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Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma

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Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. G. Bylin, G. Hedenstierna, T. Lindvall, B. Sundin.
ABSTRACT: Twenty subjects with mild asthma were exposed at rest in a body plethysmograph, to NO_2 at 0, 260, 510 and 1,000 $\mu g \cdot m^3$, for 30 min on four separate days. Bronchial responsiveness (histamine inhalation test) was measured after each exposure session. Airway resistance (Raw), thoracic gas volume (TGV) and specific airway resistance (sRaw) were measured before, during and after exposure, and the breathing pattern was monitored during the whole session. Bronchial responsiveness increased significantly after 30 min exposure to 510 $\mu g \cdot m^3$ NO_2 ($p < 0.01$). There were also tendencies to an increased bronchial responsiveness after exposure to 260 and 1,000 $\mu m \cdot m^3$ NO_2, but these changes were not statistically significant. Effects on airway resistance and breathing pattern were not demonstrated by exposure to 0–1,000 $\mu g \cdot m^3$ NO_2. We conclude that short-term NO_2 exposure at about 500 $\mu g \cdot m^3$ slightly affects human bronchial responsiveness in subjects with mild asthma.
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Nitrogen dioxide (NO_2) in high concentrations is a well-documented airway irritant [1–3], but the risk evaluation of exposure to ambient concentrations of NO_2 (range 0–1,000 $\mu g \cdot m^3$) is difficult due to few and/or inconclusive experimental and epidemiological data.

In a previous study [4], we found a small but statistically significant increase in airway resistance in healthy subjects exposed to 460 $\mu g \cdot m^3$ NO_2 and, paradoxically, a significant decrease after exposure to 910 $\mu g \cdot m^3$ NO_2. A similar trend was found in asthmatic subjects. Based on these findings, and data in the literature, we proposed that there might be a non-monotonous dose-effect relationship for NO_2-induced airway resistance, with an increase at low (about 500 $\mu g \cdot m^3$), a decrease at moderate (about 1,000 $\mu g \cdot m^3$) and an increase again at high (3,000 $\mu g \cdot m^3$ and more) concentrations of NO_2. However, in our previous study the NO_2 concentrations were, for safety reasons, presented in an ascending order only, the number of subjects was small, and concomitant changes in the thoracic gas volume (TGV) might have contributed to the alterations in airway resistance.

In the present investigation of lung physiological effects of NO_2 exposure at the concentration range 0–1,000 $\mu g \cdot m^3$ we have used a balanced presentation order of concentrations, a fairly large number of subjects and better means for revealing possible lung volume-induced changes in airway resistance. Our aim was to discover whether short-term exposure to NO_2 at ambient concentrations affects airway resistance, bronchial responsiveness, breathing pattern and subjective comfort in asthmatic subjects at rest. We also wanted to test the hypothesis of a non-monotonous dose-effect relationship for NO_2-induced airway resistance in the concentration range of 0–1,000 $\mu g \cdot m^3$ NO_2.

Subjects

Twenty asthmatic subjects (sixteen non-smokers, four ex-smokers) participated in the study (for anthropometric and clinical data, see table 1). In calculating the number of subjects, we aimed at detecting a 15% change in specific airway resistance (sRaw) at a significance level of 5% with a power of 80% provided that the precision of the sRaw measurements (coefficient of variation) was 15% or less [5].

The clinical history of the asthmatic subjects included recurrent attacks of reversible dyspnoea, with wheezing and bronchial hyperresponsiveness, during the last year (histamine inhalation test, sRaw measurements). A diagnosis of allergy was based on anamnesis, prick tests, and in some cases specific IgE

Table 1. – Anthropometric and clinical subject data

Subject no.	Age yrs	Sex	Length cm	Weight kg	Intrinsic (I) or extrinsic (E) asthma	Duration of asthma yrs
1	21	M	174	83	E animal dander	5
2	41	F	163	75	I	7
3	45	F	164	80	E animal dander	3
4	44	F	168	61	I	2
5	22	M	198	97	E animal dander	1
6	26	M	171	73	E animal dander	5
7	34	M	179	75	I	6
8	46	F	167	78	I	16
9	56	F	160	72	E birch- and grass-pollen	6
10	48	M	178	84	I	6
11	23	M	177	71	E animal dander	13
12	37	F	161	61	I	3
13	18	F	174	60	I	5
14	41	F	160	59	E grass- and mugwort-pollen, animal dander	39
15	37	M	180	77	E animal dander	3
16	37	F	165	65	E birch- and grass-pollen, animal dander	37
17	28	M	185	78	E mites	5
18	19	F	164	57	E birch-, grass- and mugwort-pollen, animal dander	19
19	27	F	157	52	E mugwort-pollen, animal dander	2
20	17	F	162	58	I	3
$\bar{x}\pm SD$	33±12	M=8 F=12	170±10	71±11	I=8 E=12	Median value=5

radioallergosorbent tests (RAST) and provocation tests. The study was performed during the spring (March–June). Subjects with birch-pollen allergy were investigated before the onset of the spring pollen season. Only two subjects had slight daily symptoms of asthma during the investigation period and sometimes had to use a bronchodilating (beta-2-stimulating) tablet or aerosol (never later than 12 and 8 h before experimental sessions, respectively).

Procedure

The subjects were exposed in an exposure chamber for 30 min on four separate days (at one week intervals) to purified air and one of three different concentrations of NO_2 (260, 510 and 1,000 $\mu g \cdot m^3$). The concentrations were presented in a variety of sequences for different subjects, giving a balanced order of presentation for the whole group. The concentration of NO_2 at each experimental session was unknown to the subject and was not disclosed until all experiments had been completed. For each subject, the experiments were performed at the same time of day.

For training purposes the subjects' airway resistance and thoracic gas volume were measured in the exposure chamber well before the series of experiments. A histamine inhalation test was performed to confirm the existence of bronchial hyperresponsiveness (a prerequisite for participation in the study).

Each experimental session started with the subjects resting for some minutes. Airway resistance (Raw) and thoracic gas volume (TGV) were then measured five times at three different lung volumes (normal, inspiration, expiration). After a short pause, monitoring of the subject's breathing pattern was started and continued throughout the experiment. Ten min before the start of the NO_2 exposure (or sham exposure to purified air) Raw and TGV were measured in the exposure chamber and again after 10, 20 and 30 min exposure. The subject was interviewed with the help of a questionnaire concerning symptoms at 5 and 25 min exposure. Ten and twenty min after the end of exposure the lung function variables were measured again. A histamine inhalation test of bronchial responsiveness was performed about 25 min after the end of exposure.

Gas dilution and exposure system

NO_2 gas (AGA, approx. 8,000 $mg \cdot m^3$), was diluted in two steps to a final concentration range of 4–1,400

$\mu g \cdot m^3$. The test gas was fed (at approx. 200 $l \cdot min^{-1}$) into a box for body plethysmography, which by installation of inlet and outlet systems was modified into a dynamic flow exposure chamber. When lung function variables were measured the gas flows to and from the body-box were bypassed and the box was air-tightened as required for the measurement technique. The gas dilution and exposure system is presented in detail elsewhere [4].

Chemical analysis

The NO_2 exposure concentrations were measured with a chemiluminiscence instrument (Monitor Labs Nitrogen Oxides Analyser, Model 8440). For calibration a NO_2-permeation tube was used (permeation rate 1,370 $\mu g \cdot m^3$ NO_2 at 1 $l \cdot min^{-1}$ air flow) and nitrogen monoxide (NO) calibration gas (Monitor Labs 8500 calibrator; AGA Special Gas, 122 $mg \cdot m^3$ NO), both referrable to standards from the US National Bureau of Standards. A calibration procedure was carried out daily. In addition, the chemiluminiscence analyses were compared with the results of a wet chemical method (TGS-ANSA, [6]); the wet chemical results being on average 98% of the chemiluminiscence results, $n = 8$.

Exposure data

NO_2 concentrations in the exposure chamber were measured close to the face of the subject. Mean concentrations of NO_2 in the body-box were about 260, 510 and 1,000 $\mu g \cdot m^3$ respectively (260 ± 6, 511 ± 6 and 997 ± 9 $\mu g \cdot m^3$ respectively; arithmetic means and standard errors, $n = 20$), and NO_2 concentrations equal to 63% of the intended value were obtained at 0.9 min and equal to 90% at 1.4 min (mean values) after the start of the exposures.

The mean ambient NO_2 concentrations (1 h mean value) during the investigation period were about 50 $\mu g \cdot m^3$ (monitored at three different stations in the city of Stockholm), maximum 1 h mean value 180 $\mu g \cdot m^3$ NO_2.

The air of the exposure chamber and the waiting-room was filtered with particulate and active coal filters (the background NO_2 concentration averaging 10 $\mu g \cdot m^3$, temperature in the chamber during the exposure 25.9 ± 0.2°C, mean difference between 5 and 25 min of exposure 0.8°C, mean relative humidity 43 ± 1% and mean difference 1.5% between 5 and 25 min of exposure).

Lung function measurements

Raw (airway resistance) and TGV (thoracic gas volume) were measured by use of a body plethysmograph (constant volume type with electronic compensation for drift due to humidity and temperature, CPI-Texas Model 2000 TB). Specific airway resistance (sRaw) was calculated as Raw × TGV. An airway conductance/lung volume curve, (five measurements of conductance and TGV at three lung volume levels, at functional residual capacity (FRC), at inspiration (about +1 *l*) and at expiration (about −0.5 *l*), was constructed by regression analysis for each subject at every experimental session in order to be able to refer all observed Raw values in a specific subject during a specific experimental session to a fixed lung volume. The Raw value, normalized with regard to lung volume, was denoted normalized airway resistance (nRaw).

Recordings of TGV and airway resistance (X-Y recorder Bryans 50,000) were made according to Du Bois and co-workers [7, 8]. Airway conductance was calculated as the reciprocal of airway resistance. The slope of the gas flow/box pressure recording was measured as the mean of 2–3 curves, between gas flow +0.5 and −0.5 $l \cdot s^{-1}$ (expiration-inspiration), and of the mouth pressure/box pressure recording between the end points, again as the mean of 2–3 curves. The data records contained no information on exposure conditions. The sRaw evaluations were therefore blind. All panting manoeuvres were made at 1 Hz, the subject being guided by a metronome. The gas flow, recorded by a pneumotachograph, was integrated electronically to provide a volume displayed on a recorder guiding the subject during the various breathing manoeuvres.

Inductive plethysmography

By inductive plethysmography (Respitrace model 300SC, two transducers, and zig-zag coils of wire were mounted at the mamillary and umbilical levels, the inductance of each coil varying in proportion to the cross-sectional area of the chest and the abdomen during breathing) a continuous recording was made of the respiratory rate, the number of sighs, and the breathing pattern, expressed as the inspiratory and expiratory times (Ti, Te).

Volumetric calibration of the transducers was performed as isovolume manoeuvres according to Konno and Mead [9].

Bronchial responsiveness test

Bronchial responsiveness was expressed as the minimum dosage of inhaled histamine aerosol that caused a doubling of the specific airway resistance measured before inhalation of histamine. In the bronchial responsiveness tests we used solutions of histamine chloride (initial concentration 0.0315 and maximal concentration 16 $mg \cdot ml^{-1}$ in physiological saline; fresh histamine solutions being prepared every day from a stock solution by Apoteksbolaget).

The histamine aerosols were inhaled during 2 min from an ultrasonic nebulizer (De Vilbis Pulmosonic Model 25, delivering 0.53 $ml \cdot min^{-1}$ at 6 $l \cdot min^{-1}$). The subject wore a nose-clip during the inhalation. Each inhalation series was preceded by a sham inhalation of saline aerosol. After each inhalation Raw and TGV were measured within 2–4 min. The histamine inhalations continued with a doubling of concentration until sRaw had increased by 100%

compared to the sRaw obtained on sham inhalation of saline.

Subjective complaints

After 5 and 25 min exposure, the subjects were interviewed with the help of a questionnaire concerning odour, eye irritation, cough, chest oppression and dyspnoea. The answers were graded from 1 (no complaints at all) to 7 (severe complaints).

Statistics

Analysis of variance was used for parametric tests of differences in lung function and breathing pattern variables. In testing the effect of one single NO_2 concentration, a 2-way analysis of variance was performed. Effects of exposure time and possible after-effects were analysed with orthogonal contrasts in connection with the 2-way analysis of variance [5].

Differences in bronchial responsiveness after NO_2 exposure were tested by Wilcoxon's signed rank test, since the form of the distribution was unclear and a non-parametric test confers less restriction in that respect. A two-tailed test was performed and values of $p < 0.05$ were considered statistically significant.

Results

Airway resistance and lung volume

The baseline values did not differ significantly from expected values for non-asthmatic individuals [10, 11] (Raw = 1.13 ± 0.08 $cmH_2O \cdot s \cdot l^{-1}$; TGV = 3.30 ± 0.16 *l*; sRaw = 3.76 ± 0.33 $cmH_2O \cdot s^{-1}$; nRaw = 1.04 ± 0.08 $cmH_2O \cdot s \cdot l^{-1}$; mean and standard error).

The group baseline values showed no significant changes during the period of the experiment. The variation range for the group sRaw was within $\pm 8\%$ and for the group TGV within $\pm 1\%$ of the grand means (3.49 $cmH_2O \cdot s^{-1}$ and 3.27 *l* respectively) of the group. The coefficient of variation of the method for sRaw and TGV, estimated by comparing the values obtained 10 and 20 min after exposure to purified air, was 15 and 4%, respectively.

The percentage changes in sRaw during and after exposure to the different concentrations of NO_2 are shown in figure 1. There was no increase in sRaw at any NO_2 concentration. Infact, there was a tendency for sRaw to decrease with time at all exposure levels, including sham exposure (purified air).

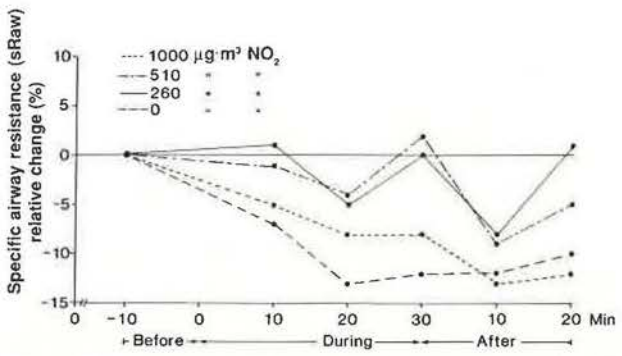

Fig. 1. Relative change (%) in specific airway resistance (sRaw) between values obtained before and at different points of time during and after exposure. Mean values.

Taking the sham exposure to purified air as a reference, exposure to 260 and 510 $\mu g \cdot m^3$ NO_2 tended to increase sRaw (10–15%), but these effects were not statistically significant. Calculations on normalized airway resistance data (nRaw) yielded the same results as for sRaw.

The mean values of TGV were not significantly affected by the NO_2 exposure (maximal difference between pre-exposure and exposure values being 0.17 *l*).

Bronchial responsiveness

All subjects reached the intended increase in sRaw of at least 100% at every histamine inhalation test series, making it possible to calculate histamine thresholds for all exposures (table 2).

The histamine threshold (100% increase in sRaw) was significantly decreased after exposure to 510 $\mu g \cdot m^3$ NO_2 ($p < 0.01$) compared to sham exposure (14/20 subjects showing a decrease). A tendency to decrease was also seen after exposure to 260 and 1,000 $\mu g \cdot m^3$ NO_2 compared to sham exposure (14/20 and 15/20 subjects respectively), but this difference was not statistically proven ($p = 0.052$ and 0.42 respectively). About half of the subjects (9/20) had a lower histamine threshold value after exposure to all NO_2 concentrations compared to purified air.

Dose-effect curves (fig. 2) for the group for the different NO_2 exposure concentrations were calculated according to SOTOMAYOR *et al.* [12] from doses of histamine causing 25, 50, 75 and 100% changes in sRaw. The increase in sRaw values after histamine inhalation was consistently higher after NO_2 exposure compared with exposure to purified air.

Breathing pattern

The respiratory rate was fairly constant during exposure to purified air (pre-exposure mean 16 breaths per min; maximal decrease during exposure 1.5 breaths per min) as well as to the different NO_2 concentrations (pre-exposure means 15.9–16.2 breaths per min; maximal decrease during exposure 1.1 breaths per min). Inspiratory and expiratory times as well as the frequency of sighs (breaths with an amplitude greater than twice the ordinary amplitude at tidal breathing), were not significantly affected at any NO_2 concentration.

Discussion

The method

We have assumed that changes in sRaw have to be about 15% or more to be of any clinical importance. Thus the number of subjects in this study would have been sufficiently large to reveal any significant effects with a probability of 80%, since the coefficient of variation for the sRaw measurements turned out to be

Table 2. – Threshold concentration of histamine ($mg \cdot ml^{-1}$) after 30 min exposure in the body-box to purified air and three different concentrations of NO_2

	NO_2 concentration $\mu g \cdot m^3$			
Subject no.	0	260	510	1,000
1	0.05	0.27	0.20	0.19
2	0.05	0.03	0.06	0.15
3	0.05	0.07	0.03	0.04
4	0.76	0.25	0.20	0.54
5	0.29	0.54	0.20	0.11
6	0.76	0.33	0.35	0.33
7	0.27	0.20	0.23	0.27
8	0.43	0.22	0.31	0.23
9	0.13	0.05	0.05	0.04
10	1.00	0.71	0.41	1.52
11	0.03	0.08	0.07	0.05
12	0.47	0.41	0.14	0.71
13	0.76	0.57	0.71	0.71
14	1.00	0.66	0.41	0.22
15	0.11	0.09	0.14	0.08
16	0.17	0.22	0.19	0.08
17	0.31	0.15	0.22	0.09
18	0.54	0.13	0.41	0.62
19	0.38	0.27	0.27	0.24
20	0.20	0.43	0.23	0.66
x̄±SE	0.39±0.07	0.28±0.05	0.24±0.04	0.34±0.08

The threshold concentration is calculated as the concentration at which the specific airway resistance is doubled compared with the value obtained after sham inhalation to saline.

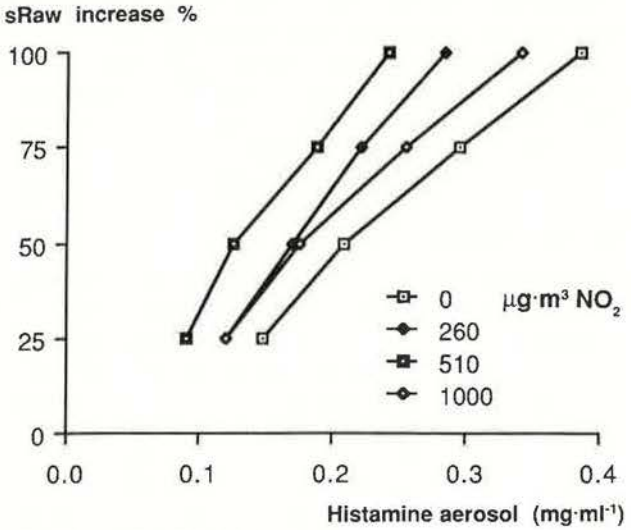

Fig. 2. Mean values of histamine causing a fixed increase (25, 50, 75 and 100% respectively) in specific airway resistance (sRaw) in twenty subjects with asthma after exposure to purified air and three different concentrations of NO_2.

15%, which was the basis for choosing the number of subjects.

All subjects had a mild form of asthma assessed from their histories, medication and lung function. The baseline airway resistance values for our study group were lower than in our previous study with asthmatic subjects [4] and in those reported by OREHEK *et al.* [13] and HAZUCHA *et al.* [14], which means that the subjects in this study had a milder form of asthma.

After the last exposure session, the subjects were asked to assess the order of the different NO_2 concentrations presented. Only five subjects identified all four concentrations correctly. Thus, although no effort was made to mask the weak odour of NO_2, only a minority of the subjects could correctly appraise the NO_2 presentation order from the smell.

The NO_2 concentrations were measured by two independent methods, chemiluminiscence technique and a wet-chemical method. Since the results of the methods correlated well, we believe that the accuracy of the NO_2 exposure concentration measurements was good.

Bronchial responsiveness

Increased bronchial responsiveness is a distinctive feature of asthma and is believed to be a pathogenetic factor for the development of airflow obstruction.

It may be argued that the threshold value of inhaled histamine gives too limited an expression of the bronchial reactivity, as it only reflects one point on a dose-effect curve based on several data-pairs of histamine concentrations and sRaw values. As can be seen from the group dose-effect curves for different NO_2 concentrations (fig. 2), the increase in sRaw values caused by a certain histamine concentration was consistently higher after NO_2 exposure compared to

exposure to purified air. Thus both methods, calculation of histamine thresholds (100% increase in sRaw) and inspection of the whole dose-effect curve, gave the impression that NO_2 exposure at the concentration of 510 $\mu g \cdot m^3$ increases bronchial responsiveness.

There are few reports on bronchial responsiveness in asthmatic subjects after short-term exposure to ambient NO_2 concentrations. OREHEK *et al.* [13] found a significant increase in bronchial responsiveness after 1 h exposure to 210 $\mu g \cdot m^3$ NO_2. HAZUCHA *et al.* [14] made a similar study at the same NO_2 concentration and found no change. Our results, at a slightly higher NO_2 concentration (260 $\mu g \cdot m^3$), concur with those reported by OREHEK *et al.* but are not statistically proven. Our results at 510 $\mu g \cdot m^3$ NO_2 are in agreement with KLEINMAN *et al.* [15] who found a slight increase in bronchial responsiveness in asthmatic subjects exposed to about 400 $\mu g \cdot m^3$ for 2 h with light intermittent exercise, and BAUER *et al.* [16] who reported that exposure to about 600 $\mu g \cdot m^3$ NO_2 potentiated exercise-induced bronchospasm and airway hyperreactivity after cold air provocation in asthmatic subjects. In a previous study, we found a significant increase in bronchial responsiveness at 910 $\mu g \cdot m^3$ NO_2 for asthmatic subjects, and in the present study the same tendency appeared, at 1,000 $\mu g \cdot m^3$ but was not proven. On the other hand, LINN *et al.* [17] found no effect on bronchial responsiveness with exposure to 600, 2,000 or even 6,000 $\mu g \cdot m^3$ NO_2 in subjects with mild asthma. The discrepancy in the results of NO_2 exposure studies is so far unexplained.

Enhanced susceptibility to inhaled histamine (or methacholine) after NO_2 exposure conforms with data from exposure studies on dogs with another oxidant, ozone, reported by HOLTZMAN *et al.* [18]. A proposed mechanism is injury of epithelial cells, infiltration of leucocytes and release of mediators, such as leukotriene B_4 [19].

The increase in bronchial responsiveness after exposure to 510 $\mu g \cdot m^3$ NO_2 was similar in magnitude to the reported spontaneous variability in bronchial reactivity in asthmatic subjects [20]. None of the subjects reported, either spontaneously or when asked, any deterioration of their asthma during the four week study period. The mean increase in bronchial responsiveness seen in this study therefore seems to be of limited clinical importance. However, in our study, as well as in the studies by Orehek *et al.* and Kleinman *et al.*, only half to two-thirds of the subjects responded more after NO_2 exposure than after exposure to purified air, suggesting that only a fraction of the asthmatic subjects were sensitive to NO_2 in these concentrations. Among these subjects the NO_2-induced increase in bronchial responsiveness was large enough to have been of clinical importance as the responsiveness in two subjects increased 4–5 times.

Non-monotonous NO_2 dose-lung effect relationship

Based on the results of a previous study of NO_2 effects and on data in the literature, we proposed [4] that there might be a non-monotonous dose-effect relationship in NO_2-induced airway resistance with increased sRaw at low NO_2 concentrations and decreased sRaw at moderately high concentrations. A similar pattern, with a slight increase in sRaw at low and unchanged or decreased sRaw at somewhat higher NO_2 concentrations, was also suggested in this study, but was not statistically proven. Furthermore, the significant changes in bronchial responsiveness were seen after exposure to the low NO_2 concentrations and not after the highest concentration. A possible non-monotonous dose-effect relationship may be explained by competing NO_2-induced effects on the airways. It is known from *in vitro* studies on alveolar macrophages [21] that cell injury caused by oxidative effects of NO_2 is rapidly counteracted by anti-oxidant systems which may reduce the toxic effects. It is an open question whether exposure to 1,000 $\mu g \cdot m^3$, but not 260 and 510 $\mu g \cdot m^3$ NO_2, for 30 min triggers defence mechanisms against NO_2-induced injury. The hypothesis of a non-monotonous dose-effect relationship in NO_2-induced effects on the airways still needs additional proof.

In conclusion, the study has demonstrated that exposure to 510 $\mu g \cdot m^3$ NO_2 for 30 min caused a slight but significant increase in bronchial responsiveness in subjects with mild asthma. The airway resistance was not adversely affected by exposure to 0–1,000 $\mu g \cdot m^3$ NO_2 and the breathing pattern was unaffected.

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RÉSUMÉ: Vingt sujets atteints d'un asthme léger ont été exposés au NO_2, au repos, dans une cabine pléthysmographique modifiée, et ce pendant 30 minutes à des concentrations de 0, 260, 510 et 1.000 $\mu g \cdot m^3$ lors de 4 jours différents. La réactivité bronchique par test d'inhalation à l'histamine a été mesurée après chaque session d'exposition. La résistance des voies aériennes, le volume gazeux thoracique, la résistance spécifique des voies aériennes, ont été mesurés avant, pendant et après l'exposition, et le type respiratoire a été suivi au cours de toute la période d'exposition. La réactivité bronchique augmente significativement après une exposition de 30 minutes à 510 $\mu g \cdot m^3$ de NO_2 ($p < 0.01$). On a noté également des tendances à une réactivité bronchique accrue après exposition à 260 $\mu g \cdot m^3$ de NO_2 et de 1.000 $\mu g \cdot m^3$, mais ces modifications ne sont pas statistiquement significatives. L'on n'a pas démontré d'effets sur la résistance des voies aériennes ou le type respiratoire après exposition à 0–1.000 $\mu g \cdot m^3$ de NO_2. Nous concluons qu'une exposition brève au NO_2, à environ 500 $\mu g \cdot m^3$, affecte légèrement la réactivité bronchique humaine chez des sujets atteints d'un asthme léger.