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Enhanced respiratory responses in children exposed to air pollution. An epidemiological study

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Abstract

The association between traffic related air pollution and respiratory symptoms was studied using a longitudinal observational design with repeated measures in 82 children attending elementary schools in Utrecht (urban) or Bilthoven (suburban). Selection of the schools was based on year mean values for black smoke (BS), indicative for traffic related air pollution: Utrecht 53 $\mu g/m^3$ and Bilthoven 18 $\mu g/m^3$. Levels of air pollutants NO, NO_2, CO and BS, as indicators of traffic related air pollution, were consistently higher in Utrecht than in Bilthoven (mean daily ratios 8, 1.5, 1.8 and 2.7, respectively). Children living in Utrecht (moderate exposure) show higher mean levels ($p<0.05$) of IL-8 (32%), urea (39%), uric acid (26%), albumin (15%), and the NO-metabolites nitrate & nitrite (21%) in nasal lavage as compared to children living in Bilthoven (low exposure). Associations were demonstrated between PEF, NO in exhaled air and inflammatory parameters in nasal lavage and ambient levels of PM_{10}, BS, NO_2 and NO. Per unit increase in air pollution levels, high exposed children living in Utrecht showed increased responses in PEF, exhaled NO and nasal release of uric acid, urea, nitrite and nitrate as compared to children living in a suburban environment (Bilthoven).
Conclusion: the children regularly exposed to moderate levels of traffic related air pollution show increased mean nasal levels of inflammatory biomarkers, and respond more pronounced to the same increments in air pollution as compared to children living in a suburban environment and exposed to low background levels of air pollution.

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Samenvatting

De associatie tussen verkeersgerelateerde luchtverontreiniging en luchtwegklachten werd bestudeerd in een longitudinale observationele studie met herhaalde metingen bij 82 kinderen van de basisschool in Utrecht (stad) of Bilthoven (dorp). De scholen waren geselecteerd op basis van jaarwaarden voor zwarte rook (ZR), hetgeen indicatief is voor verkeersgerelateerde luchtverontreiniging: Utrecht 53 $\mu g/m^3$ en Bilthoven 18 $\mu g/m^3$. Niveaus van luchtverontreiniging NO, NO_2, CO en ZR, als indicatoren voor verkeersgerelateerde luchtverontreiniging, waren in Utrecht belangrijk hoger dan in Bilthoven (gemiddelde dagelijkse ratio's waren respectievelijk 8, 1,5, 1,8 en 2,7). Kinderen die wonen in Utrecht (matige blootstelling) vertoonden gemiddeld hogere niveaus ($p<0,05$) van IL-8 (32%), ureum (39%), urinezuur (26%), albumine (15%) en de NO-metabolieten nitraat en nitriet (21%) in de neuslavage in vergelijking met kinderen die in Bilthoven wonen (lage blootstelling). Associaties werden aangetoond tussen enerzijds PEF, NO in uitgeademde lucht en ontstekingsmediatoren in de neuslavagevloeistof en anderzijds PM_{10}, ZR, NO_2 en NO in buitenlucht. Bij een gelijke verhoging van luchtverontreiniging vertoonden kinderen in Utrecht een grotere response in PEF en uitgeademde NO en in de neus een grotere toename van urinezuur, ureum, nitraat en nitriet in vergelijking met kinderen in Bilthoven.
Conclusie: kinderen veelvuldig blootgesteld aan matige niveaus van verkeersgerelateerde luchtverontreiniging vertoonden verhoogde gemiddelde niveaus van ontstekingsmediatoren in de neus en reageerden sterker op dezelfde toename van luchtverontreiniging vergeleken met kinderen wonend in een dorpse omgeving en blootgesteld aan lage achtergrondswaarden van luchtverontreiniging.

1. Introduction

A large part of the world population is exposed to ambient air pollutants inducing adverse health effects (1). Studies show increased deaths and hospitalization following exposure to increased air pollution. For instance, asthma exacerbations, increased respiratory symptoms and illness, decreased lung function, lung inflammation, increased airway reactivity and altered host defense have been described following increases in air pollutants (1). Even relatively low concentrations of air pollutants were shown to be associated with health problems and day-to-day variation in mortality (2,3). The above mentioned adverse health effects are mostly studied in populations of large American and European cities (1). As a large part of the air pollution in cities is traffic related, association between exposure to air pollution of subjects living near busy roads and their health effects has been examined (4-8). These studies, mostly performed in children, show that exposure to traffic-related air pollution, leads to impaired lung function and increased hospital admissions. To characterize respiratory health status or respiratory function in the study populations, the prevalence of symptoms of children were collected by respiratory health questionnaires (5,7) and lung function tests were performed such as spirometry (4,6). Little is known however, about the mechanism by which air pollutants impair respiratory function. Inflammatory processes in both the upper and lower respiratory tract are likely to be involved. It has been shown that inflammatory processes due to photochemical air pollution play a role in healthy adults, children (9-11) and asthmatics (12). To investigate in epidemiological studies the association between air pollution and respiratory inflammation the non-invasive nasal lavage procedure, exhaled NO, and PEF-measurement can be used to obtain information about the inflammatory status of the respiratory tract (10-13). Exhaled NO was shown to be a useful parameter to monitor respiratory diseases, such as asthma (14) and we recently demonstrated that exhaled endogenous NO is enhanced by increased levels of air pollution (13).

In the longitudinal study described herein it was investigated whether traffic related air pollution induced changes in PEF, exhaled NO and biochemical markers in nasal lavage (IL-8, albumin, uric acid, urea and NO-metabolites). We compared two populations of children visiting schools in Utrecht and Bilthoven (moderate respectively low background exposure to traffic related air pollution) during seven weeks in February until March 1998. On both locations the association between individual air pollutants and various biomarkers of effect (peak flow, exhaled NO and nasal inflammatory markers) was investigated using a mixed linear regression model.

2. Materials and Methods

2.1 Subjects

Parents of 126 Dutch children aged 8-13 years were invited in writing, providing background information, to allow their child to participate. Informed consent was given by 82 parents (65.1%), while the remainder did not complete the questionnaire or refused participation of their child. Parents were asked to fill in a modified WHO-children's questionnaire for chronic respiratory symptoms (15) extended with items such as questions on smoking habits at home. All children, of whom parents responded positively, were included, i.e. no restrictions with respect to health status criteria were applied. The study protocol was approved by the Medical Ethics Committee.

2.2 Study design

A longitudinal observational design (panel study) with repeated measures following time was used to study the association between air pollution components and respiratory symptoms. Two groups of children were compared. Children in the first group (N=38) attended a school located near a busy motorway at the border of the town Utrecht, were exposed to moderate levels of air pollution and labelled as urban or moderate exposed. Children in the second group (N=44) attended a school located in the middle of greens of the village Bilthoven and were labelled as suburban or low exposed. Selection of these schools was based on yearly mean values for ambient black smoke, indicative for traffic related air pollution and measured by air quality monitoring stations in Bilthoven (18 $\mu g/m^3$ black smoke) and Utrecht (53 $\mu g/m^3$ black smoke).
Peak flow measurement, sampling of exhaled air and nasal lavage were performed in this order seven times once a week within a two months period (February-March). Air pollution data were obtained from the Dutch National Air Quality Monitoring Network operated by the National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

2.3 Measurement of peak expiratory flow

On each sampling day peak expiratory flow (PEF) of the children was determined three times using Mini Wright Peak Flow Meters (Clement Clarke, London, U.K.) and the mean value of 2-3 measurements (difference < 10 ml/min) was used for data analysis. All children performed reproducible peak flow maneuvers during the study with an averaged PEF-value of 357 ± 56 ml/min (range: 180 to 487).

2.4 Sampling of exhaled air and measurement of NO

Samples of exhaled breath were taken following the standard method as described before (13). Children performed the maneuver while seated and wearing a nose clip. Children first breathed clean air for one minute, inhaled clean air and subsequently exhaled for 20 seconds at a pressure of 17 cm water and a flow rate of ± 500 ml/min using a flow determinator. Exhaled air samples were collected in 1000 ml Mylar bags and NO level measured afterwards via chemiluminescence by a Sievers 280B NO-analyzer (Sievers Instruments, Boulder, CO, USA).

2.5 Nasal lavage

The NAL procedure was performed according described before (10,16,17). Briefly, children bent the neck 45° backwards and elevated the palate to close the nasopharynx. Saline (4 ml, 37°C) was instilled in one nostril using a pipette, the fluid was held in the nasopharyngeal region for 10 seconds and then the child flexed the head and let the washing solution slip via a polyamide gauze (100 mesh) and funnel in a centrifuge tube kept on ice (mean recovered volume 4.5 ml). The procedure was repeated for the second nostril. After centrifugation (10 min, 250 g, 4°C) supernatants were used for assay of IL-8, albumin, uric acid, urea and nitrate & nitrite. Total cell count was not corrected for recovered volume.

IL-8 was measured by enzyme linked immuno-sorbent assay (Medgenix diagnostics, Biosource, Etten-Leur, The Netherlands) with an intra-day variability of less then 10%. Depending on 10% reproducibility albumin, uric acid and urea were assayed either in duplo or triplicate using respectively bromcresol green (18), an enzymatic colorimetric test and the Unimate 5 & 7 Urea-kit (both Hofmann-La Roche, Basel, Switzerland). After denaturation and reduction of nitrate by Klebsiella pneumoniae (19), NO-metabolites nitrite & nitrate in supernatant were assayed by the Griess method.

2.6 Ambient air pollution components

Monitoring stations located within one mile from the schools measured the following air pollutants: CO, O_3, NO, NO_2 and SO_2. PM_{10} levels were measured by another station located within 5 miles of both schools. Ambient levels of CO, O_3, NO, NO_2, BS, SO_2 and PM_{10} were expressed as mean levels from 8:00 to 11:00 (measuring time, mt), 24 h mean values of the day before (L1), three days before (L3), and mean levels of the week before (week, wk).

On each sampling day indoor and outdoor ambient air samples were collected in Mylar bags of 250 ml (ABC ballonnen, Zeist, The Netherlands) every half an hour using a permissible air pump model P-200 (Dupont de Nemours, Wilminton, Delaware, USA) and NO level assayed as described above.

2.7 Questionnaire

Of the questionnaires 87% was returned. The items concerning respiratory symptoms were clustered in three variables: coughing (upper respiratory tract), symptoms of the lower respiratory tract and allergy. Coughing was scored positive if children coughed by morning, day, night or nearly daily during winter period. Lower respiratory symptoms were indicated by shortness of breath, wheezing or dispnoea the last 12 months, or diagnosis of asthma or bronchitis ever. Allergy was scored positive in case of allergy for house dust, domestic animals, or pollen.

2.8 Statistical analysis

Daily ratios of pollutant levels in Utrecht and Bilthoven were calculated for the seven sampling days. Statistical analyses were performed with Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc., Cary, NC, USA). Associations between air pollution, nasal lavage parameters, exhaled NO and PEF were analyzed using regression analysis with adjustment for correlations between repeated measurements within subjects (Proc Mixed). Models were fit according to restricted maximum likelihood (REML) and t-type confidence intervals determined for all parameters. Covariates considered were gender, age, number of cigarettes smoked at home, having a cold on the day of sampling, coughing, history of respiratory symptoms, and allergy. Independent variables were the air pollution components at the time intervals mentioned above (cf. Ambient air pollutant components). Nasal lavage parameters were log-normally distributed and log-transformed before analysis.

Initially it was assessed that the children of both schools showed a different response to changes in air pollution so that the schools were analyzed separately. To enable comparison of the two groups the effect of air pollution on the level of biomarkers was calculated according to the range of pollutants (25-100 $\mu g/m^3$ as indicated). As such for the nasal markers the odds ratio (OR) is calculated by $OR = e^{\beta * \text{range air pollutant}}$. The associations between the various biomarkers were assessed using the same model. P-values < 0.05 were considered significant.

3. Results

3.1 Description of study population

Table 1 summarizes the characteristics of both groups studied. On both schools the number of boys exceeded the number of girls but there was no difference in age. As compared to Utrecht the prevalence of coughing and respiratory disease in Bilthoven was higher (32 versus 19%) while number of smoking parents was lower (28 versus 46%).

Table 1. Population characteristics of subjects.

	Utrecht	Bilthoven	Total
gender (boys)	19 (50)	21 (48)	40 (49)
coughing	7 (18)	14 (32)	21 (27)
respiratory disease	8 (21)	14 (32)	22 (28)
allergy	7 (18)	9 (20)	16 (20)
smoking 0 cigarettes	21 (55)	32 (73)	47 (57)
smoking 1-9 cigarettes	11 (30)	6 (14)	20 (25)
smoking ≥ 10 cigarettes	6 (16)	6 (14)	12 (15)

The percentage is given between parenthesis.

3.2 Air pollution levels

Ambient levels of air pollutants in Utrecht and Bilthoven during the study are depicted in Figs. 1 and 2. During the 90-day study period PM_{10} levels exceeded the WHO-health guideline (70 $\mu g/m^3$) on 12 days and the running 24-hour mean of 50 $\mu g/m^3$ on 24 days. In contrast to PM_{10}, NO_2 and CO levels remained below health guidelines and BS and NO levels were only moderately increased (maximal detected average daily levels of 105 $\mu g/m^3$ and 206 $\mu g/m^3$, respectively).

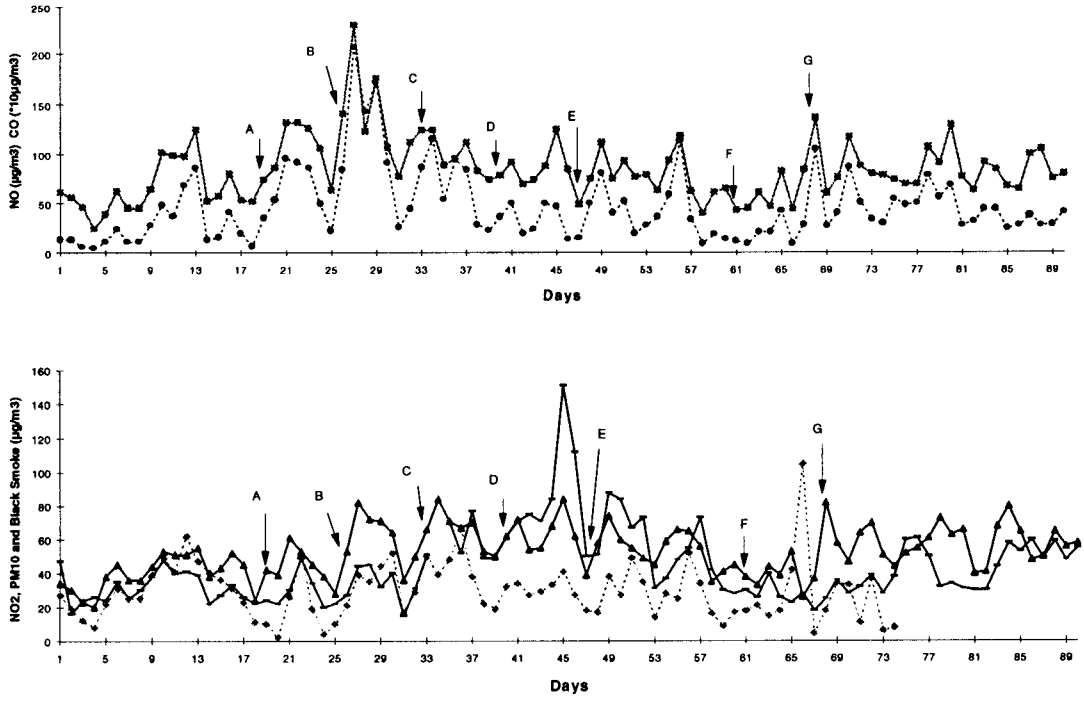

Figure 1. Levels of air pollutants in Utrecht. Daily ambient CO (■), NO (●), PM10 (-), NO_2 (▲) and Black smoke (◆). Data are presented as mean 24 hour values (12 midnight-12 midnight). Sampling days are marked by arrows and capitals (A-G).

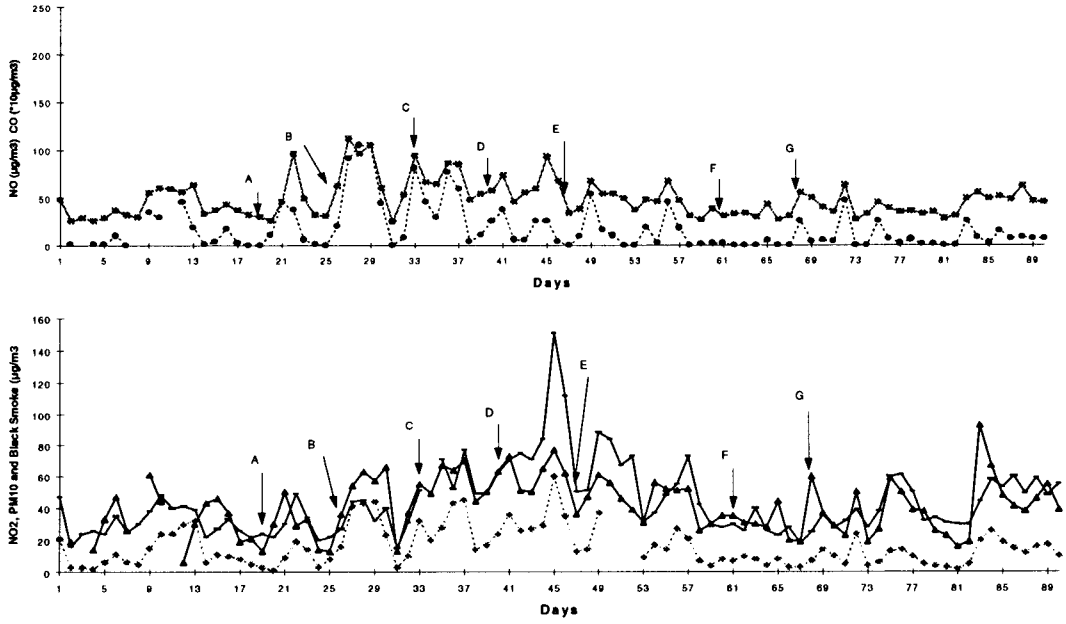

Figure 2. Levels of air pollutants in Bilthoven. Daily ambient CO (■), NO (●), PM10 (-), NO_2 (▲) and Black smoke (◆). Data are presented as mean 24 hour values (12 midnight-12 midnight). Sampling days are marked by arrows and capitals (A-G).

The averaged daily levels of the various components monitored during the study are depicted in Table 2 and show that the mean levels of the monitored air pollutants (except for PM_{10} which was monitored by the same station) were significantly higher in Utrecht than in Bilthoven during the entire period. The ratio of daily mean value of air pollutants over this period (Utrecht versus Bilthoven) was 7.8, 1.5, 1.8 and 2.7 for NO, NO_2, CO and BS, respectively (cf. Table 2). Ambient levels of SO_2 and O_3 remained very low during the study (range of daily means of 0-21 and 2-59, respectively) and were not further used for data analyses. The daily level of NO, CO, NO_2, but not BS, in Utrecht correlated well (r = 0.75-0.82) with those in Bilthoven. Levels of CO were excluded from further analysis as they closely paralleled those of NO (cf. Fig 1 and 2.).

Table 2. Air pollutant levels and ranges of mean daily values in Utrecht (urban) and Bilthoven (suburban) during the study.

air pollutant	Utrecht			Bilthoven			p-value [1]	ratio
$\mu g/m^3$	Mean	Median	Range	Mean	Median	Range		
NO_2	53	53	20-84	41	48	6-93	< 0.01	1.5
NO	46	37	5-206	17	9	0-105	< 0.0001	7.8
CO	8	8	3-23	5	5	3-9	< 0.0001	1.8
BS	29	27	2-105	16	13	1-60	< 0.0001	2.7
SO_2	7	-	1-21	5	-	0-15	< 0.0001	n.d.
O_3	21	-	2-53	28	-	2-59	< 0.05	n.d.

NB.: PM_{10} was measured by one station: mean 44$\mu g/m^3$,median 39 $\mu g/m^3$ range 15-151 $\mu g/m^3$.
[1]Student t-test daily levels Utrecht versus Bilthoven, n.d.: not determined. BS: black smoke; ratio: daily mean ratio.

3.3 Mean levels of parameters Utrecht vs. Bilthoven

Table 3 summarizes the mean value of the parameters measured in NAL fluid, PEF, and exhaled NO. Table 4 depicts the estimated differences in the values of these markers after adjustment for covariables (cf. Methods) and shows that the mean level of IL-8 (32%), urea (39%), uric acid (26%), albumin (15%), and nitrate & nitrite (21%) but not total cell number in moderate exposed children (Utrecht) is significantly (P<0.05) higher compared to low exposed children (Bilthoven).

Table 3. Geometrical means and median values of nasal markers and means for exhaled NO and PEF in Utrecht (U, urban) and Bilthoven (S, suburban).

	Geometrical mean		Median		CV (% within)		CV (% between)	
	U	S	U	S	U	S	U	S
IL-8 (ng/l)	280.4	181.8	290.9	211.5	40	49	55	66
urea (mM)	0.41	0.32	0.41	0.33	26	27	50	38
uric acid (μM)	7.37	6.79	7.96	6.96	32	31	65	56
abumin (mg/l)	79.1	66.9	74.6	64.9	20	15	36	22
cell count (10^5/ml)	1.7	1.3	1.8	1.5	96	100	272	309
NO_2 & NO_3 (μM)	25.2	22.3	25.1	20.9	35	40	54	95
exhaled NO (ppb)	34.3	36.1	27.2	48.5	38	50	79	134
PEF (ml/min)	355.4	358.8	61.3	50.1	7	4	17	14

Table 4. Estimated difference in the levels of inflammatory NAL-markers, exhaled NO and PEF of children living in Utrecht and Bilthoven.

Parameter	% difference[1]	95% Convidence interval	p-value[2]
IL-8	+ 32%	7 - 64%	< 0.05
cell count	- 11%	-38 - 32%	N.S.
urea	+ 39%	22 - 58%	< 0.001
uric acid	+ 26%	1 - 58%	< 0.05
albumin	+ 15%	6 - 24%	< 0.001
NO_2 & NO_3	+ 21%	4 - 41%	< 0.05
exhaled NO	+ 8.8 ppb	- 7 - 58 %	N.S.
PEF	- 5.3 ml/min	- 8 - 5 ml/min	N.S.

Data are adjusted for covariables (cf. Methods). [1]Values in Bilthoven as compared to Utrecht; [2] Student t-test Utrecht versus Bilthoven; N.S.: not significant.

In addition, it was observed that uric acid and IL-8 levels were significantly higher in children who were coughing and significantly lower albumin levels were observed in children who were coughing or whom parents smoke 10 cigarettes or more per day (not shown). However, children of parents smoking less than 10 cigarettes per day show a small but significant increase in albumin level. Nasal lavage of boys contained significantly less cells than nasal lavage fluid of girls, while NAL-fluid of children living in houses where more than 10 cigarettes per day are smoked contained more cells and higher levels of albumin. Smoking was related to decreased levels of NO-metabolites (not shown).

Mean exhaled NO level was 35 ± 39 ppb. Boys exhaled higher levels of NO than girls (boys: 40 ± 46; girls: 26 ± 16) but the difference was not statistically significant. In high exposed children the mean value of PEF and exhaled NO was slightly lower respectively higher as compared to low exposed children but the difference was statistically not significant (cf. Table 4). Having a cold, including runny nose on the day of sampling did not affect any of the parameter measured in all subjects (not shown).

3.4 Response to air pollution

3.4.1 Peak flow results

The presence of an interaction term in statistical analysis for PEF indicated that high exposed children responded more pronounced to increased air pollution as compared to low exposed children: an additional decrease in PEF of about 0.3 ml/min per $\mu g/m^3$ pollutant was observed in childen living in Utrecht. Table 5 shows that a significant decrease in PEF-values was found in childen living in Utrecht following increased levels of PM_{10} (lag 1 and 3), BS (lag 3), NO_2 (sampling time, lag 1 and week) and for NO (lag 3) ranging from -11 to -33 ml/min (3% to 9%) while in Bilthoven no associations were observed.

Table 5. Associations between PEF (ML/MIN), NO exhaled (PPB) and odds ratios for inflammatory nasal markers and air pollutants calculated per range pollutant in (U)trecht and (B)ilthoven.

	PEF (ml/min)		NO exhaled (ppb)		Cell count		IL8		Albumin		Uric acid		Urea		Nitrite & nitrate	
	U	B	U	B	U	B	U	B	U	B	U	B	U	B	U	B
PM10 mt	-22	17	28*	6	0.68	0.90	1.25	0.83	1.12	1.09	1.22	0.92	1.36*	0.79	0.83	0.66
PM10 L1	-13*	3	5*	1	0.97	1.24	1.00	1.48*	1.07	1.05	1.22*	0.84*	1.27*	0.92	1.49*	1.01
PM10 L3	-33*	4	9*	0	1.42	1.45	1.03	1.19	1.12*	1.08	1.27*	0.84	1.34*	0.90	1.05	0.99
PM10 wk	-18	2	3*	0	1.11	1.36	1.01	1.24*	1.06	1.04	1.14*	0.87*	1.19*	0.94	1.19*	1.00
BS mt	-2	2	16*	11*	0.72	1.10	1.32*	0.83	1.00	1.02	1.00	1.06	1.13	0.97	0.91	0.88
BS L1	13	4	10*	1	0.79	1.48	1.07	1.87*	1.04	1.08	1.23*	0.77*	1.34*	0.90	0.97	1.03
BS L3	22*	4	9*	2	0.57	0.80	1.23	0.94	0.98	1.05	0.92	1.04	0.97	0.91	1.02	0.90
BS wk	11	3	7*	3*	0.89	1.31	1.04	1.09	1.02	1.05	1.08	0.95	1.12*	0.94	0.88	0.94
NO2 mt	23*	7	3	-4	0.68	0.94	1.01	0.82	0.95	1.00	0.87*	0.83	0.85*	0.85	1.06	0.77
NO2 L1	-19*	4	8*	1	0.96	1.52	1.08	1.12	1.09	1.05	1.22*	0.82*	1.27*	0.91	1.06	0.92
NO2 L3	0	6	11*	4	0.70	0.85	1.05	0.94	1.04	1.04	1.11	0.99	1.14*	0.91	1.20	0.89
NO2 wk	-11*	4	8*	2	0.89	1.19	1.05	1.02	1.03	1.03	1.11*	0.90*	1.14*	0.93	1.03	0.93
NO mt #	1	0	10*	9*	0.96	1.04	1.14*	0.89	0.97	1.01	0.96	1.04	1.08*	100	1.05	0.93
NO L1	0	24	25*	29	0.48	1.95	1.86*	0.33	0.94	1.27	0.79	1.60	0.90	0.49	1.17	0.30
NO L3	-16*	6	16*	4	0.85	0.89	1.27*	0.86	1.03	1.05	1.08	1.08	1.14*	0.87	1.28*	0.82
NO wk	-2	2	6*	7*	0.89	1.19	1.15*	0.88	1.00	1.03	1.00	1.04	1.07	1.00	1.03	0.91

Ranges for PM_{10} and NO: 100 $\mu g/m^3$(mt and lags), 50 $\mu g/m^3$ for week BS and NO_2: 50 $\mu g/m^3$ for measurements of mt and lags; 25 $\mu g/m^3$ for week. Abbreviations: mt: sampling time from 8:00 to 11:00; L1: lag 1; L3: lag 3 (3 days) and wk: week. #: level at location of sampling. *: $p < 0.05$.

3.4.2 NO in exhaled air

Statistical analysis also showed an interaction term for exhaled NO. As depicted in Table 5 high exposed children exhaled more endogenous NO in response to increased air pollution as compared to low exposed children (3-28 ppb increase in exhaled NO per $\mu g/m^3$ pollutant). In Utrecht significant increase in exhaled NO was noted following increased exposure to PM_{10} (sampling time, lag 1, 3, and week), BS (sampling time, lag 1, 3 and week), NO_2 (lag 1, 3, and week) and ambient NO (sampling time, lag 1, 3 and week). In Bilthoven positive associations were only noted between exhaled NO and PM_{10} (sampling time), BS (sampling time and week) and NO (sampling time and week).

3.4.3 NAL-parameters

In contrast to values of exhaled NO and PEF, the values of NAL-parameters were log-normally distributed and therefore results are expressed as odd ratios (cf. Table 5). Levels of air pollution were not associated with total cell count and albumin level. The effect of air pollution on IL-8 in NAL depends on the location. Positive associations were observed between IL-8 and BS (sampling time) and NO (sampling time and all time lags) in Utrecht and PM_{10} (lag 1, week) and BS (lag 1) in Bilthoven.

The effect of air pollution on uric acid depends on the school location, as well. In Utrecht significant associations between air pollutants and uric acid were observed that were positive for PM_{10} (lag 1, 3 and week), BS (lag 1) and NO_2 (lag 1 and week) and negative for NO_2 (sampling time). Negative associations are also observed in Bilthoven for PM_{10} (lag 1 and week), black smoke (lag 1) and NO_2 (lag 1 and week). Associations between levels of urea and air pollutants were noted in Utrecht but not in Bilthoven.

Compared to childen living in Bilthoven, levels of NO-metabolites were significantly more increased in high exposed children in response to exposure to PM_{10} (lag 1 and week), NO_2 (lag 3) and NO (lag 3).

Mixed regression analysis finally showed that IL-8 is associated with total cell count, albumin, uric acid, urea and NO-metabolites. Further exhaled NO is positively associated to IL-8, and NO-metabolites while PEF was not associated with any of the other parameters (Table 6).

Table 6. Regression coefficients of associations between different biomarkers.

Predicted	IL-8	PEF	NO_2 & NO_3	urea	uric acid	albumin	cell/ml
NO exhaled	2.46 *	0.08	3.64*	-3.20	- 3.53	3.97#	1.31
IL-8		0.00	0.13*	0.60*	0.31*	1.92*	0.18*
PEF			- 0.52	4.34	3.90	- 5.67	- 0.06
NO_2 & NO_3				0.48*	0.32*	0.17*	0.00
urea					0.56*	0.87*	0.04*
uric acid						0.63*	0.02*
albumin							0.07*

Values of nasal markers were log-transformed. * $p < 0.05$, # $p < 0.10$

4. Discussion

In the longitudinal study described herein it was investigated whether traffic related air pollution induced changes in PEF, exhaled NO and biochemical markers in nasal lavage (IL-8, albumin, uric acid, urea and NO-metabolites). To our knowledge this is the first time that adverse effects of air pollution are characterized via three different methods (PEF, exhaled NO and inflammatory NAL-parameters).

To assess the effect of different ambient background levels on the relation between air pollution and health outcomes, two groups of children visiting two different schools (Utrecht, urban, moderate exposed versus Bilthoven, suburban, low exposed) were compared. Both locations differed regarding levels of traffic related air pollution. In Utrecht the yearly mean level of BS and the two month daily mean ratio of NO, NO_2, CO and BS were 1.5 to 7.8 times higher than in Bilthoven and we hypothesize that the difference in previous and actual exposure to air pollution is reflected in children by their value of PEF, exhaled NO and NAL-markers.
Indeed the levels of all NAL-markers (except total cell count) were higher in moderate exposed children as compared to low exposed children ($p< 0.05$). In addition, the levels of the NAL-markers were closely associated with each other (cf. Table 5).

Between the two locations the mean value of PEF and exhaled NO on the seven sampling days was not statistically significant. This may be due to the large inter-individual coefficient of variance of exhaled NO of 79 and 134% in moderate and low exposed children, respectively (cf. Table 3). Using mixed linear regression showed, we however show, that moderate exposed children reacted to an episode of increased air pollution by a more pronounced response regarding PEF, exhaled NO and most NAL-markers. In addition, pollutant mixtures characterized by PM_{10}, NO_2 and NO decreased PEF by 3-9% in moderate exposed children while no effect was observed in low exposed children indicating a difference in response to exposure to increased air pollution. Apparently, children living in urban environment regularly exposed to air pollution respond to episodes of air pollution by a larger impairment of lung function as compared to children living in a suburban environment. This observation is in agreement with previous studies (12,20-22) where exposure to PM_{10} and NO_2 leads to a decrease in PEF-value in a-symptomatic (21) respectively asthmatic children (20). Others (12) observed only a non-significant decrease in PEF-value following exposure of asthmatics to O_3 and BS. In asthmatics lung function is decreased by NO_2, SO_2 and acid aerosols while only O_3 and PM10 were shown to affect lung function in healthy adults and children (1). It is difficult to draw conclusions about the pollutant responsible for the health effects because each pollutant represents a mixture of polluting substances, but our study shows that, besides PM_{10}, BS, NO_2 and NO might play a role. Secondly, it cannot be excluded that the more pronounced responses observed in Utrecht are induced by polluting components in the urban environment which have not been monitored.

Exposure to air pollution increased the level of exhaled NO both in Utrecht and Bilthoven (up to 25 ppb increase in NO exhaled levels following exposure to 100 $\mu g/m^3$ NO in ambient air). The results were consistent for all pollutants in Utrecht whereas in Bilthoven associations were only found occasionally for BS and ambient NO. This is in agreement with an earlier study which showed that an increase of 120 $\mu g/m^3$ NO in ambient air at sampling time led to approximately 20 ppb increase in endogenously produced NO (13,23). Atopic children (allergic for pollen and/or house dust mite and/or pets) exhaled higher levels of endogenously produced NO than non-atopics which confirms early conclusions. (24).

Exposure to air pollution induces significant and persistent nasal epithelial alterations in healthy subjects (25) and it was reported that ozone increased albumin, uric acid, PMN's and IL-8 in NAL fluid (11). Level of the inflammation marker IL-8 was increased in NAL of compost workers with labor related acute and (sub-)chronic airways inflammation (26) and asthmatics following exposure to O_3 but not PM_{10} (27). Present results show significant increases in IL-8 following exposure to PM_{10} and BS in Bilthoven and to NO and BS in Utrecht. The mean levels of IL-8 were in Utrecht, however, significantly higher than in Bilthoven which may be caused by the prolonged exposure to high levels of air pollution. IL-8 challenge in the nasal mucosa in atopic and non-atopic subjects is known to induce a significant increase in neutrophil and eosinophil infiltration (28). Although in our study increased levels of IL-8 did not parallel the increase of cells in NAL for the separate areas, IL-8 increase was also associated with the increase of cells if both populations were taken together.

Urea and albumin are exudate markers and uric acid is an important scavenger of oxidants in the lining fluid of the respiratory tract. Uric acid in NAL has been shown to be increased by ozone (10,29). In addition, in Utrecht the level of urea and uric acid are increased following exposure to various air pollutants, especially PM_{10} and NO_2 while in Bilthoven predominantly negative associations were observed. Small changes in permeability of membranes due to inflammation or irritation may facilitate efflux of relatively small molecules, such as urea and uric acid while larger changes may also increase the efflux of macromolecules, such as albumin (16). The increase in uric acid and urea but not albumin suggests that air pollution induced only a mild inflammatory reaction in high but not low exposed children.

Because of its hydrophobic nature it is not likely that ambient NO is absorbed in the nose causing an increase in the level of nitrate & nitrite in NAL. Irritation of nasal mucosa may induce local production and release of endogenous NO which is rapidly metabolized to nitrite and nitrate. The increase in nasal NO-metabolites induced by exposure to air pollution was associated with the increase of other inflammation markers, like IL-8, indicating that increased NO-production may be considered as an inflammatory response. Likewise the increase in exhaled NO may reflect a mild inflammatory response in the lower airways induced by exposure to air pollution. This assumption is in agreement with the observed increase in exhaled NO in patients with asthma, a disease known to retain an inflammatory component. Finally, NO may shift the Th1/Th2 balance in favor of Th2 (30-32), which facilitates the establishment of allergy. As such, children living in an urban environment may become more prone to allergic reactions.

Comparison of PEF with exhaled NO and NAL-parameters learns that these parameters are not inter-related i.e. there is no association between PEF and the other parameters. This suggests that the effects of air pollution on lung function are independent from inflammatory responses. Compared to other studies (21,33,34) we have shown that the use of exhaled NO and nasal inflammatory biomarkers give additional information on the mechanism of the adverse effects of traffic related air pollution.

In conclusion, children living in an area with moderate levels of of traffic related air pollution show increased levels of inflammatory nasal markers and show an increased response in PEF, exhaled NO and inflammatory nasal markers to several indicators of traffic related air pollution. The clinical implication of these findings remains to be established.

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