Immediate and delayed effects of nitrogen dioxide exposure at an ambient level on bronchial responsiveness to histamine in subjects with asthma

V. Strand*, P. Salomonsson*, J. Lundahl**, G. Bylin*

Immediate and delayed effects of nitrogen dioxide exposure at an ambient level on bronchial responsiveness to histamine in subjects with asthma. V. Strand, P. Salomonsson, J. Lundahl, G. Bylin. ©ERS Journals Ltd 1996.

ABSTRACT: The time-kinetics of NO_2 induced effects on bronchial responsiveness are poorly known as most observations have been made shortly after exposure. The aim of this study was to measure nonspecific bronchial responsiveness, lung function and inflammatory markers at different times after NO_2 exposure in asthmatics.

Nineteen subjects with mild asthma were exposed to either purified air or 488 $\mu g \cdot m^{-3}$ (0.26 ppm) NO_2 for 30 min during intermittent exercise. Airway responsiveness to histamine, specific airway resistance (sRaw) and thoracic gas volume (TGV) were measured 30 min, 5 h, 27 h and 7 days after exposure. Peripheral blood inflammatory mediators and the expression of an adhesion molecule, (Mac-1) on granulocytes, were analysed 30 min and 27 h after exposure.

Bronchial responsiveness to histamine was significantly increased 5 h after NO_2 exposure when compared to air (median provocative dose of histamine required to cause 100% increase of sRaw (($PD_{sRaw,100\%}$) 110 μg after NO_2 exposure vs 203 μg on air). There was a tendency for an increase after 30 min, which was nonsignificant (median $PD_{sRaw,100\%}$ 100 vs 153 μg). NO_2 exposure did not affect sRaw, but TGV was significantly reduced after exposure. We found an increased expression of Mac-1 on granulocytes 30 min after NO_2 exposure when compared to pre-exposure values. No effect was seen on tryptase, eosinophil cationic protein (ECP), or myeloperoxidase (MPO).

These results suggest that exposure to an ambient level of NO_2 causes a delayed effect on bronchial responsiveness in asthmatics. The increased expression of an adhesion molecule in peripheral blood may indicate a NO_2-induced priming of human granulocytes.

Eur Respir J., 1996, 9, 733–740.

*Dept for Respiratory and Allergic Diseases, Huddinge University Hospital, Huddinge, Sweden. **Dept of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden.

Correspondence: V. Strand
Dept for Respiratory and Allergic Diseases
Huddinge University Hospital
S-14186 Huddinge
Sweden

Nitrogen dioxide is a well-known airways irritant at high concentrations, but whether exposure to NO_2 at ambient levels (below 1,000 $\mu g \cdot m^{-3}$) affects the airways has been a matter of debate. Nonspecific bronchial responsiveness has been reported to increase in asthmatics after exposure to NO_2 concentrations well below 1,000 $\mu g \cdot m^{-3}$ in several studies [1, 2], although no effect was seen in others [3, 4]. FOLINSBEE [5] recently reported, in a meta-analysis of 20 studies of asthmatics and five of normal subjects, a statistically significant increase in bronchial responsiveness on exposure to ≥200 $\mu g \cdot m^{-3}$ NO_2 in asthmatics and to ≥1,900 $\mu g \cdot m^{-3}$ in normals. There is, thus, reason to believe that NO_2 at ambient concentrations might increase bronchial responsiveness, at least in subjects with asthma.

Earlier studies of NO_2 effects have focused on bronchial responsiveness within the first hour after the end of exposure. However, the toxic effects of high concentrations of NO_2, as seen in industrial gas accidents, occurred several hours after exposure [6]. RASMUSSEN et al. [7] reported on delayed effects of NO_2 on alveolar permeability in healthy subjects. Furthermore, in bronchoalveolar lavage (BAL) studies, SANDSTRÖM et al. [8]

found a maximal influx of inflammatory cells in bronchial lavage fluid as late as 8 h after NO_2 exposure. Thus, it is of interest to know whether NO_2 also has a delayed effect on bronchial responsiveness.

The aim of this study was to investigate the time-kinetics of NO_2 induced effects on nonspecific bronchial responsiveness and lung function in asthmatics. Furthermore, expression of an adhesion molecule Mac-1, on granulocytes and inflammation markers in peripheral blood was studied in order to find signs of a possible NO_2 induced inflammatory reaction.

Material and methods

Subjects

Nineteen subjects, age 20–48 yrs, with mild asthma, participated in the study. Anthropomethric and clinical data are presented in table 1. The inclusion criterion was asthma with reversible attacks of dyspnoea and bronchial hyperresponsiveness to histamine (threshold

Table 1. – Anthropometric and clinical data

Subj No.	Age yrs	Sex	Height cm	Weight kg	Asthma duration yrs	Positive skin-prick test ≥2+	Smoking status	$sRaw$ cmH$_2$O·s^{-1}	VC % pred
1	20	F	168	73	7	B, T, D, C	LNS	4.08	129
2	33	M	178	78	12	B, T, M, R, Mi	ES	3.78	113
3	32	M	191	77	29	B, T, M, D, C, Mo	LNS	3.86	104
4	32	F	161	52	9	Mi	LNS	3.75	99
5	36	M	194	90	2	D, C, H	ES	3.21	112
6	21	M	175	71	18	D, C, H	LNS	3.87	104
7	40	M	172	67	18	B, T, D, C, Mo	ES	6.66	122
8	23	F	173	105	6	C	LNS	5.24	113
9	40	M	179	77	9	D, C	ES	3.39	114
10	45	F	162	60	6	T	ES	5.36	110
11	22	F	158	61	3	B, T, C	LNS	6.13	132
12	39	M	174	80	5	B, D, Mo	ES	4.05	82
13	26	F	160	54	1	*	LNS	5.36	98
14	26	F	177	76	11	B, C, H	LNS	6.17	111
15	37	M	177	68	11	C	LNS	4.96	99
16	48	F	152	70	29	B, T, D, C	LNS	5.65	99
17	33	F	170	82	33	B, T, M, D, C	ES	7.72	110
18	41	M	178	70	39	B, T, D, C, H	ES	5.28	123
19	23	F	164	60	7	*	ES	4.20	101
Mean	32		172	72	13			4.88	109
SD	9		11	13	11			1.23	12

Subj: subject; M: male; F: female; B: birch, T: Timothy; D: dog; C: cat, M: Mugwort; R: rabbit; Mi: house dust mite; Mo: mould; H: horse; ES: ex-smoker; LNS: lifetime nonsmoker; $sRaw$: specific airway resistance; VC: vital capacity; % pred: percentage of predicted value.

dose ≤660 µg histamine causing a doubling of the specific airway resistance, $sRaw$,100%). Lung function expressed as $sRaw$ was within normal range (<8.0 cmH$_2$O·s^{-1}) [9]. Seventeen patients had an immunoglobulin E (IgE) mediated allergy according to standard skin-prick test. The pollen allergic patients were not exposed during pollen season, and patients sensitive to animal dander did not have pets in their homes.

All subjects had occasional symptoms of asthma and then used a bronchodilating beta$_2$-agonist. Two subjects used inhaled sodium cromoglycate periodically but not during study. No oral or inhaled steroid treatment was used. All subjects were nonsmokers, 10 were lifetime nonsmokers and 9 had been ex-smokers for at least 2 yrs.

The study was approved by the Ethics Committee at Huddinge University Hospital.

Study design

The design of the study is shown in figure 1. The subjects were exposed to purified air or 488±13 µg·m^{-3} NO$_2$ (mean±SD) in an exposure chamber for 30 min on two separate days. The order of exposure was randomized (8 exposed first to NO$_2$, 11 first to air) and single-blind. The interval between exposures was 3–4 weeks. The exposures to air and NO$_2$ were performed at 8.30 or 9.30 a.m., and at the same time for each subject.

Baseline airway resistance, thoracic gas volume and bronchial responsiveness to histamine were measured on a separate screening day. On this occasion, the subject gave informed consent to participate in the study.

The experiment consisted of a 30 min exposure (NO$_2$ or air) with intermittent exercise followed by histamine inhalation tests at 30 min, 5 h, 27 h and 7 days after the end of exposure. Lung function was measured before and during exposure and before the histamine challenge. Blood samples were drawn before, 30 min and 27 h after exposure.

The procedure, in more detail, was as follows. After arrival at the laboratory, the subject rested for 15 min. A sample of venous blood was then drawn for analyses of eosinophilic cationic protein (ECP), myeloperoxidase (MPO), tryptase and Mac-1 expression on granulocytes. Specific airway resistance ($sRaw$) and thoracic gas volume (TGV) were measured and the subject rested for 10
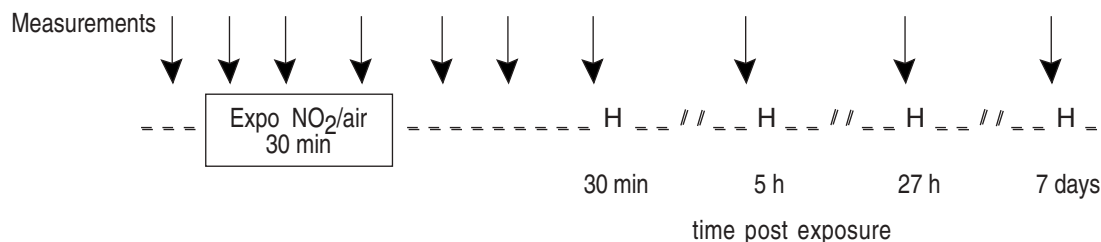

Fig. 1. – Study design. Expo: exposure; ↓: airway resistance and lung volume measurement; H: histamine inhalation challenge.

min. The subject then entered the exposure chamber and sat resting or cycling on an ergometer bicycle (2×7 min periods; from 6th to 13th and 17th to 24th minute of exposure). The workload on the bicycle was 52±21 and 51±19 W (mean±SD) during air and NO$_2$, respectively, and the corresponding values for the subjects' ventilation 35±8 and 33±5 L·min^{-1}. sRaw and TGV were measured at 4, 15 and 30 min of exposure in a body plethysmograph located in the exposure chamber. The subject was interviewed concerning symptoms after 3 and 26 min exposure, with the help of a questionnaire.

Ten and 20 min after the end of exposure, lung function was measured again. Thirty minutes after exposure, a second blood sample was drawn and a histamine inhalation test started. Five hours after the end of exposure a second histamine bronchial provocation test was performed. The following day, the subject returned for a third histamine challenge and on that occasion a third blood sample was drawn. A final bronchial provocation test was performed 7 days after exposure, and on this occasion the subject was interviewed about complaints during the past week.

Lung function measurements

Airway resistance (Raw) and TGV were measured in a body plethysmograph (Cardio-Pulmonary Instruments, Houston, TX, USA; Model 2000 TB, constant volume type). Recordings of Raw and TGV were made according to Du Bois and co-workers [10, 11]. Gas flow and box pressure, as well as mouth pressure and box pressure, were recorded on an x-y recorder (Bryans 50000).

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 2–3 slopes. The mouth/box pressure slopes were measured between the endpoints, again as the mean of 2–3 curves. All panting manoeuvres were made at about 1 Hz, the subject being guided by a metronome. Tidal ventilation was measured using a Fleisch pneumotachograph connected to a transducer (Mercury Electronics, Glasgow, Scotland, UK).

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 adjustable aerosol delivery time [12]. With this equipment, the start of the aerosolization was determined by a threshold volume of inspiration. Inhalation flow, number of nebulizations and the volume of each inhalation were displayed. The nebulization time was set to 0.5 s and the aerolization started after an inspired volume of 100 mL and ended within tidal volume. The flow rate was 0.5 mL·min^{-1}. Three concentrations of histamine diphosphate were used, 1, 8 and 64 mg·mL^{-1}. At each concentration, 2, 4 and 8 breaths were taken. After measuring baseline sRaw the subject inhaled doubling doses of histamine from an initial dose of 13.75 µg until 100% increase in sRaw was reached. sRaw and TGV were measured 3 min after each dose. Threshold provocative dose of histamine required to cause 100%

increase of sRaw (PDsRaw,100%) was calculated by linear interpolation on the dose-effect curve.

Questionnaire

After 3 and 26 min of exposure, the subject was interviewed by means of a questionnaire used previously by our group [13] comprising 16 questions concerning respiratory symptoms and annoying perceptions in the chamber, estimated by a scale with range 1–7. When returning after 7 days, the subject was asked to report symptoms within 24 h after exposure, within 7 days after exposure, and during the period between the two exposures.

Blood samples

Samples for ECP, MPO and tryptase were drawn immediately before, 30 min and 27 h after the end of exposure. ECP, MPO and tryptase were analysed by means of radioimmunoassays [14, 15]. Ethylenediamine tetraacetic acid (EDTA) blood was collected for flow cytometric immunoassay of Mac-1 expression from the subjects at the same times as above. The blood samples were haemolysed in 100 µL portions by dilution in 2 mL 4°C NH$_4$Cl-EDTA "lysing reagent" (Ortho Diagnostics Systems, Westwood, NJ, USA). After 5 min incubation at 15°C, the leucocytes were centrifugated at 300×g for 5 min followed by one wash in 4°C 0.15 M phosphate-buffered saline (PBS) supplemented with 0.1 mM EDTA and 0.02% Na-azide (PBS-EDTA).

Mac-1 expression on granulocytes was analysed routinely [16, 17] by adding 10 µL phycoerythrin (PE)-conjugated monoclonal anti-CD 11b/CD 18 (Becton and Dickinson Immunocytometry Systems, Mountain View, CA, USA) to leucocyte pellets. The suspensions were incubated at 4°C for 30 min followed by two washes in 4°C PBS-EDTA. The leucocytes were resuspended with 1 mL PBS-EDTA. Isotype matched control antibodies (PE-conjugated mouse immunoglobulin G$_2$ (IgG$_2$); Becton and Dickinson Immunocytometry Systems, Mountain View, CA, USA) were used to define the cut-off for positive fluorescence, which was the 99th percentile of the distribution of the cells labelled with respective control antibody. EDTA-blood from healthy nonallergic blood-donors was run parallel as control samples.

The leucocytes were finally examined in an Ortho Spectrum III Flow Cytofluorometer (Ortho Diagnostics Systems, Westwood, MA, USA). A discrimination frame was placed around the granulocyte field. The amount of surface antigen was described by the mean fluorescence intensity (MFI) of the cell population within the field.

Gas dilution and exposure system

NO$_2$ gas, kept in a gas bottle (Alfax, approx 8,000 mg·m^{-3} NO$_2$) was diluted in two steps to a final concentration of about 500 µg·m^{-3} NO$_2$ and fed into the exposure chamber (volume 7 m^3). The gas dilution and exposure system has been presented in more detail previously [2].

Chemical analyses

NO$_2$ concentrations in the exposure chamber were measured with a chemiluminescence instrument (Monitor Labs Nitrogen Oxides Analyser, Model 8440). For calibration, a NO$_2$ permeation tube and NO calibration gas (Monitor Labs 8500 Calibrator; AGA Special Gas, 100 ppm·m^{-3} NO) were used. A calibration procedure was run daily.

The subject's individual exposure to NO$_2$ during the week following the exposure experiment in the laboratory was measured with a personal, passive (filter badge) sampler (Toyo Roshi Kaisha Ltd, Japan; Yanagisawa and Nishimura 1982). The sampler was carried by the subject for 1 week, pinned to the clothes (at the left shoulder) during the daytime and lying on a table in the bedroom at night. The analytical technique as well as the accuracy and reproducibility of the measurements with the sampler have been presented in detail previously [18].

Exposure data

NO$_2$ concentrations in the exposure chamber were measured in the breathing zone of the subject. The mean±SD concentration was 488±13 µg·m^{-3} (range 464–515 µg·m^{-3}). During exposure to filtered air, the NO$_2$ concentration was below 10 µg·m^{-3}. The temperature in the exposure chamber was 25.2±0.6°C (mean±SD) during air and 25.5±0.6°C during NO$_2$ exposure. The corresponding values for relative humidity was 56±14 and 52±12%.

During the week following the exposure experiment, the exposure to NO$_2$ in ambient air was 11±7 µg·m^{-3} (mean±SD) (range 14–37 µg·m^{-3}) after exposure to NO$_2$ and 11±4 (5–22) µg·m^{-3} after air exposure.

Statistics

Data obtained after NO$_2$ and filtered air were compared at the different time-points (30 min, 5 and 27 h, and 7 days) by the nonparametric Wilcoxon sign rank test. Probability values of less than 5% were considered to be significant.

Results

Bronchial responsiveness

All subjects but one completed all the measurements of lung function and bronchial responsiveness. Bronchial responsiveness could not be measured 5 and 27 h after exposure to NO$_2$ in one subject (No. 8) because of symptoms of asthma.

The mean increases of sRaw, in response to inhaled histamine after exposure to NO$_2$ and filtered air, respectively, are shown in figure 2. The individual PDsRaw,100% at the different times after exposure to NO$_2$ or filtered air are shown in table 2.

PDsRaw,100% for histamine tended to decrease in the group 30 min after NO$_2$ exposure, 100 *vs* 153 µg after
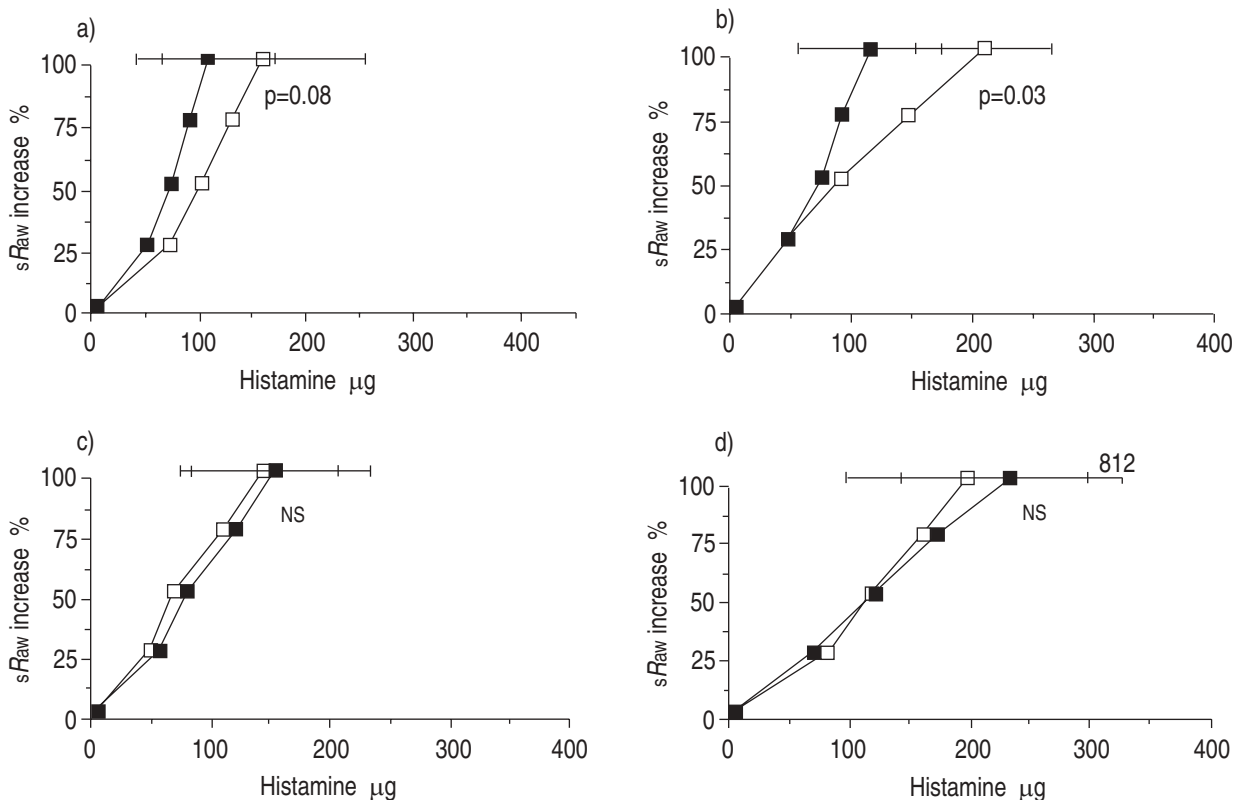

Fig. 2. – Bronchial responsiveness. Increase (%) of specific airway resistance (sRaw) in response to inhaled histamine (µg) after exposure to 488 µg·m^{-3} NO$_2$ and filtered air. Median values and 25th and 75th percentile are presented. a) 30 min after exposure (n=19); b) 5 h after exposure (n=18); c) 27 h after exposure (n=18); d) 7 days after exposure (n=19). —□— : sRaw Air; —■— : sRaw NO$_2$.

air (median) but the decrease was not significant (p=0.08). Five hours after exposure, the difference in histamine response between NO$_2$ and air was significant, (median PDsRaw,100% 110 vs 203 µg; p=0.03). There were no significant differences in response to histamine 27 h or 7 days after exposure.

The mean decrease among the 11 subjects reacting with a lower threshold dose 5 h after NO$_2$ corresponded to 0.83 doubling doses of histamine. Three of these subjects decreased their threshold dose with more than one doubling dose.

Airway resistance and lung volume

sRaw was equally increased by exercise during exposure to air (p=0.001) and NO$_2$ (p=0.001) (fig. 3). TGV was not significantly affected during exposure, but from 20 min after exposure and onwards lung volume was consistently lower after exposure to NO$_2$ compared to air, although statistically significant differences were only seen 20 min (p=0.001) and 7 days (p=0.02) after exposure (fig. 4). The change was maximal 20 min after exposure, with a 0.37 L mean reduction.

Inflammatory cells and mediators

An increased expression of Mac-1 was found on granulocytes 30 min after NO$_2$-exposure when compared to pre-exposure values (13.4±4.6 vs 10.9±4.7 MFI; p=0.01;
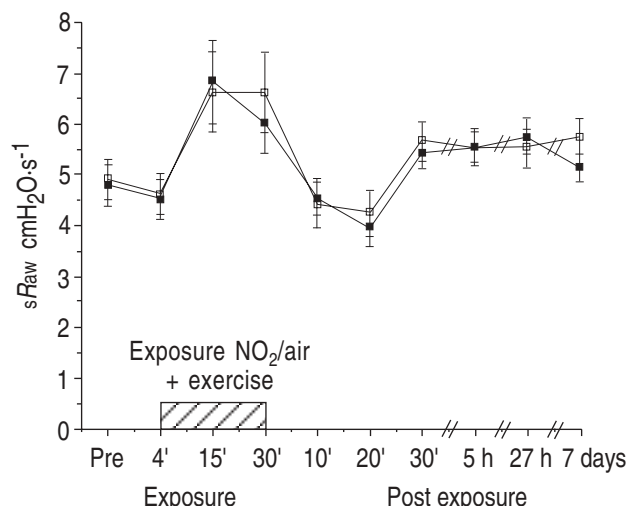

Fig. 3. – Specific airway resistance (sRaw). Changes during and after exposure to air or NO$_2$. Values are presented as mean±SEM. —□— : sRaw Air; —■— : sRaw NO$_2$.

n=15; mean±SD) (fig. 5). The Mac-1 expression was reversed to pre-exposure values 27 h after exposure (10.7±2.8 vs 10.9±4.7 MFI; NS; n=15).

There were no significant differences in ECP, MPO and tryptase levels when comparing pre- and post-exposure values. Because of inappropriate technical handling of the early samples, only the analyses of 12 subjects were complete.

Table 2. – PDsRaw,100% as a measure of bronchial responsiveness: individual dose of histamine (µg) required for 100% increase of sRaw at inclusion, 30 min, 5 h, 27 h and 7 days after exposure to air or 488 µg·m^{-3} NO$_2$ and difference between air and NO$_2$ values

Subj No.	Inclusion	30 min			5 h			27 h			7 days		
		Air	NO$_2$	Δ	Air	NO$_2$	Δ	Air	NO$_2$	Δ	Air	NO$_2$	Δ
1	512	355	473	-118	274	179	95	208	190	19	928	404	524
2	512	135	346	-211	320	131	188	213	112	100	212	330	-118
3	194	94	62	32	25	25	0	59	129	-70	88	170	-83
4	304	804	540	264	1149	787	362	1760	1067	693	812	498	314
5	124	577	71	506	9	102	-93	486	148	338	141	292	-152
6	121	40	69	-29	60	26	34	26	60	-35	63	61	2
7	165	148	146	2	203	102	101	140	49	91	282	233	48
8	49	49	49	0	59	-	-	46	-	-	35	39	-3
9	249	259	288	-29	365	110	255	97	366	-270	267	504	-237
10	495	370	421	-51	796	534	262	534	417	116	888	711	177
11	220	104	69	35	217	110	107	153	243	-90	200	228	-27
12	558	1350	908	442	848	945	-96	275	221	54	1610	2046	-436
13	107	66	25	42	37	58	-21	78	79	-1	81	80	1
14	174	207	104	103	353	186	166	371	153	218	186	73	114
15	143	171	41	130	45	50	-4	107	71	36	160	147	12
16	49	153	86	67	57	43	15	29	35	-7	99	117	-18
17	61	51	46	5	55	73	-18	37	211	-174	-	88	-
18	630	574	507	67	461	429	33	241	715	-474	1613	446	1167
19	94	111	100	11	90	117	-28	71	99	-28	108	100	8
Median	174	153	100		203	110		140	151		193	228	
P25	107	94	62		55	58		59	79		99	88	
P75	495	370	421		365	186		275	243		812	446	
Mean	251	296	229		285	223		260	243		432	346	
		NS (p=0.08)			p=0.03			NS (p=0.6)			NS (p=0.7)		

Δ: difference between air and NO$_2$ values; P25 and P75: 25th and 75th percentile, respectively. For further abbreviations see legend to table 1.
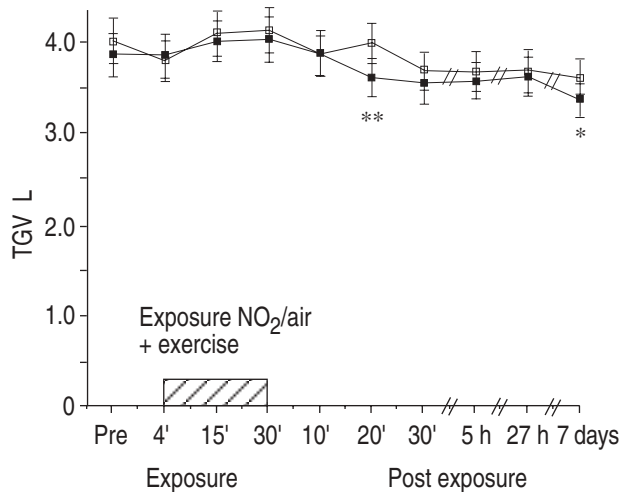
Fig. 4. – Thoracic gas volume (TGV). Changes during and after exposure to air or NO_2. Values are presented as mean±SEM. —□— : TGV Air; —■— : TGV NO_2. **: p=0.001; *: p=0.02.

Subjective complaints

When the subjects were interviewed during the exposure concerning subjective complaints, no difference between air and gas exposure could be detected except for a slightly increased smell of odour after 4 min of NO_2 exposure (mean 2.26 *vs* 1.68 during air on a scale with range 1–7).

The questionnaire was answered by 18 out of 19 subjects and revealed no difference in subjective complaints within 24 h after exposure to air or NO_2. During the week following exposure, seven subjects reported complaints after NO_2 *versus* two after air. The symptoms after NO_2 concerned increased sensitivity to irritants or exercise (four subjects), shortness of breath (2), rhinitis (1), headache (1) and hoarseness (1).

Discussion

This study showed a delayed increase in bronchial responsiveness to histamine, a small but persisting reduction in lung volume and an enhanced expression of a granulocyte adhesion protein after exposure to NO_2 in subjects with asthma.

Bronchial hyperresponsiveness is a hallmark of asthma and the degree of hyperresponsiveness correlates with severity of disease [19]. Air pollutants, such as NO_2, can enhance this responsiveness further and the duration of this increased responsiveness is of apparent interest, as a delayed effect may prolong the time when other agents can trigger asthma symptoms.

In our study, NO_2 exposure tended to increase responsiveness to histamine 30 min after exposure. Some earlier studies have shown an increase in airway responsiveness in asthmatics immediately after NO_2 exposure [1, 2], whilst, others have not [3, 4]. In a recent meta-analysis of 20 studies on NO_2 induced airway responsiveness in asthmatics, FOLINSBEE [5] concluded that NO_2 at concentrations of 200 $\mu g \cdot m^{-3}$ and above increased airway responsiveness.

There was a significant delayed effect on bronchial responsiveness 5 h after exposure to NO_2. As this delayed
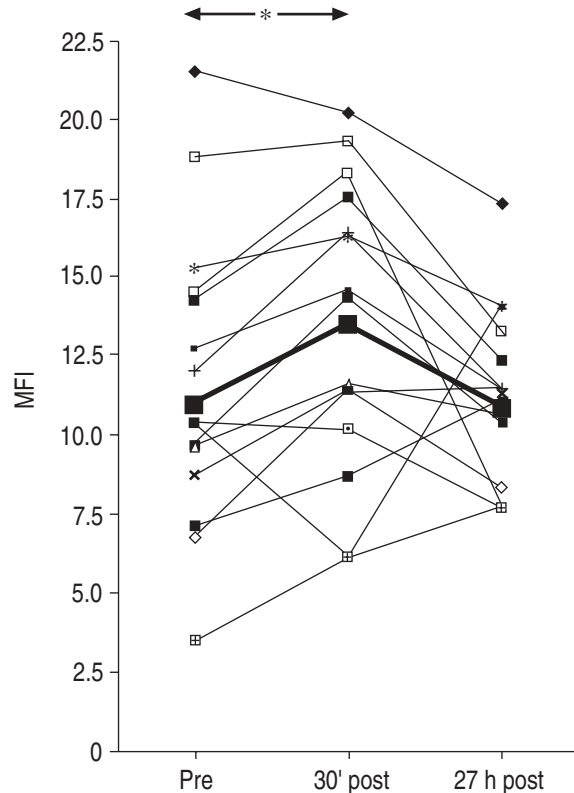

Fig. 5. – Changes in Mac-1 expression on granulocytes (n=15). Mean fluorescence intensity (MFI) before, 30 min and 27 h after exposure to 488 μg NO_2. Individual changes and mean values are presented. —■— : mean. *: p=0.01.

effect after NO_2 has not been described previously, it is necessary to consider that factors other than NO_2 might contribute to this result.

A difference in sRaw at 5 h, with a higher sRaw after NO_2, could affect the result and cause an increased responsiveness. However, sRaw values following air and NO_2 were virtually identical before histamine challenge at 5 h.

Exposure to NO_2 in ambient air during the study weeks was identical and low, according to personal monitoring both after NO_2 and after air exposure, and interference of natural exposure with the results is, therefore, not likely.

Another possibility is that the change in responsiveness 5 h after exposure was caused by histamine inhaled at 30 min after exposure. However, repeated histamine inhalation challenges within a day in asthmatics have not been reported to change sensitivity to histamine [20, 21]. More important still, the effect of NO_2 at 5 h is compared to that of sham exposure at the same time, which was also preceded by a histamine inhalation at 30 min.

Exercise can reduce bronchial responsiveness to histamine for several hours [20, 22]. In our study, a possible effect of the moderate exercise during exposure would be a decrease of bronchial responsiveness to histamine 5 h after exposure. However, the mean histamine threshold value at 5 h after sham exposure was not significantly different from that obtained at 30 min, 27 h and 7 days. When studying figure 2, it may be argued that the difference after 5 h in PD$_{s}$$R$aw,100% between NO_2 and

air is caused mainly by a shift to the right of the air curve. However, the air curve does not significantly differ from the curve obtained at 30 min. Even if it is presumed that exercise and/or histamine inhalation tends to shift the air curve to the right at 5 h, this effect is apparently counteracted by NO$_2$. To conclude, it is a real difference in bronchial responsiveness between NO$_2$ and air at 5 h. Lung volume was reduced about 5–10% immediately after exposure to NO$_2$, without any concomitant change in airway resistance, and this decrease persisted even 1 week after the exposure. However, this difference ought to be interpreted with caution, as TGV after sham exposure also tended to decrease.

The immediate effect of NO$_2$ on lung volume has been investigated in many studies in asthmatics as well as in normals, and no effect has been reported [13, 23]. Delayed effects on lung function have seldom been looked for in short-term exposure studies, although the time-course of NO$_2$ effects in gas accidents is characterized by a free interval lasting about 4–6 h followed by symptoms caused by increased microvascular permeability and oedema in the lung [6]. BEIL and ULMER [24] exposed healthy subjects to 10,000 µg·m^{-3} NO$_2$ for 14 h and found a slight increase in lung volume that had returned to pre-exposure values 10 h after the end of exposure.

In the current study, two histamine challenges were performed within a short time after the end of exposure, which might have influenced the lung volume measurements. No delayed effect on lung volume was, however, seen after sham exposure, which is in accordance with previous reports [20]. Even if histamine alone does not affect lung volume hours after inhalation it is still a possibility that NO$_2$ exposure followed by repeated histamine inhalations gives another effect on lung volume than NO$_2$ or histamine alone. A delayed effect on lung volume can, therefore, not be attributed with certainty to NO$_2$ exposure alone based on this study.

We found an increased expression of the adhesion promoting glucoprotein Mac-1 (CR3 or CD11b/CD18) on granulocytes 30 min after exposure. Mac-1 is a glucoprotein essential for chemotaxis and adhesion to the endothelium of human neutrophils [25]. Mac-1 is partly stored in secretory granules, which are the most accessible intracellular compartment [26]. During antigen induced asthma, an increased expression of Mac-1 was observed [27], and an increased readiness to mobilize this receptor has been reported in children with asthma [28]. An enhanced expression of this granulocyte adhesion protein may indicate an increased capacity or tendency to penetrate the endothelial wall. An increased number of granulocytes has been reported in BAL after NO$_2$ [29]. A small increase in adhesive capacity of the cell can also imply a priming of the granulocyte, being more easily activated when exposed to a subsequent stimuli.

The NO$_2$ induced increase in bronchial responsiveness was small and within normal variability for most of the subjects. However, 3 out of 18 patients (17%) increased their responsiveness substantially (>1 doubling dose histamine). The effect of NO$_2$ on histamine responsiveness in the laboratory makes it possible that NO$_2$ could also increase susceptibility to nonspecific irritants, cold air and also to allergens in real life. Furthermore, the delayed effect seen in the majority of our subjects means

that the time of increased susceptibility is longer than previously realized. NO$_2$ exposure during rush hours in the morning could, therefore, facilitate triggering of asthma symptoms by other agents at least up to early afternoon. The reported subjective complaints during the week after exposure in our study might be an expression of this.

The clinical importance of these laboratory findings is supported by two recent epidemiological studies in Nordic cities showing that hospital admissions and emergency visits for asthma were significantly associated with ambient NO$_2$ levels below guideline values [30, 31].

In conclusion, this study shows that short-term exposure to a relatively high ambient level of NO$_2$ causes a delayed effect on bronchial responsiveness in asthmatics. This effect might be of clinical importance for at least a part of the population with asthma. The increased expression of an adhesion molecule in peripheral blood may indicate that NO$_2$ exposure stimulates human granulocytes.

References

1. Bauer MA, Utell MJ, Morrow PE, Speers DM, Gibb FR. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 1986; 134: 1203–1208.

2. 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.

3. Hazucha MJ, Ginsberg JF, McDonell WF, *et al*. Assessment of 0.1 ppm nitrogen dioxide effects on the airways of normal and asthmatic subjects. *J Appl Physiol*: *Respirat Environ Exercise Physiol* 1983; 54(3): 730–739.

4. Linn WS, Shamoo DA, L Avol EL, *et al*. Dose-response study of asthmatic volunteers exposed to nitrogen dioxide during intermittent exercise. *Arch Environ Health* 1986; 41: 292–296.

5. Folinsbee LJ. Does nitrogen dioxide exposure increase airways responsiveness? *Toxicol Ind Health* 1992; 8(5): 273–283.

6. Hajela R, Janigan DT, Landrigan P, Boudreau S, Sebastian S. Fatal pulmonary edema due to nitric acid fume inhalation in three pulp-mill workers. *Chest* 1990; 97: 487–489.

7. Rasmussen TR, Kjaergard SK, Tarp U, Pedersen OF. Delayed effects of NO$_2$ exposure on alveolar permeability and glutathione peroxidase in healthy humans. *Am Rev Respir Dis* 1992; 146: 654–659.

8. Sandstrom T, Anderson MC, Kolmodin-Hedman B, Stjernberg N, Angstrom T. Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time-kinetics study. *Eur Respir J* 1990; 3: 138–143.

9. Cotes JE. *In*: Lung Function. Oxford, Blackwell Scientific Publ. 1979: pp. 384.

10. Du Bois AB, Bothelo SY, Bedell GN, Marshall R, Comroe JHJ. A rapid plethysmographic method for measuring thoracic gas volume: comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. *J Clin Invest* 1956; 35: 322–326.

11. Du Bois 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.

12. Nieminen MM, Lahdensuo A, Kellomaeki L, Karvonen J, Muittari A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. *Thorax* 1988; 43: 896–900.

13. 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.

14. Peterson CGB, Enander I, Nystrand I, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method: establishment of normal levels in serum and turnover *in vivo*. *Clin Exp Allergy* 1991; 21: 561.

15. Olofsson I, Olsson I, Venge P. Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol* 1977; 18: 73–80.

16. Hed J, Berg O, Forslid J, Halldén G, Lärka-Raffner G. The expression of CR1 and CR3 on nonmodulated and modulated granulocytes of healthy blood donors as measured by flow cytofluorometry. *Scand J Immunol* 1988; 28: 339–344.

17. Lundahl J, Halldén G, Hed J, Johansson SGO. A flow cytometric method to measure the stimulated mobilization and the intracellular pool of the adhesion promoting glucoprotein Mac-1. *APMIS* 1991; 99: 139–146.

18. Berglund M, Vahter M, Bylin G. Measurement of personal exposure to NO_2 in Sweden: evaluation of a passive sampler. *J Expo Anal Environ Epidemiol* 1992; 2(3): 295–307.

19. Britton JR, Burney PGJ, Chinn S, Papacosta AO, Tattersfield AE. The relation between change in airway reactivity and change in respiratory symptoms and medication in a community study. *Am Rev Respir Dis* 1988; 138: 530–534.

20. Hamielec C, Manning P, O'Byrne P. Exercise refractoriness after histamine inhalation in asthmatic subjects. *Am Rev Respir Dis* 1988; 138: 794–798.

21. Ruffin RE, Alpers JH, Crocket AJ, Hamilton R. Repeated histamine inhalation tests in asthmatic patients. *J Allergy Clin Immunol* 1981; 67(4): 285–289.

22. Schoeffel R, Anderson S, Gillam I, Lindsay D. Multiple exercise and histamine challenge in asthmatic patients. *Thorax* 1980; 35: 164–170.

23. Kerr HD, Kulle TJ, McIlhany ML, Swidersky P. Effects of nitrogen dioxide on pulmonary function in normal healthy humans and subjects with asthma and chronic bronchitis. *Environ Res* 1979; 19: 392–404.

24. Beil M, Ulmer WT. Wirkung von NO_2 im MAK-Bereich auf Atemmechanik und bronchiale Acetylcholinempfindlichkeit bei Normalpersonen. *Int Arch Occup Environ Health* 1976; 38: 31–44.

25. Larson RS, Springer TA. Structure and function of leukocyte integrins. *Immunol Rev* 1990; 114: 181–217.

26. Sengeløv H, Kjeldsen L, Borregaard N. Control of exocytosis in early neutrophil activation. *Science* 1993; 237: 1204–1206.

27. Arm JP, Walport MJ, Lee TH. Expression of complement receptors type 1 (CR1) and type 3 (CR3) on circulating granulocytes in experimentally provoked asthma. *J Allergy Clin Immunol* 1989; 83: 649–655.

28. Berends C, Hoekstra MO, Dijkhuizen B, de Monchy JGR, Gerritsen J, Kauffman HF. Expression of CD35 (CR1) and CD11b (CR3) on circulating neutrophils and eosinophils from allergic asthmatic children. *Clin Exp Allergy* 1993; 23: 926–933.

29. Helleday R, Sandström T, Stjernberg N. Differences in bronchoalveolar cell response to nitrogen dioxide exposure in nonsmokers. *Eur Respir J* 1994; 7: 1213–1220.

30. Pönke A. Asthma and low level air pollution in Helsinki. *Arch Environ Health* 1991; 46(5): 262–270.

31. Rossi OVJ, Kinnula VL, Tienari J, Huhti E. Association of severe asthma attacks with weather, pollen, and air pollutants. *Thorax* 1993; 48: 244–248.