

5. Controlled studies on humans

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In controlled studies, human volunteers are exposed to nitrogen dioxide (NO_2) gas in an exposure chamber for a short time (30 min to several hours), and possible effects on the lung can be assessed, for example, by lung function measurements, bronchial provocation tests, and analyses of bronchial lavage fluid. The concentration of NO_2 in the exposure chamber is usually chosen to be similar to, or somewhat higher than, maximal concentrations in ambient air. The effects can be studied after single or repeated short-term exposures. The subjects are healthy volunteers or individuals representing sensitive groups such as persons with asthma or chronic obstructive lung disease or the elderly. The conditions can vary from exposure of resting subjects to exposure of subjects performing different degrees of physical exercise. Other gases can be added to NO_2 for a study of the effects of combined exposure. Thus controlled exposure experiments with NO_2 can give important information on possible adverse health effects of NO_2 at ambient concentrations in normal and sensitive groups. Progress in the monitoring of inflammatory processes and improved investigating techniques (eg, bronchoalveolar lavage) have increased the possibility to study mechanisms of action of NO_2 even in human exposure experiments.

In a first series of controlled human exposure studies with NO_2 during the 1970s, the effects of fairly high (compared with ambient air) exposure concentrations ($>2000 \mu\text{g}\cdot\text{m}^{-3}$) were investigated, primarily concerning the influence on lung function. The reports published during the last decade deal more with low NO_2 concentrations ($<2000 \mu\text{g}\cdot\text{m}^{-3}$), and interest has broadened to effects on airway responsiveness, pulmonary clearance of inhaled aerosols, inflammatory response in the airways, interaction with other irritant gases and allergens, and defense against airway infections in addition to lung function measurements.

This review deals separately with data from exposure studies on normal subjects and subjects with asthma or chronic obstructive lung disease, as these groups have been shown to be more sensitive to NO_2 .

Effects of nitrogen oxides on lung function

Lung function measurements

Forced expiratory volume and vital capacity. The forced expiratory volume (FEV) is the volume of gas exhaled from the fully inspired lung over a defined time, when the subject makes a maximal expiratory effort. The time interval is usually 1 s and the index is then called $\text{FEV}_{1.0}$. When the airways are obstructed, $\text{FEV}_{1.0}$ is decreased. FEV is measured by spirometry, which is a routine method for lung function measurement that has high reproducibility. $\text{FEV}_{1.0}$ mainly reflects the function of central airways and is influenced by the degree of expiratory effort to only a limited extent.

Another index of forced expiration is peak expiratory flow rate (PEFR or PEF), which can easily be measured by the subject at home and at work. However, to a considerable extent, the test is dependent on the degree of expiratory effort and thus is not as reproducible as FEV.

Vital capacity (VC) is the maximal volume a subject can expel from the lung when instructed to continue exhalation until no more gas can be pressed out. When this maneuver is performed as a forced expiration, the index is called forced vital capacity (FVC), which often is somewhat less than the vital capacity recorded by a more leisurely expiration.

Airway resistance and lung volume. The resistance of the airways (R_{aw}) can be measured by a whole-body plethysmograph, and increased resistance is an early sign of airway obstruction. Airway resistance is dependent on the lung volume at which it is measured, and to correct for this dependence the term specific airway resistance (SRAW ; resistance times volume) is used. The plethysmographic measurement can also give lung volume. Thoracic gas volume (TGV) is the volume of the lung during tidal breathing. Adding maximal inspiration to breathing at TGV gives the total lung capacity (TLC). Airway resistance mainly reflects the function of central airways.

Forced expiratory flow and maximal expiratory flow-volume curve. During a maximal expiration from total lung capacity, the maximal flow rate is reached rapidly, and then the flow declines. From a forced expiratory spirogram, or a registration of maximal expiratory flow (MEF), together with lung

volume, different indices of flow at defined points during expiration can be derived (eg, MEF₂₅ and MEF₅₀). These measurements, which can also reflect small airway function, can sometimes give additional information on what has been gained by FEV_{1,0} and FVC. However, these indices are less reproducible and show greater normal variation than FEV_{1,0} and FVC.

Other tests that provide information about small airway function are the closing volume test and the single breath nitrogen test.

Studies on normal subjects

There are more than 40 reports of studies on healthy subjects exposed to NO₂ in the concentration range 200-10 000 µg·m⁻³. In the upper part of this concentration range, clear effects on lung function have been reported. Exposure to 9400 µg·m⁻³ (5 ppm) for 2 h increased airway resistance by about 40% in 16 subjects studied at rest (1). In a 14-h exposure of eight subjects at rest at 9400 µg·m⁻³ (5 ppm), Beil & Ulmer (1) reported an initial 30% increase in resistance (the first 30 min), followed by a slight decrease over the following 1.5 h, and then an increase again over 12 h, giving a total increase of about 60%. The resistance returned to the base-line value after 10 h, and the results over 14 h were reproduced during a second exposure day. von Nieding et al (2) exposed 11 normal subjects to 9400 µg·m⁻³ (5 ppm) for 2 h with intermittent, light exercise and reported an increase in airway resistance of about 60%. Airway resistance remained elevated during 1 h of postexposure observation.

At intermediate NO₂ concentrations, the results on lung function effects are equivocal. Beil & Ulmer (1) found an increase in airway resistance in resting subjects (about 25%) after 2 h of exposure to 4700 µg·m⁻³ (2.5 ppm), but not after 1880 µg·m⁻³ (1 ppm). Linn et al (3) did not find any effect of exposure to 7520 µg·m⁻³ (4 ppm) on 25 subjects performing both light and heavy intermittent exercise. Frampton et al (4) found no effect on lung mechanics in subjects exposed to 2820 µg·m⁻³ (1.5 ppm) or 3760 µg·m⁻³ (2 ppm) during intermittent exercise.

Studies with NO₂ concentrations below about 2000 µg·m⁻³ (1 ppm) have not shown any significant effect on lung function in normal subjects. (See, eg, references 5-9).

Studies on sensitive groups

Certain groups within the population may be especially sensitive to NO₂, such as persons with respiratory disease, children, and the elderly. Asthma is a disease characterized by increased reactivity to a variety of inhaled materials, such as cold air, dust, and air pollutants. Chronic obstructive bronchitis is also usually associated with increased airway responsiveness, and patients with this disease have a reduced pulmonary reserve.

Patients with asthma. During the last decade there has been a growing interest in the effects of NO₂ exposure on asthmatic subjects. There are about 25 reports, and almost all of them concern the concentration range 200-1100 µg·m⁻³. There are only two reports on exposure of asthmatics at concentrations higher than 1200 µg·m⁻³ (10, 11), and in both studies no effect was seen (at 5600 and 7500 µg·m⁻³, respectively).

Most of the reports on the NO₂ exposure of asthmatic subjects in the NO₂ concentration range of 200 - 1200 µg·m⁻³ show no effects on lung function (6, 9, 11-15). However, statistically significant, although small, effects have been reported in some studies. Bauer et al (16) exposed 15 asthmatics to 560 µg·m⁻³ during 30 min, including 10 min of light exercise, and found a greater decrease in postexercise FEV_{1,0} after NO₂ exposure than without such exposure (10.1% compared with 4.1%). Avol et al (17) reported that 59 asthmatics exposed to 560 and 1120 µg·m⁻³ during 2 h with 6 x 10 min of moderate exercise showed no change in lung function attributable to NO₂. Later, the same authors (18) exposed 34 adolescents to 560 µg·m⁻³ for 3 h, including light exercise, and after 60 min of exposure found a significant decrease in FEV_{1,0}, FVC and PEF (-3.4, -4.0, and -5.6%, respectively) and an increase in SRaw of 17%. These changes vanished during the following 120 min of exposure, and after 180 min of exposure the lung function had returned to base-line levels. Roger et al (19) reported the results of 12 asthmatics exposed to 560 µg·m⁻³ during 110 min, including three periods of 10-min moderate exercise. The FEV_{1,0} decreased 11% after the first exercise period, compared with a 7% decrease after filtered air exposure. The differences between NO₂ and air persisted for the rest of the exposure time, and after the second and third exercise period significant changes also occurred in the FVC and SRaw. In a later study with a similar design, 21 asthmatic subjects were exposed for 75 min to 280, 560, and 1120 µg·m⁻³ during intermittent moderate exercise, but no effect on FEV_{1,0} or SRaw was seen (19).

Patients with chronic obstructive bronchitis. Patients with chronic obstructive bronchitis showed an increase in SRaw after 5 min of exposure to NO₂ concentrations above 3770 µg·m⁻³ (2 ppm), but no effect was seen below about 2800 µg·m⁻³ (20). Morrow et al (21) reported that 20 patients with chronic obstructive lung disease exposed to 560 µg·m⁻³ for 4 h, including intermittent light exercise, showed a progressive decline in FVC with a maximal decrease (-8.2%) at the end of the exposure. The FEV_{1,0} decreased -4.8% after 4 h of exposure. Linn et al (22) found no significant changes in lung function in 22 patients with chronic obstructive bronchitis exposed to 940, 1880, and 3720 µg·m⁻³ (0.5, 1.0, and 2.0 ppm) for 1 h with very light, intermittent exercise.

Effects of nitrogen dioxide on airway responsiveness

Airway responsiveness measurements

Airway responsiveness is the capacity of the airways to react to inhaled substances with cough, bronchoconstriction, and increased mucus production. Persistently increased airway responsiveness, or bronchial hyperresponsiveness, is a cardinal feature of asthma, and the degree of nonspecific bronchial hyperresponsiveness is fairly well correlated to the severeness of asthma (23).

Nonspecific airway responsiveness can be assessed by different airway challenges (eg, methacholine, histamine, cold air). The dose of provoking agent causing a defined bronchoconstriction measured by FEV_{1.0}, PEF or SRaw is determined. In the same way, the specific airway responsiveness to allergens can be measured after the inhalation of nebulized allergens.

Studies on normal subjects

There are seven studies reporting the effect of NO₂ exposure on the bronchial responsiveness of normal persons. Beil & Ulmer (1) reported a significant increase in responsiveness to inhaled acetylcholine in 16 normal subjects exposed to NO₂ at 9400 µg·m⁻³ (5.0 ppm) for 14 h or 14 100 µg·m⁻³ (7.5 ppm) for 2 h. Mohsenin (24) found increased reactivity to methacholine in 18 normal subjects exposed to 3700 µg·m⁻³ (2 ppm) during 1 h at rest. Frampton et al (4) reported on 15 normal subjects exposed to 2800 µg·m⁻³ (1.5 ppm) for 3 h during intermittent moderate exercise, and the responsiveness to carbachol increased after NO₂ exposure.

Exposure concentrations of NO₂ of less than 2000 µg·m⁻³ have not been shown to affect airway responsiveness in normal subjects (9, 6, 24, 25, 27).

Folinsbee (28), in a meta-analysis of all published reports on the NO₂ exposure of normal subjects, showed a statistically significant effect of NO₂ on bronchial responsiveness in normal subjects at concentrations above 1880 µg·m⁻³ (1 ppm). All but one of the published studies were performed with the subjects at rest.

Studies on asthmatic subjects

There are nearly 20 reports on asthmatic subjects exposed to concentrations of NO₂ varying between 200 and 2000 µg·m⁻³ for about 0.5 to 4 h, followed by measurement of nonspecific airway responsiveness immediately or within 1 h after the end of exposure (table 5.1).

Orehek et al (29) reported that exposure to a low NO₂ concentration (190 µg·m⁻³) for 1 h at rest increased reactivity to carbachol in a group of 20 asthmatic subjects. Ahmed et al (25, 26) found a trend towards increased responsiveness to carbachol in 20 asthmatics after exposure to 190 µg·m⁻³ at rest, but

the difference was not statistically significant. In three other studies at the same concentration level (9, 12, 30), no influence of NO₂ on airway responsiveness was seen.

Jörres & Magnussen (31), using sulfur dioxide as the challenging substance instead of histamine or methacholine, found a significant increase in responsiveness after NO₂ exposure of 14 subjects with asthma at rest to 470 µg·m⁻³. Bauer et al (16) reported on an increase in airway responsiveness to cold air inhalation in 12 asthmatics exposed to 560 µg·m⁻³ during intermittent moderate exercise, and Bylin et al (12) found an increase in responsiveness to histamine after exposure to 510 µg·m⁻³ during rest. When Bylin et al (12) exposed the same 20 subjects to 1000 µg·m⁻³ during rest, no effect on airway responsiveness was seen. Using these results and observations of NO₂-induced slight changes in airway resistance at NO₂ concentrations of 260, 510 and 1000 µg·m⁻³ as a basis, the authors proposed a hypothesis of a nonmonotonic NO₂-lung effect relationship.

Bylin et al (6) found an increased responsiveness to histamine in eight asthmatics exposed for 20 min to NO₂ at 910 µg·m⁻³ at rest, and Mohsenin (32) likewise found a significant effect on responsiveness to methacholine in 10 asthmatic subjects exposed to 940 µg·m⁻³ for 1 h at rest.

However, besides these reports showing an effect of NO₂ on airway responsiveness, there is also a considerable number of studies at the same concentration levels in which no significant effect was seen (9, 11, 13, 18, 19, 30, 33, 34).

Folinsbee (28), in a meta-analysis of all published reports on NO₂ exposure in asthmatics (251 subjects) compared airway responsiveness after exposure to air and NO₂ in each individual subject. He determined the direction of change in responsiveness (ie, increase or decrease) and grouped the NO₂ concentrations into the following three classes: 94-375 µg·m⁻³ (0.05-0.20 ppm; no studies in this group are in fact reported below an exposure concentration of 190 µg·m⁻³), 375-560 µg·m⁻³ (0.20-0.30 ppm), and >560 µg·m⁻³ (0.30 ppm). The fraction of subjects with an increased responsiveness in the whole group was 0.59, which was a statistically significant effect ($P < 0.01$). When analyzing subjects exposed during rest and exercise separately, Folinsbee surprisingly found that the overall increase in airways responsiveness was almost entirely explained by the results from the studies of resting subjects. Among all of the resting subjects the fraction of "reactors" was 0.69, and a statistically significant effect was seen in all of the concentration groups. The fraction of "reactors" among the resting subjects in the different concentration groups was 0.65, 0.76, and 0.73 (94-375, 375-560, >560 µg·m⁻³, respectively). Subjects exposed during exercise showed no significant effect, neither in the whole group (fraction 0.51) nor

Table 5.1. Changes in airway responsiveness in nitrogen dioxide (NO₂) exposure studies on subjects with asthma. (BHR = bronchial hyperreactivity). See also Folinsbee (28) for a more-detailed summary.

Reference	No of subjects	NO ₂ (µg·m ⁻³)	Duration of exposure (min)	Exercise	Time after end of exposure	Result*	Notes
25, 26	20	190	60	No	-	-	Trend toward increased BHR (13 of 20 subjects "reacted", P=0.07)
17	37	560	120	Yes	60	-	Trend towards increased BHR after 560 µg·m ⁻³ , not after 1130 µg·m ⁻³
18	34	560	30	Yes	60	-	Young asthmatics
16	12	560	30	Yes	60	+	-
6	8	910	20	No	20	+	-
12	20	260	30	No	25	-	Significant increase after
	20	510	30	No	25	+	510 µg·m ⁻³ , trend towards increase
	20	1000	30	No	25	-	after 260 and 1000 µg·m ⁻³
9	15	190	60	No	20	-	-
31	14	470	30	No	25	+	Provocative agent sulfur dioxide
32	11	470	30	Yes	60	-	-
13	31	380	120	Yes	-	-	Trend towards increase (2 of 3 subjects "reacted")
11	21	560	60	Yes	-	-	-
	21	1880	60	Yes	-	-	-
	21	5640	60	Yes	-	-	-
33	10	940	60	No	-	+	-
15	20	560	225	Yes	-	-	-
29	20	190	60	No	-	+	-
30	20	190	120	Yes	-	-	-
	20	380	120	Yes	-	-	-
	20	1500	120	Yes	-	-	-
19	19	280	80	Yes	60	-	-
	19	560	80	Yes	60	-	-
	19	1130	80	Yes	60	-	-
34	9	560	30	Yes	60	-	Provocative agent sulfur dioxide

* + = positive, - = negative

in the concentration group (fraction of "reactors" varying between 0.49 and 0.59).

The time course of NO₂ effects on airway responsiveness is not fully known. All of the reviewed studies concern effects observed during the first hour after exposure. According to preliminary data (35), an increased responsiveness to histamine was observed in 19 asthmatic subjects 5 h after exposure to an NO₂ concentration of 490 µg·m⁻³ during intermittent light exercise. Twenty-seven hours after exposure the airway responsiveness had returned to the preexposure level.

Information on the dose-effect relationship in NO₂-induced changes in airway responsiveness is still incomplete. During exercise, the dose of NO₂ to the lung is greater due to an increased tidal vol-

ume and respiratory rate, but the effect on bronchial responsiveness is less than after exposure at rest. A possible explanation is that exercise per se influences the mechanism responsible for increased responsiveness in NO₂-exposed subjects and attenuates the response. Another hypothesis is that higher doses of NO₂ cause changes in the bronchial mucosa (eg, edema) and lead to a decreased accessibility of receptors in the airways to the bronchoconstricting agent (histamine, methacholine). A third explanation is that NO₂, at concentrations around 1000 µg·m⁻³, might have mild bronchodilating effects. The last possibility has some experimental support (6, 36).

The possible interaction between NO₂ and inhaled allergens on bronchial responsiveness has hitherto only been addressed in a few studies, although a re-

cent report (37) indicates that such an interaction may exist between ozone and ragweed allergen.

There are only two studies (26, 38) on the effects of NO_2 exposure on airway responsiveness to allergen inhalation. No effect was seen in either study, but the exposure concentration in both studies was low ($200 \mu\text{g}\cdot\text{m}^{-3}$), and the study of Orehek et al (38) comprised only three subjects with asthma (together with four who had allergic rhinitis). No conclusion can therefore be drawn.

Effects on alveolar permeability and bronchoalveolar lavage fluid

Exposure of 14 normal subjects to an NO_2 concentration of $4300 \mu\text{g}\cdot\text{m}^{-3}$ (2.3 ppm) during 5 h at rest gave a significant decrease (22 %) in alveolar permeability 6 h after the end of exposure (39). Furthermore, a decrease in glutathione peroxidase activity was observed 18 h after the end of exposure, a result indicating reduction due to increased lipid peroxidation caused by NO_2 . The authors concluded that the data support the assumption that NO_2 also has delayed effects on humans.

There are some recent studies of changes in cells and mediators in bronchoalveolar lavage fluid after NO_2 exposure of humans. Mohsenin & Gee (40) reported that the exposure of normal subjects to 6000 to $8000 \mu\text{g}\cdot\text{m}^{-3}$ resulted in a decrease in alpha-1-protease inhibitor activity in bronchoalveolar lavage fluid. Johnson et al (41), exposing subjects to $3000 \mu\text{g}\cdot\text{m}^{-3}$, found no effect on alpha-1-protease-inhibitor activity. Sandström et al (42, 43) exposed normal subjects to $8000 \mu\text{g}\cdot\text{m}^{-3}$ and then performed bronchoalveolar lavage 4, 8, 24, or 72 h postexposure. Increased numbers of mast cells and lymphocytes were found at 4, 8, and 24 h postexposure. Eosinophil and neutrophil counts in the lavage fluid were not affected by NO_2 exposure. Sandström et al also studied repeated exposures to $8000 \mu\text{g}\cdot\text{m}^{-3}$ during 12 d (seven exposures) and found a decrease in the numbers of mast cells, as well as in the number of T-suppressor cells, B-lymphocytes, and NK cells (42, 43).

The bronchial lavage technique may give further valuable information on whether, and to what extent, NO_2 exposure causes an inflammatory reaction in the airways at ambient concentration levels.

Subjective symptoms and odor threshold

In exposure studies performed at ambient concentrations of NO_2 ($<1000 \mu\text{g}\cdot\text{m}^{-3}$) subjects have not experienced any subjective complaints of dyspnea, cough, or airway irritation. The odor threshold for NO_2 is reported to be about $100 \mu\text{g}\cdot\text{m}^{-3}$ (6).

Summary and concluding remarks

Lung function

Controlled exposure studies have shown that NO_2 can cause bronchoconstriction in *normal subjects* at about $9000 \mu\text{g}\cdot\text{m}^{-3}$, probably also at concentrations of about 4000 – $5000 \mu\text{g}\cdot\text{m}^{-3}$, but below $2000 \mu\text{g}\cdot\text{m}^{-3}$ no significant effects on lung function have been reported. For *asthmatics* there are some studies indicating statistically significant, although slight, effects on spirometry and airway resistance at NO_2 concentrations of about $500 \mu\text{g}\cdot\text{m}^{-3}$, but there are also other studies at the same concentration level reporting no effects. The reason for the conflicting results is unknown and makes the data difficult to evaluate. Differences in asthma severity among subjects, adaptation to NO_2 in subjects living in heavily polluted areas, and study design (eg, rest or exercise) have been discussed as possible sources of variation. Patients with *chronic obstructive bronchitis* have a further reduction of their already reduced lung function after some hours of exposure to $560 \mu\text{g}\cdot\text{m}^{-3}$.

Airway responsiveness

Since the report of Orehek et al (29) on increased airway responsiveness to carbachol in asthmatics after NO_2 exposure of $200 \mu\text{g}\cdot\text{m}^{-3}$, there has been considerable debate as to whether NO_2 has such an effect or not since subsequent studies have shown conflicting results. Now the published reports from several studies have made it possible to create a data base for statistical analysis with an appropriately high power. The recently published meta-analysis by Folinsbee (28) shows that there is a statistically significant effect on bronchial responsiveness in asthmatics at NO_2 concentrations above $190 \mu\text{g}\cdot\text{m}^{-3}$ and in normal subjects at concentrations above $2000 \mu\text{g}\cdot\text{m}^{-3}$.

Discussion

The effect of main concern regarding exposure to NO_2 is the increase of bronchial responsiveness seen in asthmatics at high ambient concentrations. At ambient concentrations NO_2 can also exert bronchoconstricting effects on sensitive groups, such as subjects with chronic obstructive bronchitis and asthma.

The health effects of an acute and transient increase in bronchial responsiveness to nonspecific stimuli in normal people are not well known. One interpretation is that the defense mechanisms of the human bronchi can well cope with this NO_2 -induced damage and consequently should not be regarded as an adverse effect. Another interpretation, although speculative, is that repeated NO_2 exposure may not only induce a transient shift in bronchial responsiveness, but also a persistent one (ie, it may contribute to the pathogenesis of bronchial asthma).

Asthmatics have a persistently increased bronchial responsiveness and a further increase in that respon-

siveness is expected to have clinical implications for accentuated symptoms from cold air inhalation and exercise and possibly a need for additional or supplemental medication. For people with asthma, an increased airway responsiveness therefore must be regarded as an adverse effect. People with asthma make up a considerable proportion of the population (about 5%), and it is therefore necessary to judge NO_2 concentrations of $200 \mu\text{g}\cdot\text{m}^{-3}$ as the lowest level of effect when concern for public health is borne in mind.

There are several uncertainties in the judgement of $200 \mu\text{g}\cdot\text{m}^{-3}$ as the lowest level of effect. One is that it is not clear that the most sensitive subjects have been included in the reported studies. To avoid the potentially conflicting effects of concomitant medication and varying base-line values of lung function, exposure studies usually use asthmatic subjects with a mild type of disease. Patients with more severe asthma might be more sensitive to NO_2 . Another problem is whether the maximum effects of NO_2 are the acute ones already studied or whether there is a possible delayed response that has not yet been investigated. It might also be expected that other pollutants and the breathing of cold air interact with NO_2 . These factors speak in favor of adopting a safety margin to the lowest level of effect to protect the most sensitive groups in society.

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