM. Short-term association between air pollution and emergency room visits for asthma in Barcelona. *Thorax* 1995; 50: 1051–1056.

2. Rossi OVJ, Kinnula VL, Tienari J, Huhti E. Association of severe asthma attacks with weather, pollen, and air pollutants. *Thorax* 1993; 48: 244–248.
3. Bascom R, Bromberg PA, Costa DL, *et al.* Health effects of outdoor air pollution, state of the art. *Am J Respir Crit Care Med* 1996; 153: 477–498.
4. Bylin G. Health risk evaluation of nitrogen oxides; controlled studies on humans. *Scand J Work Environ Hlth* 1993; 19(1): 37–43.
5. Bylin G, Hedenstierna G, Lindvall T, Sundin B. Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. *Eur Respir J* 1988; 1: 606–612.
6. Folinsbee LJ. Does nitrogen dioxide exposure increase airways responsiveness? *Toxicol Ind Hlth* 1992; 8(5): 273–283.
7. Strand V, Salomonsson P, Lundahl J, Bylin G. Immediate and delayed effects at ambient levels of nitrogen dioxide on bronchial responsiveness to histamine in subjects with asthma. *Eur Respir J* 1996; 9: 733–740.
8. Jorres R, Magnussen H. Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO₂ or 0.5 ppm SO₂. *Eur Respir J* 1990; 3: Suppl. 2, 132–137.
9. Hazucha M, Folinsbee L, Seal E, Bromberg P. Lung function response of healthy women after sequential exposures to NO₂ and O₃. *Am J Respir Crit Care Med* 1994; 150: 642–647.
10. Koenig JQ, Covert DS, Smith MS, van Belle G, Pierson WE. The pulmonary effects of ozone and nitrogen dioxide alone and combined in healthy and asthmatic adolescent subjects. *Toxicol Ind Hlth* 1988; 4(4): 521–532.
11. Strand V, Rak S, Svartengren M, Bylin G. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. *Am J Respir Crit Care Med* 1997; 155: 881–887.
12. Tunnicliffe WS, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 1994; 344: 1733–1736.
13. Ihre E, Zetterström O. Increase in non-specific bronchial responsiveness after repeated inhalation of low doses of allergen. *Clin Exp Allergy* 1993; 23: 298–305.
14. Rak S, Löwhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol* 1988; 82: 470–480.
15. DuBois AB, Bothelo SY, Bedell GN, Marshall R, Comroe JHJ. A rapid plethysmographic method for measuring thoracic gas volume; comparison with a nitrogen wash-out method for measuring functional residual capacity in normal subjects. *J Clin Invest* 1956; 35: 322–326.
16. DuBois AB, Bothelo SY, Comroe JHJ. A new method for measuring airway resistance in man using body plethysmograph; values in normal subjects and in patients with respiratory disease. *J Clin Invest* 1956; 35: 327.
17. Pedersen OF, Miller MR, Sigsgaard T, Tidley M, Harding RM. Portable peak flow meters: physical characteristics, influence of temperature, altitude, and humidity. *Eur Respir J* 1994; 7: 991–997.
18. Nieminen MM, Lahdensuo A, Kellomaeki L, Karvonen J, Muttari A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. *Thorax* 1988; 43: 896–900.
19. Bylin G, Lindvall T, Rehn T, Sundin B. Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. *Eur J Respir Dis* 1985; 66: 205–217.
20. Berglund M, Vahter M, Bylin G. Measurement of personal exposure to NO₂ in Sweden: evaluation of a passive sampler. *J Expo Anal Environ Epidemiol* 1992; 2(3): 295–307.
21. Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 1994; 344: 1668–1671.
22. Kulle T, Sauder L, Kerr H, Farrell B, Bermel M, Smith D. Duration of pulmonary function adaptation to ozone in humans. *Am Ind Hygiene Assoc J* 1982; 43(11): 832–837.
23. Bylin G, Cotgreave I, Gustafsson L, *et al.* Health risk evaluation of ozone. Criteria Document. *Scand J Work Environ Hlth* 1996; 22(3): 52–71.
24. Sandström T, Helleday R, Bjerner L, Stjernberg N. Effects of repeated exposure to 4 ppm nitrogen dioxide on bronchoalveolar lymphocyte subsets and macrophages in healthy men. *Eur Respir J* 1992; 5: 1092–1096.
25. Wang JH, Duddle J, Devalia JL, Davies RJ. Nitrogen dioxide increases eosinophil activation in the early-phase response to nasal allergen provocation. *Int Arch Allergy Immunol* 1995; 107: 103–105.
26. Infante-Rivard C. Childhood asthma and indoor environmental risk factors. *Am J Epidemiol* 1993; 137(8): 834–844.
27. Ackermann-Lieblich U, Leuenberger P, Schwartz J, *et al.* Lung function and long term exposure to air pollutants in Switzerland. *Am J Respir Crit Care Med* 1997; 155: 122–129.
28. Castellsague J, Sunyer J, Sáez M, Murrillo C, Antó JM. Effect of air pollution in asthma epidemics caused by soya-bean dust (Abstract). *Eur Respir J* 1992; 5: Suppl. 15, 413.