Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen

M. Svartengren*,**, V. Strand***, G. Bylin***, L. Järup**,+,#, G. Pershagen**,+

Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen. M. Svartengren, V. Strand, G. Bylin, L. Järup, G. Pershagen. ©ERS Journals Ltd 2000.

ABSTRACT: The aim of this study was to assess whether air pollution in road tunnels would promote asthmatic reactions in persons with mild allergic asthma.

Twenty volunteers with mild allergic asthma were exposed, inside a car, for 30 min in a Stockholm city road tunnel. As a control, the subjects were exposed to much lower pollution levels in a suburban area. Four hours after the exposure, the subjects inhaled a low dose of allergen. Asthmatic reaction during the early phase was measured as the increase in specific airway resistance 15 min after allergen inhalation and during the late phase as the decrease in lung function forced expiratory volume in one second 3–10 h after allergen inhalation. Asthma symptoms and drug use were monitored up to 18 h after allergen inhalation.

The median nitrogen dioxide level during exposure was 313 µg·m^{-3} (range 203–462). The median levels of particles with 50% cut-off aerodynamic diameters of 10 (PM10) and 2.5 µm (PM2.5) were 170 (range 103–613) and 95 (range 61–218) µg·m^{-3}, respectively. Subjective symptoms during tunnel exposure were not pronounced. However, subjects exposed to tunnel NO$_2$ levels of ≥300 µg·m^{-3} had a significantly greater early reaction, following allergen exposure, as well as lower lung function and more asthma symptoms during the late phase, compared to control. Also, subjects with PM2.5 exposure ≥100 µg·m^{-3} had a slightly increased early reaction compared to control.

In conclusion, exposure to air pollution in road tunnels may significantly enhance asthmatic reactions to subsequently inhaled allergens.
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*Division of Occupational Medicine, Dept of Public Health Sciences, Karolinska Institute, Stockholm, **Dept of Environmental Health, Stockholm County Council, Stockholm, ***Dept of Respiratory and Allergic Diseases, Huddinge University Hospital, Huddinge and Dept of Medicine, Karolinska Institute, Stockholm, and +Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden. #Dept of Epidemiology and Public Health, Imperial College School of Medicine, London, UK.

Correspondence: M. Svartengren, Division of Occupational Medicine, Dept of Public Health Sciences, Karolinska Institute at Karolinska Hospital, S-171 76 Stockholm, Sweden. Fax: 46851773766.

Motor vehicles are the dominant source of air pollution in many cities, including Stockholm. The adverse health effects of short-term exposure to car exhaust are often inferred from studies of separate components such as nitrogen oxides and particulates. Nitrogen dioxide (NO$_2$) increases bronchial responsiveness to both nonspecific agents [1] and allergens [2–4] in asthmatic subjects. Experimental studies suggest that asthmatics are also more sensitive to particles than healthy subjects [5]. Increased numbers of hospital visits due to asthma or other respiratory illnesses have been associated with daily variations in the concentrations of particles with a 50% cut-off aerodynamic diameter of 10 mm (PM10) [6–9] and NO$_2$ [10, 11].

The health effects of short-term exposure to motor vehicle exhaust have mainly been assessed by means of controlled chamber studies [12]. These studies involve controlled exposure to a single pollutant, or sometimes a combination of two or more pollutants. Controlled studies have also been performed using "diluted diesel exhaust" [13, 14]. However, real life exposure is to a mixture and more complex. Furthermore, temperature and humidity during real exposure often differ from that in the laboratory environment.

The aim of this study was to assess whether air pollution in road tunnels would promote asthmatic reactions in persons with mild allergic asthma. Respiratory symptoms and pulmonary function were investigated in such subjects during and after a brief stay in a tunnel during the early morning rush hour. Furthermore, an assessment was made of the influence of the tunnel exposure on the subject's sensitivity to subsequent allergen inhalation, several hours later.

Methods

Study design

Twenty subjects with allergic asthma were studied on two separate occasions, at least 4 weeks apart, between December 1996 and February 1997, *i.e.* outside the pollen season. The weather in Stockholm during the study period was fairly cold with a mean temperature of -1.4°C (range -12.4–8.6) and relative humidity of 76% (range 32–95). The subjects were randomly allocated to start with tunnel or control exposure. For the tunnel exposure the test subjects sat for 30 min during the morning rush hour (08:00–09:00 h) in a car parked at the roadside in the Söderleden road tunnel. This tunnel has a length of ~1,500 m and is used by

~35,000 vehicles·day^{-1}. Ventilation of the tunnel is brought about by the movement of the vehicles themselves, and can be assisted by fans and ventilation towers. The windows of the car were closed and the ventilation fan inside the car was on during exposure. The ventilation system was not equipped with a pollen filter. For the control, subjects stayed at a hotel near Huddinge University Hospital, in southern Stockholm, in an area with low levels of air pollution. Exposure measurements were taken just outside the hotel. Respiratory symptoms and pulmonary function were measured immediately before, during and after the 30-min tunnel exposure and likewise during the control experiment. Four hours after the exposure, i.e. at ~13:00 h, the test subjects were challenged with a low dose (see below) of inhaled allergen. Asthmatic reactions during the early and late phase were monitored by means of hourly pulmonary function measurements and symptom registration until bedtime, i.e. ~23:00 h, which corresponds to a 10-h follow-up of the asthmatic reaction to allergen.

Subjects

Subjects were recruited from the Huddinge hospital outpatient clinic for respiratory and allergic diseases and by advertising in newspapers. All subjects gave informed consent to participation in the study. The study was approved by the Ethical Committee at Huddinge University Hospital.

Twenty subjects, 10 females and 10 males, aged 19–55 yrs, with mild seasonal asthma and allergy to pollen (13 birch, seven timothy grass) participated. Lung function (forced expiratory volume in one second (FEV_1)) at inclusion was 99 ± 12 % of the predicted (mean±SD). Anthropometric and clinical data are given in table 1. The subjects were included if they had a diagnosis of asthma based on reversible attacks of dyspnoea during the pollen season and bronchial hyperresponsiveness to histamine. Allergy to either birch or timothy grass pollen was confirmed via a positive skin-prick test with a weal diameter of >3 mm [15], and a positive bronchial challenge with the relevant allergen. Six subjects showed a late asthmatic reaction with a maximal decrease in FEV_1 of >15%, 3–10 h after allergen challenge. The study subjects used inhaled β_2-agonists as needed and 11 used inhaled steroids during the pollen season (May–August). However, none of them received inhaled or oral steroids during the study period. All subjects were nonsmokers (14 neversmokers and six exsmokers for ≥2 yrs). All subjects were also sensitized to animals (dog 19, cat 15, and horse nine) and three to dust mites according to a skin-prick test. No subject had pets in their homes. Seven subjects had domestic gas appliances, which may be an important source of NO_2 exposure.

Exposure measurements

In the tunnel, NO_2/oxides of nitrogen (NO_X) sampling was carried out inside and outside the car, which was parked in the tunnel during the whole of the exposure period. Air samples from the two measuring points were alternately sucked into a chemiluminescence instrument (AC 31M chemiluminescent nitrogen oxides analyser;

Environment SA, Poissy, France) for 30 s with 60 s washout in between. Sampling of the PM_{10} and particles with a 50% cut-off aerodynamic diameter of 2.5 μm ($PM_{2.5}$) fractions was performed inside the car only, simultaneously using two instruments (TEOM 1400; Rupprecht& Patashnick, Albany, NY, USA). The NO_2/NO_X and particulate fraction data were recorded and stored in a computer as 15-min means. At the control site, NO_2/NO_X sampling was carried out outdoors at a height of 3 m and PM_{10} and $PM_{2.5}$ sampling at a height of 2 m using the same methodology as in the tunnel.

Individual exposures to NO_2 during the exposure and control day were also measured with personal passive (filter badge) samplers (Toyo Roshi Kaisha, Tokyo, Japan). The analysis technique as well as the accuracy and reproducibility of measurements using the sampler have been presented in detail elsewhere [16]. The measuring time started just before the subjects entered the tunnel and lasted 24 h.

Symptom registration

The study subjects were asked to indicate their general respiratory status on a visual analogue scale, 0–10, before they entered the car within the tunnel and at the end of exposure. During the stay in the tunnel, they also answered 15 questions covering different symptoms of the respiratory tract as well as general symptoms. For the control exposure, similar registrations were performed in the morning, when the subjects arrived at the hospital. These questions have been used previously in chamber exposure studies [3]. A scale with discrete values from 1–7 was used, 1 indicating no symptoms and 7 indicating very strong symptoms.

After the allergen challenge, the subjects kept a diary of respiratory symptoms and medication. Separate scales were used for night- and daytime symptoms of asthma and symptoms of the nose and eyes. Two combination variables were created. One variable was computed as the sum of asthma symptoms during the evening, night and morning after the exposure. The other combination variable was calculated as the total number of β_2-inhalations during the day, night and morning after the exposure.

Whole body plethysmography

Specific airway resistance (sR_{aw}) and thoracic gas volume (TGV) were measured in a constant-volume body plethysmograph (Model 2000 TB; Cardio-Pulmonary Instruments, Houston, TX, USA), according to DUBOIS and colleagues [17, 18]. The gas flow/box pressure slopes were measured at a gas flow of 0.5– -0.5 L·s^{-1} (expiration/inspiration) as the mean of two or three slopes. The mouth pressure/box pressure slopes were measured between the end points, again as the mean of two or three curves. All panting manoeuvres were performed at ~1 Hz, the subject being guided by a metronome.

Histamine bronchial challenge

Histamine provocation tests at inclusion were performed using an automatic inhalation-synchronized dosimeter jet nebulizer (Spire Elektro 2; Respiratory Care Center,

Table 1. – Anthropometric and clinical data

Subject No	Sex	Age yrs	Asthma duration yrs	Smoking status	FEV1 % pred	Domestic gas appliances	Histamine dose at inclusion mg	Inhaled allergen	Allergen dose inhaled[†] SQ units	LPR Maximal fall in FEV1[#] %
1	M	38	8	Ex	115	-	223	B	55	3
2	F	30	17	Ex	110	-	178	B	35	11
3	F	26	25	Never	94	-	326	T	28	11
4	M	45	40	Never	88	+	64	B	28	22
5	M	32	6	Never	73	-	58	B	21	27
6	F	23	18	Never	107	-	23	T	14	-5
7	F	36	11	Never	93	-	76	B	28	13
8	M	26	20	Never	108	+	58	T	55	58
9	M	23	19	Ex	106	-	34	B	21	26
10	M	30	20	Never	108	+	461	B	35[§]	-2
11	M	24	20	Ex	114	+	461	B	330	21
12	M	19	8	Never	105	-	97	T	165	0
13	F	27	6	Never	108	-	737	B	330	14
14	M	33	32	Never	95	+	20	B	28	12
15	F	33	13	Never	73	-	137	B	21/14[§]	3
16	F	41	13	Never	107	+	478	T	35	5
17	M	55	2	Never	99	-	699	B	165	22
18	F	54	50	Never	89	-	39	B	28	9
19	F	24	6	Never	88	-	175	T	660	4
20	F	27	8	Never	108	+	699	T	83	5
All		32±10	17±12		99±12		156 (58–461)		35 (28–103)	11 (3–22)

Data for all subjects are presented as mean±SD or median (interquartile range). *: causing 100% increase in specific airway resistance at inclusion; [†]: 4 h after tunnel/control exposure; [#]: 3–10 h after allergen challenge at inclusion (late phase reaction); [§]: reduced allergen dose, see *Asthmatic reaction during the early phase*. FEV1: forced expiratory volume in one second; SQ: standardized quality; M: male; F: female; B: birch pollen; T: timothy grass pollen. +: present; -: absent.

Hämeenlinna, Finland) with an adjustable aerosol delivery time according to a previously described method [3, 19]. Doubling doses of histamine diphosphate at three concentrations, 1, 8 and 64 mg·mL^{-1} were administered from an initial dose of 14 μg until a 100% increase in sRaw was reached. sRaw and TGV were measured 3 min after each dose. The provocation dose causing a doubling of the sRaw was calculated by linear interpolation on a logarithmic scale.

Allergen challenge and allergen inhalation

Four hours after tunnel or control exposure, an allergen challenge was carried out. The subject rested for 15 min in the laboratory, and then sRaw and TGV were measured using the whole body plethysmograph described above.

The allergen challenge at the inclusion test and the allergen inhalation during the study were provided *via* the dosimeter jet nebulizer described above. Standardized freeze-dried birch or timothy grass pollen allergen extracts (Aquagen, Copenhagen, Denmark) were used at a maximum of four concentrations, 1,000, 4,000, 16,000 and 64,000 standardized quality (SQ) allergen units·mL^{-1}. For each concentration, two and four breaths could be taken, and, if needed, this was followed by eight and 16 breaths at the highest concentration. sRaw and TGV were measured 15 min after each dose. After measurement of baseline sRaw, the subject inhaled doubling doses of allergen, starting from an initial dose of 14 SQ units until a 100% increase in sRaw was reached. The dose causing a doubling of the sRaw for the allergen was calculated by linear interpolation on a logarithmic scale. The inhaled allergen dose during the study was set to 40% of this dose or the dose step above (table 1). The median allergen dose used

was 34 SQ units (range 7–590). For safety reasons, the inhalation started with 20% of the inclusion dose. Another 20% was then inhaled provided that the sRaw increase, after 15 min, was <50% compared to baseline. If the sRaw increase after the first dose was 50–75% compared to baseline, the second allergen dose was reduced to 10% of the inclusion dose. Fifteen minutes after the second dose, sRaw and TGV were measured again. These measurements are referred to as the early phase asthmatic reaction.

Spirometry

At inclusion, pulmonary function was followed hourly in all subjects. The best value of FEV1 was called "ever best", and FEV1 obtained thereafter were expressed as a percentage of this "ever best" FEV1. On tunnel and control exposures days, subjects were asked to measure peak expiratory flow (PEF) and forced vital capacity every hour from 08:00 h, using a portable computerized spirometer (Diary Card spirometer®; Micromedical, Chatham, UK). After the allergen inhalation, the subjects went home and continued to measure FEV1 hourly for ≥10 h. The subjects were instructed to inhale bronchodilators if needed or if the FEV1 decreased by ≥30%. The maximal percentage fall in FEV1 3–10 h after allergen challenge is referred to as the late phase asthmatic reaction.

Statistical methods

The statistical package SPSS (SPSS Inc., Chicago, IL, USA) for Windows (6.1.3) was used for all statistical analyses. The differences in pulmonary function, subjective symptoms and concentrations between tunnel exposure and control were analysed using the Wilcoxon′s

signed-rank test. Differences in responses between subjects exposed to high and low levels of pollution were compared using the Wilcoxon rank sum test. *A priori* dichotomizations of 300 $\mu g \cdot m^{-3}$ NO_2 and 100 $\mu g \cdot m^{-3}$ PM2.5 were chosen based on expected pollution levels within the tunnel. Rank correlation analysis was used to estimate Spearman correlation coefficients. A p-value of <0.05 using two-tailed tests was considered statistically significant.

Results

Air pollution exposure

Concentrations of NO_2 in the tunnel outside and inside the car were similar. The median NO_2 concentration during rush hour 08:00–09:00 h, Monday to Friday) was 289 $\mu g \cdot m^{-3}$ (range 179–688) inside the car and 11 $\mu g \cdot m^{-3}$ (range 0–51) at the hotel. PM10 and PM2.5 levels inside the car during rush hour were 126 $\mu g \cdot m^{-3}$ (range 75–907) and 93 $\mu g \cdot m^{-3}$ (range 60–262) respectively, and the levels near the hotel were 7 $\mu g \cdot m^{-3}$ (range 2–17) and 5 $\mu g \cdot m^{-3}$ (range 2–11).

Individual exposures in the car are given in table 2. The median NO_2 concentration was 313 $\mu g \cdot m^{-3}$ (range 203–462). The NO_2 concentration in the car was highly correlated with the concentrations of NO_X in the car (r=0.91), NO_2 in the tunnel (r=0.83), and NO_X in the tunnel (r=0.77). Furthermore, there was a significant association between NO_2 concentration measurements in the car and the difference in the badge measurements between tunnel exposure and the control day (r=0.47, p=0.036).

The 24-h median exposure to NO_2 measured with the personal passive sampler (badge) was 29 $\mu g \cdot m^{-3}$ (range 20–50) during tunnel exposure and 22 $\mu g \cdot m^{-3}$ (range 10–38) during control exposure. The difference was statistically significant (p<0.001). Domestic gas appliances contributed a median of 8 $\mu g \cdot m^{-3}$ to the 24-h median concentrations (p=0.011).

The PM10 and PM2.5 concentrations in the car were 170 $\mu g \cdot m^{-3}$ (range 103–613) and 95 $\mu g \cdot m^{-3}$ (61–218), respectively. PM10 and PM2.5 concentrations were highly correlated (r=0.91). However, there were only very weak associations between NO_2 levels and PM10 or PM2.5 concentrations (r<0.18).

Effects during air pollution exposure

Questionnaire data showed that, in the tunnel, several symptom scores were increased. The greatest differences were found for noise, smell and cough irritation (median difference 3.5, 3.0 and 1.0). The values for self-perceived general respiratory status were significantly higher (worse) in the tunnel (median 2.05, range 0–8.2 on the visual analogue scale) than before tunnel exposure (median 1.0, range 0–5.0, p<0.004) or at the control site (median 0.6, range 0–7.2, p<0.006). Higher NO_2 1evels were significantly associated with nasal congestion (rS=-0.44, p=0.050). After dichotomization at a NO_2 exposure of 300 $\mu g \cdot m^{-3}$, no significant differences were found.

FEV1 in the car was determined in the sitting position, which might slightly decrease the values compared to other measurements, which were all performed with the subjects in a standing position. The FEV1 in the car (mean 3.47, median 3.33, range 1.46–5.26 L) was not different from the morning value (mean 3.48, median 3.38, range 1.51–5.13 L, p=0.84), but lower compared to later on in the morning (mean 3.56, median 3.38, range 1.63–5.51 L, p=0.002) and just before allergen challenge (mean 3.56, median 3.36, range 1.69–5.38 L, p=0.001). There was an association between NO_2 and FEV1 change in the car (rS=0.48, p=0.042) but no association between PM2.5 concentration and pulmonary function at any time before allergen challenge.

Asthmatic reaction during the early phase

All subjects completed the allergen inhalation test. However, in subject No. 10, the allergen dose was reduced from 40 to 20% of the inclusion dose at both exposures. Furthermore, in subject No. 15, the total allergen dose at control exposure was reduced to 30% because of symptoms of asthma, and the patient was excluded from comparisons of the effects of allergen inhalation.

Lung function results are given in table 3. There were no differences in sRaw or TGV, before allergen inhalation, between tunnel and control exposure. Median sRaw increased 44% (from 5.0 to 7.2 cmH$_2$O·s) after allergen inhalation following the tunnel exposure, and 31% (from 5.1 to 6.7 cmH$_2$O·s) for control exposure. Median TGV increased from 3.5 to 3.7 L (p<0.001) after tunnel plus allergen exposure compared to 3.4 to 3.2 L (p=0.94) after control plus allergen exposure. The increases in both sRaw

Table 2. – Individual exposure to NO_2 or particles

Subject No	24 h badge		30-min exposure in car	
	Tunnel NO_2 $\mu g \cdot m^{-3}$	Hotel NO_2 $\mu g \cdot m^{-3}$	NO_2 $\mu g \cdot m^{-3}$	PM2.5 $\mu g \cdot m^{-3}$
1	29	12	462	87
2	35	16	462	87
3	26	21	203	68
4	38	25	279	78
5	25	27	203	68
6	27	22	324	128
7	29	30	324	128
8	50	30	355	101
9	31	24	410	91
10	31	21	275	108
11	47	18	355	101
12	20	10	237	77
13	22	21	271	76
14	37	38	303	77
15	29	10	298	61
16	21	18	275	108
17	22	24	313	139
18	25	12	313	139
19	26	22	329	218
20	33	26	321	144
Median	29	22	313	95
Minimum	20	10	203	61
Maximum	50	38	462	218

PM2.5: particles with 50% cut-off aerodynamic diameter of 2.5 μm.

and TGV after tunnel plus allergen exposure were significantly greater than after control plus allergen exposure (p=0.025 and p=0.01, respectively). A similar tendency with a greater effect for tunnel plus allergen exposure (median 4.5, range -2.2–19.1%) than for control plus allergen (median 1.6, range -4.7–17.7%) was also found for fall in FEV_1 (p=0.11).

In order to assess the influence by level of exposure in the tunnel of the two measured air pollutants, the group was divided into two parts. *A priori* dichotomizations were chosen based on expected pollution levels within the tunnel. Twelve of the subjects had tunnel NO_2 exposure levels of ≥ 300 $\mu g \cdot m^{-3}$. These subjects exhibited a significantly greater early phase reaction after allergen challenge following tunnel exposure (median sRaw increased 44% from 5.3 to 7.7 $cmH_2O \cdot s$) than following control exposure (median sRaw increased 24% from 5.1 to 6.4 $cmH_2O \cdot s$). The seven individuals with NO_2 exposure levels below 300 $\mu g \cdot m^{-3}$ did not differ from control (p=0.61). In the group as a whole, there was no overall association between NO_2 exposure and difference in sRaw change caused by allergen challenge between tunnel and control (rS=0.36, p=0.14) (fig. 1a).

Ten of the subjects had PM2.5 exposure levels of ≥ 100 $\mu g \cdot m^{-3}$. These subjects had a marginally greater early reaction after allergen challenge following tunnel exposure (median sRaw increased 36% from 4.9 to 6.6 $cmH_2O \cdot s$) than after control exposure (median sRaw increased 22% from 5.1 to 6.3 $cmH_2O \cdot s$) (p=0.038). There was no difference between PM2.5 exposure below and above 100

$\mu g \cdot m^{-3}$. There was no overall association between PM2.5 exposure and the early reaction (r=0.14, p=0.53) (fig. 1b).

Asthmatic reaction during the late phase

The test subjects had significantly more asthma symptoms during the evening after the tunnel exposure than after the control exposure (p=0.016). Furthermore, the combined asthma symptoms during the evening, night and morning after the exposure tended to be worse (p=0.085). These symptoms were significantly correlated with NO_2 exposure (fig. 2a), (rS=0.72, p=0.001) but not PM2.5 exposure (fig. 2b) (rS=0.32, p=0.18).

The combined asthma symptoms variable was significantly increased compared to control (p=0.028), when NO_2 exposure was >300 $\mu g \cdot m^{-3}$. The group with NO_2 exposure above 300 $\mu g \cdot m^{-3}$ had significantly more asthma symptoms during the exposure evening compared to control (p=0.026) and more symptoms during the night after allergen inhalation compared to the group with exposure <300 $\mu g \cdot m^{-3}$ (p=0.024). Corresponding analyses for PM2.5 with a dichotomization at 100 $\mu g \cdot m^{-3}$ showed a slight but nonsignificant effect for the combined asthma symptom variable.

The mean number of β_2-agonist inhalations during the 20 h following tunnel exposure was low, 0.8, compared to 0.7 for the control exposure. The combined medication variable based on the number of β_2-agonist inhalations indicating positive association with NO_2 exposure (rS= 0.56, p=0.014). The group with NO_2 exposure >300 $\mu g \cdot m^{-3}$

Table 3. – Asthmatic reaction during the early phase

Subjects No	Tunnel exposure				Control exposure			
	sRaw $cmH_2O \cdot s$		TGV L		sRaw $cmH_2O \cdot s$		TGV L	
	Before allergen	After allergen	Before allergen	After allergen	Before allergen	After allergen	Before allergen	After allergen
1	5.0	8.3	3.6	4.2	4.0	6.2	4.1	3.9
2	4.7	7.0	3.0	3.6	5.0	5.9	3.2	3.2
3	5.1	6.9	2.9	3.0	5.0	7.7	3.2	3.2
4	8.3	13.2	4.3	5.4	9.7	10.0	4.3	4.5
5	6.2	10.4	2.6	2.9	6.0	8.5	2.7	2.6
6	4.8	7.2	2.6	2.8	5.2	5.2	2.7	2.5
7	6.7	12.9	2.7	2.9	5.9	8.7	2.4	2.2
8	5.9	16.7	4.8	6.1	6.1	12.5	4.9	5.7
9	6.8	8.1	3.5	3.7	5.9	6.7	4.2	3.5
10[+]	4.9	6.1	4.5	4.8	5.1	6.1	4.7	4.5
11	4.4	5.4	4.1	4.2	4.1	5.2	4.4	5.0
12	6.3	8.1	4.3	4.4	4.2	8.3	4.1	4.5
13	4.6	6.0	2.0	2.1	4.8	5.0	2.3	2.2
14	4.9	7.0	4.2	4.5	5.6	8.7	3.8	4.0
15[#]	7.3	10.0	2.3	2.5	9.9	15.5	2.8	3.2
16	4.9	5.8	3.7	3.7	5.6	7.4	3.8	4.1
17	4.4	5.1	3.0	3.0	4.1	4.8	3.1	3.1
18	6.1	10.7	2.0	2.3	6.7	8.9	2.2	2.3
19	5.8	8.3	2.8	3.3	4.3	6.4	3.2	3.0
20	4.7	5.6	4.1	3.9	4.0	4.7	3.4	3.2
Median	5.0	7.2	3.5	3.7	5.1	6.7	3.4	3.2
Minimum	4.4	5.1	2.0	2.1	4.0	4.7	2.2	2.2
Maximum	8.3	16.7	4.8	6.1	9.7	12.5	4.9	5.7

*: 15 min after allergen challenge; [+]: provocation dose of 20% of inclusion dose used for both exposures; [#]: subject excluded because different provocation doses were used for the tunnel (40% of inclusion dose) and control exposure (30% of inclusion dose). sRaw: specific airway resistance; TGV: thoracic gas volume.
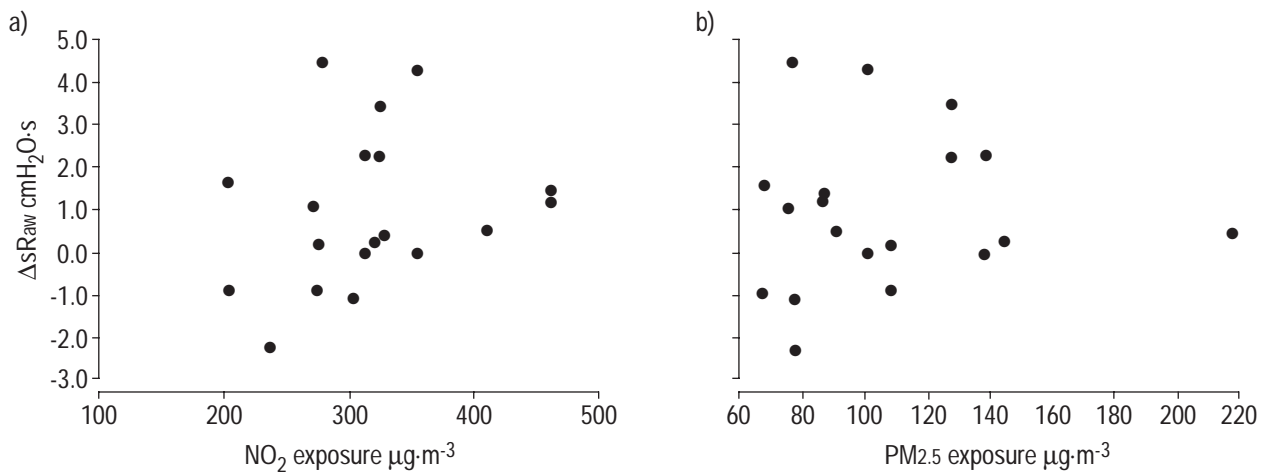
Fig. 1. – Asthmatic reaction during the early phase. Changes in specific airway resistance sRaw (ΔsRaw) caused by allergen inhalation after tunnel exposure. Data are expressed as the difference from the values obtained on the control day. Negative values correspond to a greater effect on the control day compared to tunnel exposure. Individual differences between tunnel and control as a function of exposure to: a) nitrogen dioxide (rS=0.36, p=0.14); and b) particles with 50% cut-off (PM2.5) (r=0.14, p=0.58) within the car.

used significantly more β_2-agonist inhalations compared to the group with exposure <300 $\mu g \cdot m^{-3}$ (p=0.044). No such tendency could be seen for PM2.5.

In total, 18 subjects were followed with hourly FEV1 determinations during the late phase. On the day of the tunnel exposure, the maximal decrease in FEV1, at any time 3–10 h after allergen challenge, ranged 1.9–45.4% (median 8.8%) compared to the (best) prechallenge FEV1, and the average late FEV1 values were from 2.2% higher to 23.5% lower (median 4.3% lower) compared to the prechallenge FEV1. On the control day, the maximal "decrease" in FEV1 ranged from 6.5 higher to 30.4% lower (median 7.0% lower) with the mean FEV1 being 6.5% higher 23.2% lower (median 2.6% lower) than the prechallenge FEV1. However, none of these changes differed significantly between the tunnel exposure day and the control day. However, among those with NO₂ exposure >300 $\mu g \cdot m^{-3}$, the maximal decrease in FEV1 during the late phase was significantly greater (p=0.04) control (median 8.5% versus 6.8%), and also tended to be greater (p=

0.06) than that found in the group with exposure <300 $\mu g \cdot m^{-3}$ NO₂. Figure 3a presents the effect differences in the maximal late phase reaction between tunnel and control as a function of NO₂ exposure within the car (rS=0.28, p=0.26). There was a tendency for a greater late phase reaction in the group with PM2.5 exposure >100 $\mu g \cdot m^{-3}$ compared to control (p=0.051). There was no overall association between PM2.5 exposure and late phase change in FEV1 reaction (fig. 3b).

Discussion

The levels of NO₂ and particulates measured in the road tunnel were higher than those observed in streets with heavy traffic in Stockholm, confirming earlier data [18]. The concentrations of NO₂ inside the car were only slightly lower. The duration of exposure in a road tunnel depends on the length of the tunnel as well as the traffic situation, and is probably often shorter than in the present
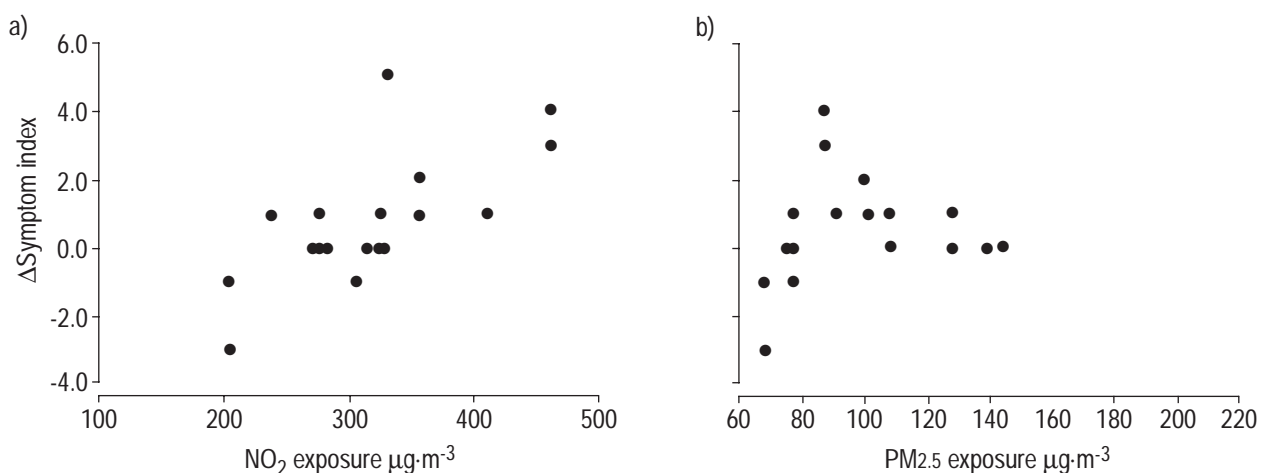

Fig. 2. – Asthma symptoms index during the late phase. Changes (Δ) in symptom index during the evening, night and morning after allergen inhalation. Data are expressed as difference from the values obtained on the control day. Negative values correspond to a greater effect on the control day compared to tunnel exposure. Individual differences between tunnel and control as a function of exposure to: a) nitrogen dioxide (rS=0.72; p=0.001); and b) particles with 50% cut-off aerodynamic diameter of 2.5 μm. (PM2.5) exposure (rS=0.32, p=0.18) within the car.
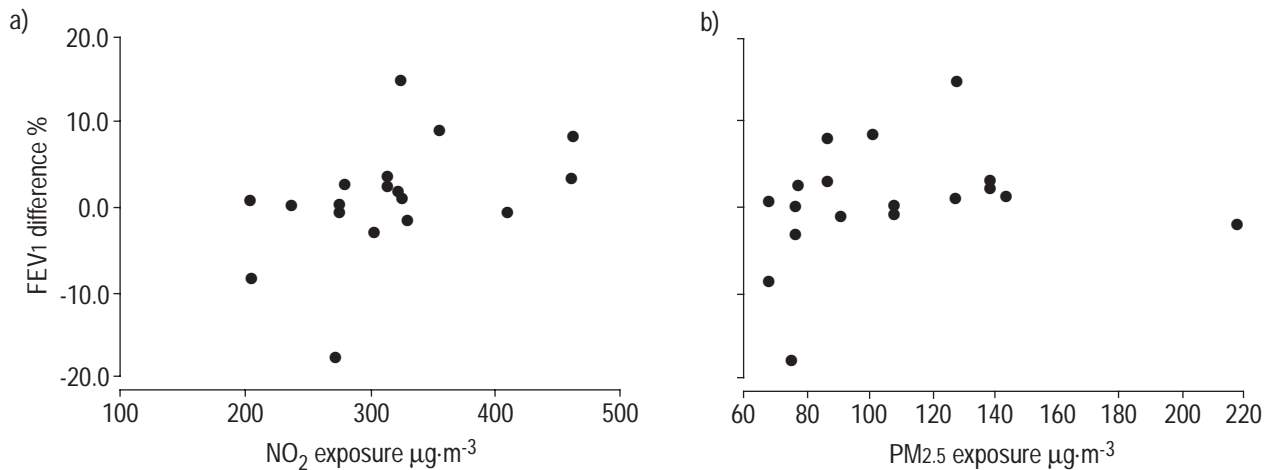
Fig. 3. – Asthmatic reaction during the late phase. Maximal late phase change in forced expiratory volume in one second (FEV1) (from best value). Data are expressed as the difference from the values obtained on the control day. Negative values correspond to a greater effect on the control day compared to tunnel exposure. Individual differences between tunnel and control as a function of exposure to: a) nitrogen dioxide (rS=0.28, p=0.26); and b) particles with 50% cut-off aerodynamic diameter of 2.5 mm (PM2.5) (rS=0.18, p=0.46) within the car.

study. However, a 30-min exposure time is plausible in long tunnels or tunnel systems, and when traffic congestion occurs because of accidents or the rush hour. Moreover, this exposure time facilitates comparisons with earlier controlled chamber exposure studies [3, 4]. Data from the personal NO_2 samplers indicate that the contribution from the tunnel exposure to total daily exposure is clearly discernible. Correlations between the concentrations of NO_2 and particulates in the tunnel were weak, possibly due to the strong influence of the wet road surface on the particulate levels, leading to reduced resuspension of settled dust.

During the stay in the tunnel, the effects related to the airways, such as irritation with increased tendency to cough, breathing discomfort and chest tightness, were small or moderate. Pulmonary function, measured as FEV1 in the tunnel was similar to that in the morning, but there was an association between NO_2 concentration and pulmonary function during the following morning hours. It must be taken into account that all subjects in the present study had bronchial asthma of a mild type with normal pulmonary function. People with asthma of a more brittle or severe type may have reacted differently.

For obvious reasons, it was not possible to blind the experimental design, and the subjects might have been more eager to report symptoms after the stay in the tunnel. However, not only was the magnitude of the symptoms greater during the tunnel day compared to control but also symptoms and the use of β_2-agonist inhalations were related to the exposure level of NO_2 in the tunnel. The exposure concentrations were known to neither the subjects nor the personnel in the research group, when the experiment was performed. Furthermore, the consistency of the results is noteworthy concerning subjective symptoms, medication and changes in pulmonary function after allergen inhalation.

The health effect of main concern observed in the present study is the enhanced asthmatic reaction to allergen occurring several hours after the tunnel exposure. This finding is in agreement with earlier observations in controlled human exposure studies. In the study by STRAND et al. [3], a single exposure to 500 $\mu g \cdot m^{-3}$ NO_2 followed by

inhalation of a high dose of allergen resulted in an enhanced fall in PEF during the late asthmatic reaction and a tendency to increased early reaction to allergen. In a later study [4] of similar design with repeated exposure to 500 $\mu g \cdot m^{-3}$ NO_2 followed by a low dose of allergen, there was an increase in both the early and late phase reactions. There was also a suggestion of increased symptoms of asthma during the night. In the current study, pulmonary function in persons with high NO_2 exposure was affected during the early and late phase. They also had more asthma symptoms and used more medication during the late phase. The concentration of NO_2 in the tunnel was, on average, considerably lower than that in the chamber studies, but an enhancing effect still appeared. This may be due to combined exposure to other air pollutants from car exhaust in the tunnel.

The associations between exposure and outcome variables were generally weaker for particulates than for NO_2. A marginal effect of PM2.5 concentrations >100 $\mu g \cdot m^{-3}$ on the early asthmatic reaction as well as an association between PM2.5 concentration and asthma symptoms during the late phase was found in the present study. The latter was, however, highly dependent on one extreme data point. There might be important interactions between PM2.5 and NO_2, but the limited number of subjects in the present study makes such an evaluation difficult.

To the authors' knowledge, there are no controlled human studies on the effects of particles on allergen responsiveness. Several epidemiological studies have also reported adverse health effects at rather low levels of PM10. For example, a US study showed an increase in hospital admissions at a PM10 level of as low as 24 $\mu g \cdot m^{-3}$ [21]. Relatively low levels of particulate matter (PM10 <60 $\mu g \cdot m^{-3}$) may give rise to acute health effects and increasing need for medication in children [22]. It is not possible to evaluate whether interactions with allergens also played a role in these effects as relevant data were not reported. There is some evidence that exposure to the smaller fraction of particulate matter (PM2.5), may be more important than that to PM10 for acute respiratory symptoms [23] and even for death from respiratory diseases [24].

Unvented domestic gas appliances may contribute to NO_2 exposure [25]. The seven subjects with gas stoves at home showed a higher NO_2 exposure, measured using the personal sampler, on the tunnel exposure day. However, they did not differ in responsiveness to allergen (early and late phase and asthma symptom index) from the group without domestic exposure to NO_2.

The allergens used in the present study were extracted from birch and timothy grass pollen, but it is likely that similar effects would be seen with other allergens (*e.g.* from cat, dog or mites) present all year round. TUNNICLIFFE *et al.* [2] showed that exposure to 800 µg·m^{-3} NO_2 for 1 h amplified both the early and late reaction to mite allergen. The allergen dose used in the present study corresponds to natural exposure and can be obtained in a day during peak pollen season (ALK, Copenhagen, Denmark, personal communication). Similar effects on allergen responsiveness after NO_2 exposure have been reported after lower, as well as higher, allergen doses [3, 4].

It is thus reasonable to assume that exposure to air pollutants for half an hour in a road tunnel can increase the bronchial response to allergens several hours after the exposure in individuals with allergic asthma. The findings suggest that exposure to car exhaust initiates a pro-inflammatory or inflammatory process in the bronchial mucosa. This state persists for ≥ 4 h and gives an extra impetus to the allergic reaction with accompanying deterioration of lung function. Such an interpretation, implying a pro-inflammatory effect of exposure to NO_2 and particles, is supported by data obtained in human and animal exposure experiments [12]. If this interpretation is correct, it is also likely that the inflammation increases bronchial responsiveness to not only allergens but also nonspecific agents such as cold air and tobacco smoke, as well as exercise. The increase in bronchial responsiveness in the present study occurred without changes in lung function during exposure or in the interval before allergen exposure. There are other studies reporting similar findings with no observed effect on lung function during NO_2 exposure, but which induced an increase in airway responsiveness to agents like histamine, methacholine or ozone [2, 26, 27]. This makes it difficult for the exposed individual to be aware of the risk.

In conclusion, short-term exposure to air pollution in a road tunnel during the rush hour enhanced the asthmatic response to allergen inhaled several hours later, although pulmonary function during the exposure was not affected. The adverse effect was related to the exposure levels of nitrogen dioxide and, to some extent, to particles, and merits attention from a public health perspective as large groups of the population with asthma may be at risk.

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