

Asthmatic bronchial hyperresponsiveness varies with ambient levels of summertime air pollution

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ABSTRACT: It is widely believed that the mechanisms of action of outdoor air pollutants are the same as those found in the laboratory, although few studies have attempted to clarify this issue. This study investigates the relationship of asthmatic bronchial hyperresponsiveness (BHR), a marker of airway inflammation, and pulmonary function to ambient levels of summertime air pollution.

Thirty eight nonsmoking adult asthmatic subjects underwent repeated measurement of methacholine BHR, using Yan's method, at differing levels of air pollution (O₃, SO₂, NO₂, smoke) during summer 1993.

A total of 109 evaluable tests were performed: 31 subjects completed three or more challenge tests, and seven managed two. Levels of all pollutants remained within current World Health Organization (WHO) Guidelines for Health. Changes in BHR were found to correlate significantly with changes in the levels of 24 h mean SO₂, NO₂ and smoke; 48 h mean NO₂ and smoke; 24 h lag NO₂; although the effect was only small, accounting for approximately 10% of the variability in within-subject BHR between visits. Twenty four hour lag NO₂ was also associated with forced vital capacity (FVC).

In conclusion, in subjects with asthma, methacholine bronchial hyperresponsiveness varies with ambient levels of summertime air pollution. This suggests that changes in airway inflammation underlie the increased respiratory morbidity known to accompany pollution episodes.

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Over the past two decades, environmental chamber studies have played an important role in characterizing the response of normal and asthmatic volunteers to a variety of commonly occurring air pollutants. These studies have been instrumental in the identification of at-risk groups, the determination of thresholds of effect, and the elucidation of mechanisms of action. However, the artificial circumstances under which exposure protocols are performed may not adequately reflect the conditions under which exposure takes place in the natural environment. In particular, the duration of exposure is often only short, the concentration of pollutant remains constant, and in order to demonstrate effects at ambient pollutant levels, vigorous programmes of exercise are often included in the protocol. In contrast, exposure to outdoor pollutants may be more prolonged, pollutant levels tend to vary on a daily basis, and estimates of exposure are complicated by individual movement in and out of doors. Despite these discrepancies, the findings from epidemiological studies are in broad agreement with those from the chamber but effects have tended to occur at lower concentrations of pollution, possibly as a result of the action of ambient co-factors or pollutant interactions [1, 2].

We have previously demonstrated that relatively low levels of summertime ozone (O₃) and sulphur dioxide (SO₂) air pollution are independently associated with peak expiratory flow rate (PEFR) measurements, respiratory symptoms and medication use in subjects with asthma and chronic bronchitis [3]. Evidence from laboratory exposures to these pollutants suggests that these effects are mediated by differing mechanisms of action: inhalation of SO₂ produces a transient reflex bronchoconstriction, easily inhibited by the administration of a beta-adrenoreceptor agonist [4]; whereas, exposure to O₃ induces airway inflammation (in itself capable of producing bronchoconstriction) and causes stimulation of pain receptors leading to a decrease in lung volumes [5, 6].

It is widely believed that similar mechanisms of action are in operation in the outdoor environment, although few investigations have attempted to clarify this issue. It is, however, important to determine the mechanisms of action of pollutants in the outdoor environment, since, where effects are mediated by changes in airway calibre alone, removal from pollution can be expected to be followed by rapid resolution of symptoms. Changes in airway inflammation are, on the other hand, likely to

manifest as more persistent symptoms but, in addition, might be important in lowering the threshold for effects and in modifying the response to a variety of other environmental factors [7, 8]. This has important implications to health but in particular to the rising number of asthma sufferers [9], who are exposed to a constantly rising concentration of vehicle exhaust pollution [10].

In the laboratory, asthmatic airway inflammation is best measured by direct sampling of the bronchial mucosa and harvesting of cells and inflammatory mediators with fiberoptic bronchoscopic biopsy/bronchoalveolar lavage [11, 12]. In the epidemiological setting, airway inflammation is indirectly reflected by the response to inhaled nonspecific bronchoconstrictors with which it is known to correlate [11, 12].

This study sets out to investigate whether asthmatic bronchial hyperresponsiveness (BHR) and basic pulmonary function vary with ambient levels of summertime air pollution in an area where air pollution has been found previously to be a problem.

Methods

Study setting

The entire study was performed in the urban-industrialized, medium-sized British towns of Runcorn and Widnes (total population of 120,000 inhabitants), which straddle the river Mersey in the Northwest of England. The study area is characterized by a number of potential local sources of air pollution, including a variety of chemical manufacturing and processing plants, a network of urban roads, a motorway 6 km to the southeast, and a 2,000 megawatt, nondesulphurized, coal-fired electrical generating power station (Fiddlers Ferry) to the east. In addition, up to 60,000 vehicles of commuter traffic pass through the centre of each town daily.

Study population

In each town, a suitable general practitioner (GP) surgery was identified, from which to enrol subjects (Brookvale Practice in Runcorn and Appleton Surgery in Widnes), and whose catchment area lay close to the pollution monitoring sites. The names of all nonsmoking asthmatic subjects, aged 18–70 yrs, who were contactable by telephone and were living and working within 4 km of their respective pollution monitoring site were collected from the asthma registers of both practices. All subjects had previously been found by their GP to give typical histories of asthma and, in addition, had demonstrated greater than 15% reversibility to inhaled bronchodilator and/or at least 20% daily PEFV variability (maximum PEFV - minimum PEFV / maximum PEFV) \times 100 on at least one occasion during a 2 week period of measurement.

A total of 205 potential study subjects were identified and all were sent letters of information jointly signed by the principal investigator and respective GP. Subjects were requested to return a slip of paper in the prepaid envelope provided, indicating whether they were or were not interested in taking part.

Study protocol

The study was approved by the Research and Ethics Committees of both Halton Borough Council and the University Hospitals of South Manchester and recruitment commenced on the 17th July 1993. Each responder to the information letter was contacted by telephone and their current asthma status was assessed by the principal investigator (with training in respiratory medicine). If they were clinically stable and no changes had been made to their asthma medication in the previous 3 months, and providing they were lifelong nonsmokers or ex-smokers of more than 1 yr and were not taking either regular oral steroids, long-acting oral/inhaled bronchodilators, or inhaled anticholinergics, an appointment was made for them to attend their respective GP for testing.

Prior to entry, each subject received a full explanation of the study and gave their consent. In order to blind subjects to the study objectives and, thereby, minimize selection biases, participants believed they were enrolling in a study designed to investigate whether bronchial hyperresponsiveness and simple measures of lung function varied with time, and what factors determined individual response to methacholine. Basic demographic details and medication use were recorded on a separate piece of paper and subjects then underwent skin-prick testing to four common allergens: *Dermatophagoides pteronyssinus*, cat, trees, and grasses (E. Merck Ltd, Alton, Allergopharma, Germany; Bio Diagnostics, Upton-Upon-Severn, UK) with histamine and saline solutions as controls. All skin-prick tests were performed by the principal investigator and were read after 15 min. A positive response was defined as a skin weal diameter of at least 3 mm more than the saline control.

The age and height of all subjects were measured and predicted values for forced expiratory volume in one second (FEV₁) were then determined. Baseline pulmonary function was measured, corrected for body temperature, atmospheric pressure and water saturation (BTPS), on a dry bellows spirometer (Vitalograph, Buckingham, UK), and FEV₁ and forced vital capacity (FVC) were recorded as the best of three blows within 150 mL or 5% of one another. Prior to testing, all subjects were required to do without any short-acting inhaled bronchodilators for 6 h. Providing baseline FEV₁ was within 60% of predicted, the FEV₁/FVC ratio was greater than 50%, and there were no absolute contraindications, subjects then underwent a methacholine bronchial challenge using the method of YAN *et al.* [13].

Methacholine bronchial challenge

Following the measurement of baseline spirometry, three inhalations of normal saline were administered from a handheld De Vilbiss No. 40 glass nebulizer (De Vilbiss, Somerset, UK) and FEV₁ was recorded 1 min later taking the best of a further two blows within 100 mL of each other. Starting from 0.05 μ mol, sequential doubling doses of methacholine were inhaled from different, individually calibrated De Vilbiss No. 40 nebulizers to a maximum of 12.5 μ mol methacholine, or until FEV₁ fell by 20% or more from baseline. A positive response was defined as the cumulative provocative dose

of methacholine causing a 20% drop in the postsaline FEV₁ (PD₂₀FEV₁). This was estimated using linear interpolation between log doses. All positive responders were given an inhaled β_2 -agonist (salbutamol 200 μg) via a spacer device and were allowed to return home once their FEV₁ had increased to 90% baseline.

Pollution monitoring

Halton Borough Council has routinely monitored SO₂ and smoke air pollution in the area since the 1960s but in 1990 purchased new equipment that uses differential absorption spectroscopy (OPSIS®, Sweden) to provide a continuous analysis of a variety of pollutants. A beam of light at visible and ultraviolet frequencies is transmitted to a receiver approximately 500 m away. The receiver contains an optical fibre system connected to a spectrometer, which scans the light beam and determines which frequencies have been absorbed by gases traversing the beam. Since pollutant gases each absorb light at a characteristic frequency, the atmospheric concentration of the selected gases can be determined. The spectrometer is linked to a computerized recording system, which stores pollutant levels continuously. The system can, therefore, be used to determine pollution levels at a particular time-point or levels can be averaged over a time period. In this study, 24 h mean levels were used.

Two receivers are situated in the Halton District, one at a height of 32 m (Municipal Building in Widnes) and the other at 12 m (Runcorn Town Hall). Both receive light traversing a representative area of each town from sources placed 12 m above the ground and at a distance of 500 m away. The system is, therefore, ideal for measuring background concentrations of commonly occurring urban air pollutants but cannot be used to provide information on particulate matter. The system has a detection limit of 0.5 $\mu\text{g}\cdot\text{m}^{-3}$, is accurate to within 1.0 $\mu\text{g}\cdot\text{m}^{-3}$, and is comparable with standard measurements of pollution [14].

Daily mean concentrations (for any 24 h period) of NO₂, SO₂ and O₃ were monitored continuously by OPSIS®. In addition, daily mean background concentrations of particulate matter were collected using the blacksmoke method [15], at ground level outside the buildings where OPSIS® measurements were made and at a distance of 50 m from the nearest road. Particles were collected onto a single filter paper for each 24 h period, starting at 0900 h each day. All pollutant exposure variables were expressed as $\mu\text{g}\cdot\text{m}^{-3}$. Daily mean temperature was also recorded (°C). All measurements were kindly performed by a team of dedicated environmental scientists based at Halton Borough Council.

Pollen collection

Daily levels of grass and tree pollen were collected using a standard Burkard trap 15 km to the southwest by the local pollen station (data kindly supplied by C. Murphy of "The Geochem Group", Chester, UK) and results are expressed as grains·m⁻³ for the 24 h period commencing at 0900 h each day.

Repeated bronchial challenge tests

Those subjects with a PD₂₀FEV₁ of less than 12.5 μmol methacholine ("reactors") were recruited into the current study to investigate whether asthmatic bronchial hyperresponsiveness (BHR) varies with air pollution, and their recruitment PD₂₀FEV₁s were carried forward for inclusion into the main study analysis.

This involved performing repeat bronchial challenge tests at differing levels of pollution. During the 48 h prior to testing, subjects were required not to leave the study area. Each subject was assigned to the same challenge test operator using the same equipment, and was tested at the same time on each visit to minimize effects due to differences in operator technique and diurnal variation in clinical disease. The principal investigator used 4 day local meteorological forecasts (supplied by the local Meteorological Office) complemented by privileged access to pollution data, to predict changes in the levels of pollution and organize recruitment for testing. Analysis of the OPSIS® data from the previous summer had revealed a strong relationship between the levels of NO₂ and wind speed ($r=0.74$; $p<0.001$), and a less strong association for SO₂ ($r=0.42$; $p<0.001$). In contrast, levels of O₃ were primarily influenced by temperature. In general, a rise in pollution was expected during anti-cyclonic weather conditions, and a fall in pollution with low pressure weather fronts.

Each individual's progress was monitored throughout the study to ensure that their BHR was measured on at least two separate occasions when the 24 h mean levels of total pollution (NO₂ + SO₂ + O₃ + smoke) differed by an arbitrarily defined magnitude of 2. BHR testing, therefore, took place on days when pollution was expected to be relatively "high" and on days when it was expected to be relatively "low". All recruitment PD₂₀FEV₁s were performed at a time when pollution levels were "low" and, in order to minimize any learning effects, half of the group were randomly assigned to have their second measurement of PD₂₀FEV₁ performed when pollution was expected to be "high" and the other half when pollution was expected to be "low". Those subjects tested on sequential "lows" were tested again where possible when pollution was "high", whilst those on a "low" and "high" were again randomly tested either on a "low" or "high" day. Appointments were made on the telephone by the principal investigator, who kept all subjects and other investigators blinded to pollution levels. The principal investigator played no part in the testing of subjects.

Statistical analysis

A log-linear relationship was identified between all lung function measures and pollutant levels, grass pollen concentrations and temperature. The previous 24 h mean and 48 h mean of each pollutant (NO₂, SO₂, O₃) and temperature was individually calculated to allow for the different times that subjects presented for testing. To allow for a lagged effect, the 24 h mean for the period commencing 48 h and ending 24 h before each challenge was also calculated (24 h lag). It was not possible to allow for the differences in time of testing when

calculating individual exposures to smoke pollution and pollen, as data were collected for a 24 h period commencing at 0900 h each day. The 24 h mean exposure variable, therefore, represents the average exposure from 0900 h the previous day to 0900 h on the day of testing. The 48 h mean represents the average exposure over a period of 2 days commencing at 0900 h 2 days prior to testing and ending at 0900 h on the day of testing. Twenty four hour lag was again calculated starting 2 days before testing and ending at 0900 h on the day before testing.

The hypothesis that asthmatic methacholine BHR or simple spirometry (FEV₁ or FVC) varies with ambient levels of summertime air pollution was tested using univariate nested (hierarchical) analysis of variance [16]. The analysis was limited to an investigation of within-subject variation of the dependent variable (BHR, FEV₁ or FVC); the first step of the analysis was, therefore, to remove from the total variation that component due to differences between subjects. The proportions of the within-subject variation in each of the dependent variables accounted for by each of the exposure variables in turn were then estimated, and the results expressed as Pearson correlation coefficients. To better illustrate the size of the relationship between BHR and pollution, the percentage changes in BHR associated with a 10 unit increase in each pollutant were also estimated and are reported with their 95% confidence limits. The level of significance was set at 5%.

Individual plots of BHR against the 24 h mean of each of the four pollutants were also examined to investigate for the presence of any heterogeneity of response. A "responder" was defined as any subject demonstrating a consistent increase in BHR as levels of pollution rose, whereas "nonresponders" showed no such relationship.

Data were processed on a portable IBM computer using the statistical package GLIM 3.77 (Generalized Linear Interactive Modelling).

Results

Recruitment

The overall response rate was 187 (91%), and 63 subjects (29%) agreed to take part. Those subjects not wishing to take part did not differ in either sex or age. Recruitment commenced on the 17th July 1993 and continued until the 12th of August 1993. Forty "reactors" were identified, and a further 20 subjects had a PD₂₀FEV₁ of >12.5 µmol methacholine ("nonreactors"). In three subjects, baseline prechallenge FEV₁ was <60% predicted, which precluded challenge testing, although each had >15% reversibility following inhaled β₂-agonist (salbutamol 200 µg).

Demography

Of the original 40 reactors, 38 completed the study. Two subjects developed infective exacerbations of their asthma requiring changes to their regular medication and

Table 1. — Demographic characteristics of 38 reactors completing the study

Characteristic	
Subjects n	38
Sex M/F	13/25
Age yrs [#]	40 (18–70)
FEV ₁ % pred [#]	88 (60–134)
FEV ₁ /FVC % [#]	75 (50–88)
PD ₂₀ FEV ₁ µmol*	1.68 (0.05–11.2)
Distance from OPSIS® km [#]	2.5 (0.1–4.0)
Atopic ⁺	33 (87)
HDM atopy ⁺	25 (66)
Cat atopy ⁺	23 (61)
Grass atopy ⁺	24 (63)
Tree atopy ⁺	11 (29)
Inhaled steroid ⁺	26 (68)

[#]: group mean, and range in parenthesis; *: geometric mean, and range in parenthesis; +: absolute value, and percentage in parenthesis. M: male; F: female; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; PD₂₀FEV₁: cumulative provocative dose of methacholine causing a 20% fall in FEV₁; % pred: percentage of predicted value; HDM: house dust mite; OPSIS®: equipment using differential absorption spectroscopy to continuously analyse a variety of pollutants.

were, therefore, excluded from further study. Demographic data on the 38 reactors completing the study are summarized in table 1.

Challenge tests

From 17th July to 22nd September 1993, a total of 109 evaluable challenge tests were performed. Twenty nine subjects performed three tests; two completed four tests; and seven managed only two. Four reactors developed nonreactor status on one occasion each from recruitment PD₂₀FEV₁s of 11.17, 10.30, 2.44 and 1.04. Median (range) time between study visits was 18 (4–63) days. Thirty four of the 38 subjects had their BHR recorded on at least two separate occasions when the proportional change in the level of 24 h mean total pollution (NO₂ + SO₂ + O₃ + smoke) was greater than 2 (mean 3.1; 95% confidence interval 3.0–3.2).

The 109 challenge tests were performed on 18 separate days, but 2 days in particular contributed to nearly 50% of readings: 24 tests were performed on 31st July (Day 15), when pollution levels were "low", and 27 tests on 18th August (Day 33), when pollution levels were "high". Levels of grass pollen were identical on both of these days (9.6 grains·m⁻³) and temperatures were very similar (14.3 and 14.9°C, respectively). The temporal association between daily total pollution concentration and the number of subjects tested each day is displayed graphically in figure 1.

Pollution levels

The levels of all pollutants remained within current WHO Guidelines for Health [17] for the duration of the study. Maximum individual exposures for each pollutant were: SO₂ 103.7 µg·m⁻³; NO₂ 77.5 µg·m⁻³; and smoke

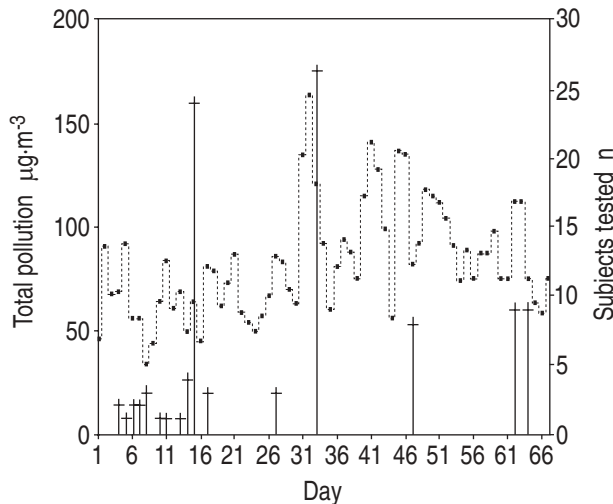


Fig. 1. — Temporal association between daily total pollution* and number of subjects tested. *: sum of daily mean ($O_3 + SO_2 + NO_2 + \text{smoke}$). - - ■ - -: daily total pollution; + : subjects.

$28 \mu\text{g}\cdot\text{m}^{-3}$ (WHO Guidelines for Health: 125, 150 and $125 \mu\text{g}\cdot\text{m}^{-3}$, respectively). Guidelines for O_3 are usually given as a 1 or 8 h average (240 and $120 \mu\text{g}\cdot\text{m}^{-3}$, respectively), and maximum 1, 8 and 24 h averages in this study were 61, 42 and $24.5 \mu\text{g}\cdot\text{m}^{-3}$, respectively.

Pollen data

Levels of tree pollen remained very low ($<2 \text{ grains}\cdot\text{m}^{-3}$) for the duration of the study and were not, therefore, subjected to further analysis. The maximum concentration of grass pollen occurred early in the study (48 $\text{grains}\cdot\text{m}^{-3}$) but had fallen to below $10 \text{ grains}\cdot\text{m}^{-3}$ by 31st July onwards. The temporal association of daily grass pollen levels with the number of subjects undergoing BHR testing each day is illustrated in figure 2.

Temperature

Maximum daily mean temperature reached 18.1°C (range $11.3\text{--}18.1^\circ\text{C}$).

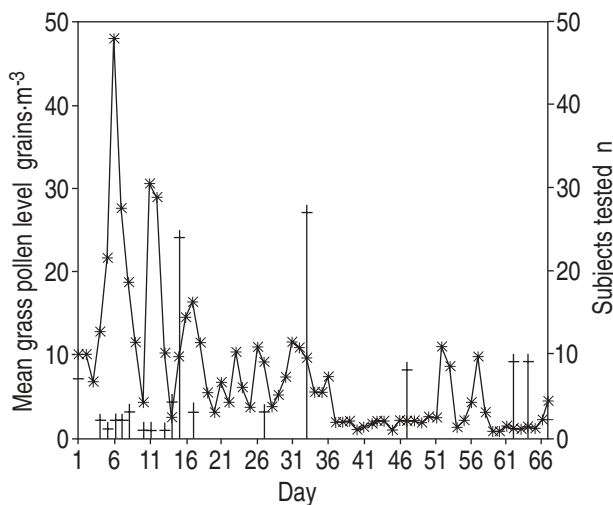


Fig. 2. — Temporal association between daily mean grass pollen levels and number of subjects tested. —*: daily grass pollen level; + : subjects tested.

Exposure variables

A high degree of intercorrelation between the various explanatory variables was observed, although no associations with O_3 were detected. In general, pollutants were positively correlated with one another but negatively with temperature and grass pollen. Correlation coefficients were broadly similar between 24 h mean, 48 h mean and 24 h lag values, although the relationship between SO_2 and either NO_2 or smoke was weaker at 24 h lag than 24 h mean, whereas that between temperature and either smoke or NO_2 increased. Pearson correlation coefficients for 24 h lag values are given in table 2.

A high degree of correlation was also found between the 24 h mean, 48 h mean and 24 h lag of each pollutant (all $r>0.70$; $p<0.001$). For each individual, the maximum change in the level of each of the 18 exposure variables was calculated (highest-lowest) and group mean (SD) results are detailed in table 3.

Associations between BHR and pollution, pollen or temperature

Twenty four hour mean levels of NO_2 , smoke and SO_2 were all significantly negatively associated with BHR, *i.e.* increasing levels of pollution heightened airway responsiveness ($r=-0.322$, $r=-0.293$ and $r=-0.185$, respectively; $p<0.01$, $p<0.02$ and $p<0.05$, respectively). Twenty four hour mean grass pollen concentrations were also significantly associated, although the direction of change was towards an improvement in BHR with increasing values ($r=0.329$; $p<0.01$).

NO_2 , smoke and grass pollen were significant at 48 h mean exposure ($r=-0.334$, $r=-0.242$ and $r=0.247$, respectively; $p<0.01$, $p<0.05$ and $p<0.05$, respectively) but at 24 h lag, only the association of NO_2 remained significantly related with BHR ($r=-0.254$; $p<0.01$). Furthermore, temperature gained significance at 24 h lag ($r=0.258$; $p<0.05$). No associations were found for O_3 . Table 4 summarizes the Pearson correlation coefficients for the relationship between BHR and pollution, pollen or temperature.

Pulmonary function

No effects of pollution on FEV1 were detected, although FEV1 was significantly correlated with temperature at all exposures ($r=0.279$, $r=0.289$ and $r=0.268$, respectively;

Table 2. — Intercorrelations between the 24 h lag values of the six independent variables (expressed as Pearson correlation coefficients)

	O_3	SO_2	NO_2	Smoke	Temp	Grass pollen
O_3	1.0	0.13	0.08	0.03	0.16	0.12
SO_2		1.0	0.65***	0.48***	0.13	-0.14
NO_2			1.0	0.89***	-0.57***	-0.25*
Smoke				1.0	-0.34**	-0.11
Temp					1.0	0.35**

Temp: temperature. Test of significance: *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 3. – Group mean values of the maximum change in each of the six independent variables experienced by each of the 38 subjects for each of the three exposure variables

Exposure	O ₃ µg·m ⁻³	SO ₂ µg·m ⁻³	NO ₂ µg·m ⁻³	Smoke µg·m ⁻³	Grass pollen grains·m ⁻³	Temp °C
24 h mean	9.7 (4.6)	30.2 (10.3)	46.6 (10.9)	21.1 (6.5)	10.7 (9.2)	3.1 (1.7)
48 h mean	9.9 (4.6)	22.3 (9.8)	31.1 (9.3)	13.8 (4.0)	12.2 (9.9)	3.3 (1.6)
24 h lag	8.6 (4.7)	18.2 (11.6)	19.5 (8.8)	17.1 (4.2)	14.4 (11.4)	3.0 (1.6)

Values are presented as mean, and SD in parenthesis. Temp: temperature.

Table 4. – Pearson correlation coefficients between log_e BHR and each of the three log_e exposure variables for pollution, pollen and temperature

Exposure	O ₃	SO ₂	NO ₂	Smoke	Grass pollen	Temp
24 h mean	0.029	-0.185*	-0.322**	-0.293*	0.329**	0.103
48 h mean	0.093	-0.073	-0.334**	-0.242*	0.247*	0.193
24 h lag	0.145	0.139	-0.254**	-0.154	0.079	0.258*

Temp: temperature. Test of significance: *: p<0.05; **: p<0.01.

p<0.02, p<0.02 and p<0.05, respectively) and with 48 h mean and 24 h lag values of grass pollen (direction of change was towards an improvement in FEV₁ with rising levels of grass pollen; r=0.319 and r=0.244, respectively; p<0.01 and p<0.05, respectively).

FVC was significantly associated with 24 h lag NO₂ (r=-0.168; p<0.05), and a borderline significance was found for 24 h mean NO₂ (r=-0.161; p<0.06). No other associations were detected for either FEV₁ or FVC.

BHR and pulmonary function

No significant associations or trends were found between BHR and either prechallenge FEV₁ or FVC (r=0.072, p>0.10; and r=0.11, p>0.10, respectively).

Magnitude of effect of pollution on BHR

The percentage changes in BHR (95% confidence interval) associated with a 10 unit increase in each pollutant are reported in table 5. The numbers of patients studied are too small for these estimates to be used for clinical prediction outside of the current study design but are reported here to indicate the general size of the relationships identified. As a marker for the maximum pollutant effect on BHR, 24 h mean NO₂ was used to estimate an approximate increase in within-subject BHR (95% confidence interval) of 0.54 (0.14–0.94) doubling doses of methacholine for a 45 µg·m⁻³ increase in NO₂ (*i.e.* in

moving from 25–70 µg·m⁻³), as of all the pollutants studied, it tended to explain more of the variation in within-subject BHR.

A similar change in 24 h lag NO₂ was associated with a very modest fall in within-subject FVC of 20 mL (0.6%) from 3,650 mL to 3,630 mL (95% confidence interval 0–41 mL). For a change in temperature of 3°C (13–16°C), within-subject FEV₁ increased from 2,590 to 2,629 mL, representing a 39 mL (1.5%) change (95% confidence interval 7–92 mL).

Heterogeneity of response

On reviewing the individual raw data, 24 subjects were identified as responders and 14 as nonresponders. The same 24 individuals responded to each of the three pollutants, demonstrating significant associations, but no responders to ozone were detected. Individual plots of BHR against 24 h mean NO₂ concentration are given in figures 3 and 4 for responders with mild and moderate/severe BHR, respectively.

Responders tended to be younger than nonresponders (38 vs 44 yrs, respectively) but the difference did not reach statistical significance. Females were equally distributed between the two groups (67 vs 64%). Mean distance from OPSIS® (2.4 vs 2.2 km) and geometric mean PD₂₀FEV₁ (1.87 vs 1.54 µmol, respectively) were also similar in the two groups.

Atopy to any of the four allergens tended to be more common in the responders (92%) than nonresponders

Table 5. – Percentage change in BHR for each 10 µg·m⁻³ increase in pollution for each exposure variable

	O ₃	SO ₂	NO ₂	Smoke
24 h mean	0.28 (-16.6 to 20.6)	-6.3* (-13.6 to 0.6)	-6.1** (-10.9 to -1.1)	-4.3* (-8.0 to -1.1)
48 h mean	1.2 (-20.4 to 28.6)	-2.9 (-12.8 to 8.2)	-9.2** (-15.7 to -2.3)	-5.2* (-9.2 to -1.9)
24 h lag	2.6 (-22.1 to 34.9)	7.4 (-4.5 to 20.8)	-13.2** (-23.2 to -1.8)	-7.1 (-15.9 to 1.7)

The 95% confidence intervals are presented in parentheses. Test of significance: *: p<0.05; **: p<0.01.

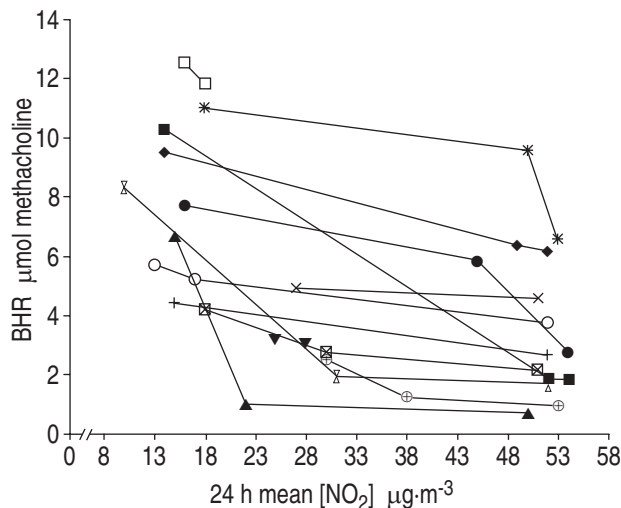


Fig. 3. — Individual plots of bronchial hyperresponsiveness (BHR) against 24 h mean nitrogen dioxide concentration $[\text{NO}_2]$ in 13 responders with mild BHR (at baseline of 2.5–12.5 μmol methacholine).

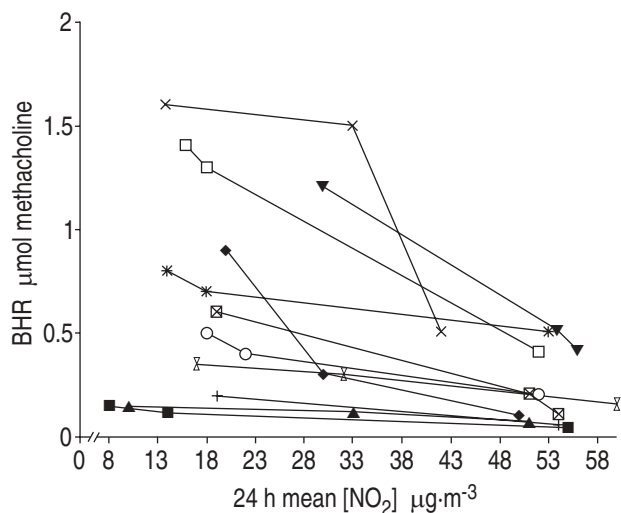


Fig. 4. — Individual plots of bronchial hyperresponsiveness (BHR) against 24 h mean nitrogen dioxide concentration $[\text{NO}_2]$ in 11 responders with moderate/severe BHR (BHR at baseline of <2.0 μmol methacholine). Note the difference in BHR scale compared to fig. 3, mild BHR.

(71%) but the difference did not reach significance (Chi-squared test). Similarly, no significant difference was detected in the proportion of grass pollen atopics occupying the responder compared with the nonresponder group (16 out of 24 or 67% vs 8 out of 14 or 57%, respectively) although twice as many grass pollen atopics were found to be responders compared to nonresponders (16 out of 24 or 67% vs 8 out of 24 or 33%, respectively). The proportion of individuals in the responder group on inhaled steroids (17 out of 24 or 71%) was also similar to that in the nonresponder group (9 out of 14 or 64%).

Discussion

This study has shown that asthmatic methacholine BHR varies significantly with background ambient concentrations of summertime NO_2 , SO_2 and smoke air pollution. The association is, however, only modest when levels of

pollution are low, accounting for approximately 10% of the variation between visits of within-subject BHR. Other factors are, therefore, more important determinants of response, although attempts were made to minimize these where possible.

The study was of a double-blind design in order to minimize selection and testing biases, although the principal investigator was aware of the likely levels of pollution on the days that testing was performed but played no part in the testing of subjects. All subjects were assigned to the same challenge test operator, who used the same equipment on each visit, and were tested at the same time and same place each day to minimize effects due to differences in operator technique and diurnal variation in disease. Efforts were also made to vary the order in which an individual's BHR was tested with respect to the level of pollution, in order to minimize any learning effects through repeated measures. Those subjects experiencing infective exacerbations of their asthma were excluded from analysis, as viruses in particular can potentiate the response to nonspecific bronchoconstrictors [18]. Finally, the study was limited to either life-long non-smokers or to those who had not smoked for at least 1 yr to avoid any confounding by cigarette smoke [19], and was designed to coincide with the end of the grass pollen season.

The study ran for a total of 68 days and, although BHR measurements were spread over 18 of these, two days in particular (31st July and 18th August) contributed to almost 50% of the total data collected. How these two days may have influenced the results is difficult to predict as other unmeasured confounding variables may have been in operation. This does, however, seem unlikely, as one would have to assume that the same factors had no influence on each of the remaining 16 days. It is more probable that the fortuitous similarity in temperature and pollen counts on these days allowed the effects of a modest change in pollution to be more easily identified.

For the duration of the study, grass pollen levels were generally very low but were shown to vary significantly with BHR, although the direction of change was towards an improvement in BHR with increasing grass pollen concentrations. This is contrary to the expected potentiation of BHR known to occur during the pollen season [20], and most likely resulted from confounding by other factors. This would appear to be the case, as grass pollen levels were significantly and negatively correlated with NO_2 pollution. Daily mean temperatures were also generally low for the duration of the study, reflecting the ordinariness of the summer. Consequently, effects tended to be minimal though not necessarily causally related, as confounding by pollution, pollen or other meteorological variables may have been in operation. Ozone levels were also lower than in previous years and presumably below the threshold required for effects to occur.

Unfortunately, it was not possible to collect information on fungal spores, which are known to provoke asthma and other allergic diseases in sensitive subjects [21, 22]. However, the prevalence of atopy to fungal spores in asthmatics is low, accounting for 13% of subjects in one study [23]. This suggests that only a minority of individuals in our study group may have been at risk from fungal spore effects but whether the numbers involved

would have been sufficient to achieve statistical significance remains open to question.

The lack of a general pollution effect on spirometry contrasts with the findings of an earlier study [3], although differences in study design may have been important, particularly as the levels of pollution were similar in the two studies. In the study by HIGGINS *et al.* [3], subjects were allowed to make peak expiratory flow rate measurements in a variety of indoor and outdoor environments, whereas participants in the present study were required to rest indoors for a short period of time prior to testing. We believe that this may have provided the correct conditions for a degree of reflex bronchodilation to occur, resulting in the loss of any transient pollution effects. However, a single association was detected for FVC and 24 h lag NO₂ but was only small, amounting to a 0.6% fall in FVC for each 45 µg·m⁻³ increase in pollution. This finding is in broad agreement with a similar study in children where pulmonary function was measured on repeated occasions before and after moderate episodes [24]. Concentrations of NO₂ were not measured but associations were detected for SO₂ and TSP pollution with which it is likely to have correlated.

The lack of an association between BHR and spirometry values suggests not only that BHR can change independently of airway calibre but also that the observed changes in BHR are probably not all that important clinically. Formal assessment of respiratory symptoms prior to each bronchial challenge was not, however, performed because of the possible problem of recall bias. We elected, therefore, to use objective measures of respiratory health which are known to correlate well with the clinical severity of disease [25]. The results suggest that greater changes in pollution, such as those occurring at the start of a pollution episode, are required to worsen pulmonary function to the extent that symptoms develop. Had effects both on BHR and pulmonary function been detected, then adjustment of BHR values would have been necessary as the response to bronchoconstrictors is known to correlate with prechallenge spirometry [26].

In this study, measurement of background ambient concentrations of pollution were used to estimate personal exposure, although it is recognized that there are limitations to this approach, particularly as the majority of people spend over 95% of their day indoors [27], where the concentrations of outdoor pollutants are generally lower but may rise to significant levels under exceptional conditions [28]. Our results from this and a previous study suggest that measurement of background concentrations of pollution offer a practical alternative to personal sampling methods, which currently provide information on a limited number of pollutants and are subject to varying degrees of inaccuracy.

In common with most epidemiological studies on air pollution, we have observed a high degree of intercorrelation between the different explanatory variables and have not, therefore, attempted to apportion blame to a specific pollutant. The evidence from environmental chamber studies linking NO₂ exposure to the potentiation of asthmatic BHR is inconsistent [29–32], whereas the effects of smoke and SO₂ on BHR are yet to be studied. Furthermore, measurements of other pollutants, such as acid aerosols and particulate matter with an aerodynamic diameter

≤10 µm (PM₁₀) were not performed in this study. Such pollutants are likely to have correlated with the measured levels of NO₂, SO₂ and smoke, although concentrations of acid aerosol and PM₁₀ would have been most closely related to O₃ and smoke, respectively. For the duration of the study, however, disappointingly low levels of O₃ were encountered, making it increasingly unlikely that significant acid aerosol production took place.

Whether the results of this study are representative of all nonsmoking asthmatics remains open to question, particularly in view of the low response rate (which is most likely to be related to the requirement for study subjects to undergo repeated tests of bronchial reactivity) and the presence of a distinct heterogeneity in the response of participating subjects. This heterogeneity of response appears to be unrelated to basic demographic details but may reflect variation in individual sensitivity to pollution, as in the laboratory exposures of asthmatic subjects to O₃ or SO₂ are known to produce a spectrum of physiological effects [33, 34].

The results of this study suggest that at levels below current WHO Guidelines for Health, changes (rather than the crossing of absolute thresholds) in the concentrations of certain traffic-related air pollutants are capable of potentiating airway inflammation in nonsmoking asthmatic subjects, and that these effects can be detected by repeated measurement of methacholine bronchial hyperresponsiveness. Potentiation of asthmatic bronchial hyperresponsiveness may, therefore, underlie the increase in respiratory morbidity known to accompany pollution episodes [35].

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