ORIGINAL ARTICLE

Intrauterine and early postnatal exposure to outdoor air pollution and lung function at preschool age

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ABSTRACT

Background Effects of prenatal and postnatal exposure to air pollution on lung function at preschool age remain unexplored. We examined the association of exposure to air pollution during specific trimesters of pregnancy and postnatal life with lung function in preschoolers.

Methods Lung function was assessed with spirometry in preschoolers aged 4.5 years (n=620) participating in the INfancia y Medio Ambiente (INMA) cohort. Temporally adjusted land use regression (LUR) models were applied to estimate individual residential exposures to benzene and nitrogen dioxide (NO_2) during specific trimesters of pregnancy and early postnatal life (the first year of life). Recent and current (1 year and 1 week before lung function testing, respectively) exposures to NO_2 and nitrogen oxides (NO_x) were also assessed.

Results Exposure to higher levels of benzene and NO_2 during pregnancy was associated with reduced lung function. FEV_1 estimates for an IQR increase in exposures during the second trimester of pregnancy were −18.4 mL, 95% CI −34.8 to −2.1 for benzene and −28.0 mL, 95% CI −52.9 to −3.2 for NO_2. Relative risk (RR) of low lung function (<80% of predicted FEV_1) for an IQR increase in benzene and NO_2 during the second trimester of pregnancy were 1.22, 95% CI 1.02 to 1.46 and 1.30, 95% CI 0.97 to 1.76, respectively. Associations for early postnatal, recent and current exposures were not statistically significant. Stronger associations appeared among allergic children and those of lower social class.

Conclusions Prenatal exposure to residential traffic-related air pollution may result in long-term lung function deficits at preschool age.

Key messages

What is the key question?

- Does exposure to outdoor air pollution during the prenatal and the early postnatal period impact lung function at preschool age?

What is the bottom line?

- Exposure to higher levels of benzene and NO_2 during the second trimester of pregnancy was associated with clinically relevant deficits in lung function at preschool age.

Why read on?

- This is the first prospective population-based study evaluating the impact of air pollution acting through different windows of susceptibility for lung development including specific trimesters of pregnancy and first years of life on lung function at preschool age.

INTRODUCTION

Adverse effects of air pollutants on lung function in school-age children and adolescents have been extensively highlighted in both cross-sectional[1–4] and longitudinal studies.[5–9] However, susceptibility exposure windows during *in utero* lung development and impact on preschool lung function remain unexplored. In humans, respiratory airways development occurs during the second and third trimesters of pregnancy and continues until 3 years of age.[10 11] During these early stages of development and rapid growth, immature lungs may be highly vulnerable to permanent harmful effects of environmental factors including air pollutants.[12 13]

Preschool children represent one of the major challenges in lung function assessment; however, evaluating lung function in this age group is important for clinical reasons and also due to the considerable growth and development of the respiratory system that occurs. To date, few studies have assessed lung function at preschool age—most of them assessing airway resistance—in relation to exposure to air pollution early in life with inconsistent results.[7 8] Furthermore, very little work has been done on assessing the impact of exposure to air pollution during the prenatal period on lung function later in life. Only a small study conducted among 176 preschoolers of non-smoking mothers showed significant deficits in spirometric lung function parameters at age 5 years in relation to higher 48 h personal measurements of fine particulate matter during pregnancy.[14]

The limited epidemiological evidence on prenatal and early postnatal exposure to air pollutants on lung function effects warrants further investigation to understand the full impact of air pollution on lung development and growth. Furthermore, new evidence on adverse effects of air pollution exposure on lung function at preschool age, a more objective measurement, will support previous findings on associations of air pollution with subjectively reported respiratory symptoms.[15] Here, we

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aimed to examine the associations between exposure to outdoor air pollution during specific trimesters of pregnancy and postnatal life with lung function at preschool age.

METHODS

Study population

The INfancia y Medio Ambiente (INMA) Project is a population-based mother–child cohort study set up in several geographic areas in Spain.[16] For the present study, data came from two areas of study: Sabadell and Gipuzkoa. Pregnant women (n=1295, 657 from Sabadell and 638 from Gipuzkoa) were recruited at their first routine antenatal care visit in the public health centre or referral hospital, from 2004 to 2008. Inclusion criteria were: ≥16 years of age, intention to deliver at the reference hospital, no problems of communication, singleton pregnancy, and no assisted conception. A total of 1175 (91%) children had available data on exposure to air pollution, and were eligible (602 from Sabadell and 573 from Gipuzkoa) (see online supplementary figure S1). The study was approved by the ethical committees of the centres involved in the study, and written informed consent was obtained from the parents of all children.

Residential air pollution exposure assessment in pregnancy and postnatal lifetime periods

We developed area-specific land use regression (LUR) models of benzene and nitrogen dioxide (NO_2) to estimate residential-based exposures during specific trimesters of pregnancy and early postnatal life (during the first year of life) as previously described.[15 17] Ambient levels of benzene and NO_2 were measured with passive samplers (Radiello, Fundazione Salvatore Maugeri, Padua, Italy) distributed over the study areas according to geographic criteria, taking into account the expected pollution gradients and the distribution of the residences of the women. The samplers remained exposed during several periods of 1 week each as previously described.[18 19] Further information is given in online supplementary table S1. Measurements taken in the different sampling campaigns were averaged to represent annual mean levels in each study area. Potential predictor variables, such as traffic indicators, surrounding land use, topography, and population density were derived in the geographic information system (GIS) ArcGIS 9.1 (ESRI, Redlands, California, USA). Multiple linear regression models were built using a supervised forward stepwise procedure. Traffic-related variables, altitude and land uses (urban, industrial, or agricultural), were the main predictor variables in the final LUR models. Models explained 75% and 51% of the variability in measured NO_2 levels, and 72% and 44% of the variability in measured benzene levels, depending on the study area (see online supplementary table S1). Residential addresses were geocoded (Sabadell area n=608 and Gipuzkoa area n=573) using mapping applications from the regional governments. LUR models were applied to predict outdoor levels of both pollutants at the residential addresses. NO_2 estimates were temporally adjusted by using the daily NO_2 levels obtained from the monitoring network stations covering the study areas. Due to the lack of benzene measurements in many stations, and high missing data in those stations measuring benzene, we used the pollutant that exhibited the highest correlation with benzene for temporal adjustment (see online supplementary table S1), as in previous studies.[15 17 18] For women and infants who changed their residential address during the study period, we calculated an average exposure estimate weighted by the time spent at each residence. We derived individual exposures to benzene and NO_2 during pregnancy by multiplying the LUR estimate by the ratio between the average concentrations measured at the fixed stations over the woman's pregnancy period and over the whole air pollution sampling period. We applied the same procedure to estimate exposures during each trimester of pregnancy and the first year of life.

To assess recent and current individual exposure to outdoor air pollution, we estimated NO_2 and nitrogen oxides (NO_X) levels at home addresses using LUR modelling developed within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project framework.[20 21] Briefly, home addresses of participants were geocoded at postal address including residential changes from pregnancy to age 4.5 years. Information on exact dates when participants switched address was also collected. LUR models were based on real air pollution measurements spread out within the study area together with GIS variables on traffic, population density, land use, elevation and topography to predict annual concentration levels at unmeasured locations. Data from routine monitoring stations were used to temporally adjust the long-term exposures for each address to the exactly temporal window desired. We estimated the spatio-temporal exposure at each address and period for which each participant lived in. Recent and current exposure to air pollution were estimated as average of temporally adjusted spatial exposure at children's current address during 1 year and 1 week, respectively, before the lung function testing.

Lung function assessment

At age 4.5 years, 967 children with prenatal air pollution assessment were invited for lung function testing, and 817 (84%) participated. A trained nurse performed the pulmonary function tests. Spirometry test was performed by using a portable spirometer (EasyOne, NDD Medical Technologies, Zürich, Switzerland) with computerised data acquisition software in a portable computer after regular calibration. Lung function was measured according to American Thoracic Society and European Respiratory Society guidelines.[22] A total of 197 children had no technically acceptable testing and were excluded. Finally, 620 children had at least 1 acceptable manoeuvre and were eligible. The following lung function parameters were investigated: FVC, mL, FEV_1, mL, forced expiratory flow between 25% and 75% of FVC ($FEF_{25–75}$, mL/s) and peak expiratory flow (PEF, mL/s). The best FVC and best FEV_1 were recorded, whereas $FEF_{25–75}$ and PEF were derived from the best curve, defined as the greatest sum of FVC and FEV_1. A reproducible test was defined as FVC and FEV_1 agreeing within 100 mL between the best two blows (n=378, 46%).[22] Percent-predicted lung function parameters were calculated adopting the European Respiratory Society Global Lung Function Initiative 2012 prediction equations.[23]

Potential confounders and effect modifiers

Based on previous knowledge, the following variables were considered *a priori* in the analyses: child' sex, child's age, height and weight at assessment, child's ethnic background (white children vs other children), birth weight, preterm delivery (<37 weeks of gestation), older siblings at birth, day-care attendance during the first year of life, maternal age at birth, parity (0 vs 1 or more), maternal educational level (primary or less, secondary, university degree), maternal social class (occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system) (I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled), maternal prepregnancy Body Mass Index

based on height and prepregnancy self-reported weight (kilograms per square metre, kg/m^2), maternal and paternal smoking in pregnancy (yes vs no), postnatal environmental tobacco smoke (ETS) exposure during the first year of life and during the last 12 months (yes vs no), duration of breastfeeding (0, <16, 16–24 and >24 weeks), type of cooker at home (electric vs gas), pets and dampness at home, and lower respiratory tract infections (LRTI) during the first year of life.

Child's sex, child and parental allergic history, and current wheezing and asthma at the time of lung function assessment were evaluated as potential effect modifiers. Children and parents were considered as allergic if they reported to suffer from allergic asthma, atopic dermatitis, eczema or allergic rhinitis. We classified children as having current wheezing based on a positive answer to 'Has ever your child experienced whistling or wheezing from the chest, but not noisy breathing from the nose in the last 12 months?' Current asthma at the time of lung assessment was defined as a positive answer to either to 'Has a doctor ever diagnosed your child with asthma?' or 'Has ever your child used medication for wheezing during the last 12 months?'

Statistical analysis

Linearity of dose-response relationship between levels of air pollutants and lung function parameters was assessed using adjusted generalised additive models by graphical examination and likelihood ratio testing. Separate multiple linear regression models were run to estimate the associations between levels of residential air pollutants during each specific trimester of pregnancy, early postnatal (during the first year of life), and recent and current exposures with lung function parameters (ie, FVC, FEV_1, PEF and $FEF_{25–75}$) at age 4.5 years. Base models were adjusted for area of study, child's sex, and child's age, height and weight at assessment, and ethnic background. Fully adjusted models further included all variables that had at least marginally significant association with air pollutant levels ($p<0.1$) or modified the coefficient of air pollutant levels by at least 5%. Moreover, multiple log-binomial regressions were conducted to estimate associations between levels of air pollutants and clinically low lung function, defined as FEV_1 <80% of the predicted value. The measures of associations are presented as the mean difference (mL) in each lung function parameter (linear regression) or the relative risk (RR) for clinically low FEV_1 (log-binomial regression), with 95% CIs, for an IQR increase (difference between 25th and 75th percentile) in exposure, to be able to compare the effect of pollutants on lung function. We also estimated lung function changes for a given increase in exposure (1 μg/m^3 for benzene and 10 μg/m^3 for NO_2). Analyses were conducted by using Stata software, V.12.0 (Stata-Corp, College Station, Texas, USA).

RESULTS

From the 1295 women enrolled in the study at the beginning of pregnancy, we obtained data on exposure to both air pollution and lung function assessment at 4.5 years for 620 (48%) of their children (see online supplementary figure S1). Descriptive statistics of the study population, and distributions of lung function parameters are presented in tables 1 and 2, respectively. Lung function parameters at age 4.5 years did not differ between areas of study (all p values >0.35). Compared with excluded participants, mothers of those who were included in the present analysis were older and had higher social class and education level, and children showed higher day-care attendance in the first year of life and higher prevalence of LRTI and wheezing in infancy, but did not differ in other main baseline characteristics (see online supplementary table S2).

Table 3 shows the distributions of intrauterine and postnatal exposure to residential air pollutants. Prenatal and postnatal levels of NO_2 and NO_x were higher in the predominantly urban Sabadell area than in the Gipuzkoa area (see online supplementary table S3). Levels of each pollutant were moderately to highly correlated between trimesters of pregnancy (Pearson coefficients 0.73–0.82), and highly correlated between the entire prenatal period and the first year of life (Pearson coefficient=0.84 for benzene and 0.93 for NO_2) (see online supplementary ables S4 and S5). Benzene and NO_2 were moderately correlated (Pearson coefficients 0.25–0.55).

A linear inverse relationship was found between residential levels of benzene and NO_2 during pregnancy and parameters in spirometry at age 4.5 years (figure 1). Exposure to higher levels of benzene and NO_2 in pregnancy was associated with reduced lung function parameters in spirometry (table 4). FEV_1 estimates for an IQR increase in exposure during the second trimester of pregnancy were −18.4 mL, 95% CI −34.8 to −2.1 for benzene; and −28.0 mL, 95% CI −52.9 to −3.2 for NO_2. Similar estimates were found using temporally unadjusted air pollutant levels, although statistical significance was weaker (see online supplementary table S6). Estimates for benzene were similar between areas of study, while estimates for NO_2 were stronger in Gipuzkoa than in Sabadell area (see online supplementary table S7). Although levels of air pollutants during the first year of life were inversely associated with parameters in spirometry at age 4.5 years the estimates were slightly weaker and not statistically significant (table 4). A 1 μg/m^3 increase in benzene and a 10 μg/m^3 increase in NO_2 exposure during pregnancy were associated with significant deficits in FEV_1 at age 4.5 years (estimates for exposures during the second trimester were −51.9 mL, 95% CI −97.9 to −5.9 for benzene; and −17.4 mL, 95% CI −32.8 to −2.0 for NO_2) (see online supplementary table S8). Deficits in average lung function associated with higher levels of exposure to benzene and NO_2 in pregnancy translated into deficits in percent-predicted lung function estimates. An IQR increase in benzene and NO_2 exposure during the second trimester of pregnancy were associated with a decrease in the percent-predicted FEV_1 by 1.6% (95% CI −3.2 to 0.0) and 2.7% (95% CI −5.1 to −0.3), respectively (see online supplementary table S8). Recent and current exposures to residential air pollution levels of NO_2 and NO_x were not associated with significant deficits in lung function (table 5).

Moreover, risk of clinically low lung function (<80% of predicted FEV_1) increased with exposure to higher levels of benzene and NO_2 during pregnancy. RR of low lung function for an IQR increase in benzene and NO_2 during the second trimester were 1.22, 95% CI 1.02 to 1.46 and 1.30, 95% CI 0.97 to 1.76, respectively (table 6).

After restricting the analyses to children with reproducible spirometry manoeuvres, estimates for the associations between levels of air pollutants and lung function were essentially the same, although statistical significance was attenuated (see online supplementary table S10). In stratified analyses, we did not find any evidence of different associations for girls and boys. Associations of levels of NO_2 during pregnancy tended to be stronger in girls than in boys, but none of the interaction terms were statistically significant (see online supplementary table S11). No differences of the association between levels of air pollutants and lung function parameters were found according to allergic parental status (see online supplementary table S12) and child's asthmatic status (see online supplementary table S13).

Table 1 Description of study population characteristics

Variables	n	Children eligible at birth (n=1175)	n	Study population (n=620)
Male sex, n (%)	1172	591 (50.3)	620	323 (52.1)
Age at assessment (years.), mean (SD)	847	4.5 (0.16)	611	4.5 (0.2)
Height at assessment (cm), mean (SD)	845	106.2 (4.4)	611	106.2 (4.3)
Weight at assessment (kg), mean (SD)	846	18.3 (2.6)	611	18.4 (2.7)
Ethnic background, n (%)	1165		616	
White		1118 (96.0)		600 (97.4)
Others		47 (4.0)		16 (2.6)
Birth weight, mean (SD)	1163	3272.2 (447.5)	618	3266.4 (426.9)
Preterm (<37 weeks), yes n (%)	1163	40 (3.4)	613	17 (2.8)
Siblings at birth, yes n (%)	1175	511 (43.5)	620	254 (41.0)
Day-care attendance, yes n (%)	1046	404 (38.6)	603	251 (41.6)
Maternal age at birth, mean (SD)	1174	30.8 (4.0)	619	31.1 (3.7)
Parity	1173		618	
0		648 (55.2)		358 (57.9)
1+		525 (44.8)		260 (42.1)
Maternal education level, n (%)	1170		617	
Primary or less		247 (21.1)		117 (19.0)
Secondary		452 (38.6)		226 (36.6)
University		471 (40.3)		274 (44.4)
Maternal social class, n (%)	1175		620	
I+II (high)		295 (25.1)		177 (28.6)
III (mid)		336 (28.6)		194 (31.3)
IV+V (low)		544 (46.3)		249 (40.1)
Maternal prepregnancy BMI, (kg/m^2) n (%)	1175		620	
<18.5		55 (4.7)		31 (5.0)
18.5–25		846 (72.0)		455 (73.4)
25–30		197 (16.8)		94 (15.2)
>30		77 (6.5)		40 (6.5)
Maternal smoking in pregnancy, yes n (%)	1141	162 (14.2)	604	85 (14.1)
Father smoking in pregnancy, yes n (%)	1144	399 (34.9)	606	204 (33.7)
ETS				
0–14 months, yes n (%)	1041	155 (14.9)	598	91 (15.2)
36–48 months, yes n (%)	865	408 (47.2)	608	288 (47.4)
Maternal allergic history*, yes n (%)	1174	333 (28.4)	619	178 (28.8)
Father allergic history*, yes n (%)	1174	279 (23.8)	619	160 (25.8)
Predominant breastfeeding (wks), n (%)	1044		589	
0		202 (19.4)		105 (17.8)
>0–16		337 (32.3)		199 (33.8)
16–24		386 (37.0)		213 (36.2)
>24		119 (11.4)		72 (12.2)
Type of cooker in the home at 14 months, n (%)	1145		607	
Gas (natural gas or butane)		424 (37.0)		224 (36.9)
Electric		708 (61.8)		375 (61.8)
Others		13 (1.1)		8 (1.3)
Pets at home at 14 months, yes n (%)	1045	260 (24.9)	602	156 (25.9)
Dampness at home at 14 months, yes n (%)	1047	89 (8.5)	603	43 (7.1)
LRTI from birth to 14 months, yes n (%)	1127	415 (36.8)	620	250 (40.3)
Wheezing from birth to 14 months, yes n (%)	1128	381 (33.8)	620	225 (36.3)
Current† wheezing at 4 years, yes n (%)	871	155 (17.8)	613	121 (19.7)
Medication use for wheezing at 4 years, yes n (%)	841	100 (11.9)	596	79 (13.3)
Current‡ asthma at 4 years, yes n (%)	836	116 (13.9)	592	92 (15.5)
Allergic status at age 4 years*, yes n (%)	802	202 (25.2)	569	147 (25.8)

*Suffering from allergic asthma, atopic dermatitis, eczema or allergic rhinitis.
†During the last 12 months.
‡Doctor-diagnosis of asthma or medication use for wheezing at 4 years of age.
BMI, Body Mass Index; ETS, environmental tobacco smoke; LRTI, lower respiratory tract infections.

Table 2 Descriptive lung function parameters in preschoolers aged 4.5 years (n=967)

	Area of study	
	Sabadell n=495	Gipuzkoa n=472
Spirometry		
No	62 (12.5)	88 (18.6)
Yes	433 (87.5)	384 (81.4)
Children with at least 1 acceptable manoeuvre	332 (67.1)	288 (61.0)
Number of acceptable manoeuvres		
1	69 (20.8)	51 (17.7)
2	84 (25.3)	60 (20.8)
3	118 (35.5)	108 (37.5)
4	15 (4.5)	40 (13.9)
5	19 (5.7)	16 (5.6)
6	12 (3.6)	7 (2.4)
7	9 (2.7)	5 (1.7)
8	6 (1.7)	1 (0.3)
Reproducible testing*	204 (47.2)	174 (45.2)

Summary of measures (mean±SD)	N	Sabadell	N	Gipuzkoa
FVC (mL)	332	1000.0 (217.7)	288	1000.6 (184.9)
Predicted FVC (mL)	329	1076.9 (114.9)	277	1080.0 (101.4)
Predicted FVC (%)	329	93.2 (18.2)	277	92.6 (15.3)
FEV_1 (mL)	332	922.0 (188.5)	288	927.3 (167.2)
Predicted FEV_1 (mL)	329	1003.4 (96.4)	277	1006.6 (86.4)
Predicted FEV_1 (%)	329	92.1 (16.7)	277	92.1 (15.0)
FEV_1/FVC ratio (as %)	332	92.7 (7.3)	288	92.9 (5.8)
Predicted FEV_1/FVC ratio (as %)	329	93.3 (0.01)	277	93.7 (0.8)
PEF (mL/s)	332	2000.8 (493.9)	288	1972.7 (471.9)
$FEF_{25–75}$ (mL/s)	332	1271.9 (372.1)	288	1325.9 (360.6)
Predicted $FEF_{25–75}$ (mL/s)	329	1482.8 (94.6)	277	1485.9 (89.3)
Predicted $FEF_{25–75}$ (%)	329	86.0 (25.1)	277	89.7 (24.1)

*FVC and FEV_1 agreeing within 100 mL between the best two blows.
PEF, peak expiratory flow; FVC, forced vital capacity

However, stronger associations were found among allergic children (see online supplementary table S14). Additionally, estimates were essentially the same after excluding infants whose mothers smoked during pregnancy, preterm deliveries and low birthweight newborns (see online supplementary table S15). Stratification by maternal social class showed stronger associations of air pollutants with FEV_1 among children of mothers of lower social class (classes III–V) compared with those of high social class (classes I–II) (see online supplementary table S16). Similarly, estimates were stronger among children of mothers with low education levels (primary or less and secondary) compared with those with high education levels (university) (see online supplementary table S17).

DISCUSSION

In this population-based prospective study, higher levels of residential outdoor air pollutant (ie, benzene and NO_2) during pregnancy were associated with clinically significant deficits in lung function at preschool age. Associations were robust after adjusting for a large number of potential confounding factors. Associations between early postnatal life (during the first year of life), recent and current exposures to outdoor air pollutants with lung function at preschool age were not statistically significant.

To our knowledge, this is the first study examining effects on lung function as early as at preschool age, in relation to residential exposure to traffic-related air pollutants through different windows of susceptibility including specific trimesters of pregnancy and postnatal lifetime periods. Both, lung volumes (FVC, FEV_1) and flow measures (PEF and $FEF_{25–75}$), showed deficits in relation to higher levels of air pollutants in pregnancy, with stronger associations for the second trimester. FEV_1 is a marker of airway obstruction, and flow measures such as PEF and $FEF_{25–75}$ are considered markers of small-airway function[24 25] which is particularly sensitive to oxidant air pollutants including ozone[26 27] and tobacco smoke.[28] The magnitude of deficits here reported seems plausible and similar to those previous studied, and translated into higher risk of clinically defined low lung

Table 3 Distribution of estimated residential outdoor air pollutants

	n	Minimum	p25	Median	Mean	p75	Maximum
Benzene ($\mu g/m^3$)							
Entire pregnancy	618	0.32	0.66	0.77	0.83	0.96	2.78
First trimester	618	0.23	0.62	0.81	0.83	0.98	2.59
Second trimester	618	0.24	0.62	0.80	0.83	0.98	2.87
Third trimester	618	0.24	0.65	0.81	0.84	0.99	2.90
First year of life	612	0.33	0.70	0.84	0.87	1.01	3.56
NO_2 ($\mu g/m^3$)							
Entire pregnancy	620	5.68	17.40	25.50	25.60	31.66	66.33
First trimester	620	5.59	16.76	24.30	25.80	33.48	76.20
Second trimester	620	5.68	16.96	24.23	25.63	33.10	70.28
Third trimester	620	5.68	16.88	23.87	25.87	33.26	69.15
First year of life	614	7.14	19.84	27.87	27.59	33.59	70.92
Recent exposure*	620	2.55	16.26	24.91	29.25	41.67	91.26
Current exposure†	570	1.95	16.73	26.37	31.78	43.05	136.34
NO_x ($\mu g/m^3$)							
Recent exposure*	620	2.44	31.62	45.81	51.77	68.74	209.51
Current exposure†	570	1.48	29.99	46.96	56.85	67.11	397.73

*One-year average before lung function testing.
†One-week average before lung function testing.
NO_2, nitrogen dioxide; NO_x, nitrogen oxides

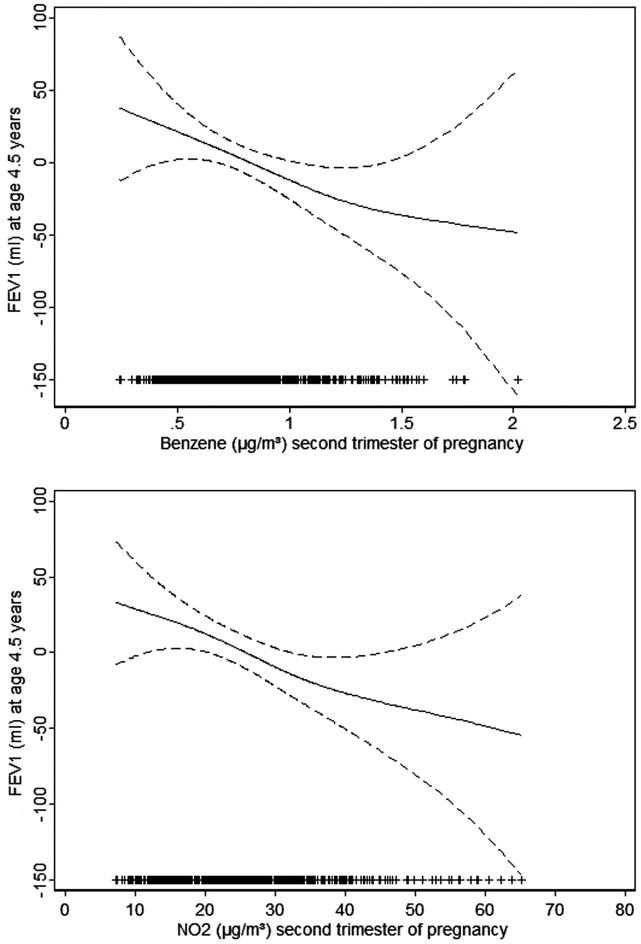

Figure 1 The relation (and 95% confidence levels) of air pollutant levels during the second trimester of pregnancy with FEV_1 in preschoolers aged 4.5 years. General additive models adjusted for area of study, child's sex, and child's age, height and weight at assessment and ethnic background, birth weight, maternal social class, maternal education level, maternal smoking in pregnancy and paternal smoking in pregnancy, environmental tobacco smoke 0–14 months, and lower respiratory tract infections 0–14 months. The symbols (+) on the X-axis indicate air pollutant observations.

function. Gauderman *et al* reported that children aged 10–18 years, and living in the most polluted community, had a growth deficit in FEV_1 of approximately 100 mL (~7% for girls, ~4% for boys), as compared with those living in the cleanest community (exposure range 4–38/ppb NO_2).[5] In a more recent analysis, Gauderman *et al* found that children aged 10 years living within 500 m of a freeway had deficits in 8-year growth of FEV_1 (−81 mL, 95% CI −143 to −18) compared with children living at least 1500 m from a freeway.[6] Rojas-Martinez *et al* reported that NO_2 and O_3 levels were associated with annual growth in FEV_1 in schoolchildren of Mexico City. Decreases in annual growth in FEV_1 per IQR of exposure ranged from −16 mL for O_3 (IQR, 11.3 ppb) in boys to −32 mL for NO_2 (IQR, 12.0 ppb) in girls.[29] Jedrichowski *et al* have shown that exposure to higher levels of $PM_{2.5}$ (>52.6 mg/m^3) during pregnancy was associated with reduced FVC (−91 mL) and FEV_1 (−87 mL) in preschoolers aged 5 years.[14] Despite high correlation between prenatal and postnatal levels of air pollutants, our results suggest that *in utero* exposures and, more specifically during the second trimester of pregnancy, may be more relevant for long-term adverse consequences for lung function than exposures later in life. Our results are in agreement with a previous study that found deficits in offspring lung function at preschool age in relation to maternal short-term exposure to traffic-related air pollutants (ie, $PM_{2.5}$) in the second trimester of pregnancy.[14] Mechanisms underlying the associations of air pollution exposure in pregnancy with reduced lung function in offspring are unknown. Interestingly, respiratory airways development occurs during the second and third trimesters of pregnancy, and continues until 3 years of age.[10 11] Thus, it is biologically plausible that harmful conditions acting during this crucial period of lung development might have more relevant long-lasting pathophysiological consequences in the lung.

Sensitivity analyses showed that associations between exposure to outdoor air pollutants and lung function at preschool age were not confounded by maternal smoking during pregnancy, either mediated by preterm delivery or low birth weight. Additionally, we did not find any evidence of differential effects according to the child's sex, asthmatic status and allergic parental status, although stronger estimates appeared in allergic children as previously suggested.[30] Additionally, we found stronger deficits of lung function in relation to higher levels of air pollutants among preschoolers from middle and low socioeconomic groups, which suggest that socioeconomic status may act as a potential effect modifier of the harmful effects of air pollution on lung function as previously indicated.[31] Although the reasons for these differences are not entirely clear, there are some plausible explanations. Several studies have documented that atopy occurs in close association with bronchial hyperresponsiveness, both in asthma patients and in random population samples, which could act synergistically with traffic-related air pollutants. Lower social class households are more likely to be located in areas of poor air quality and higher traffic exposure, and lower social position may make some groups more susceptible to health threats because of factors related to their disadvantage.

The population-based and prospective design of the study set up as early as the first trimester of pregnancy are main strengths of this study. We investigated the potential effects of exposure to residential air pollution during specific periods of pregnancy, and the first year of life on offspring lung function, to identify susceptible exposure windows early in life. We used temporally adjusted LUR models to estimate individual exposures during specific time periods; despite their spatial accuracy, LUR estimates are still a proxy for personal exposure, which may be influenced by individual time-activity patterns.[32] Additionally, a large number of potential confounding mediators, and effect-modified factors were considered in the analyses.

This study has some limitations. Loss of follow-up may be a potential source of bias; compared to excluded participants, mothers of those who were included in the present analysis were older and had higher social class and education levels, and children showed higher day-care attendance in the first year of life and higher prevalence of LRTI and wheezing in infancy, but did not differ in other main baseline characteristics. While these differences may have some impact on the generalisability of results, it should not affect their internal validity. We did not measure particulate matters considered good markers of traffic-related pollution. However, NO_2 is a widely used marker of traffic-related air pollution, and benzene levels can reflect industrial activities and are considered as a surrogate for a mixture of predominantly traffic-driven pollutants. Air pollution exposures during pregnancy and first year of life tend to be highly correlated, which limits the interpretation of estimates

Table 4 Associations of lung function parameters in preschoolers aged 4.5 years with exposure levels of air pollutants *in utero* and early postnatal (during the first year of life)

	FVC (mL)			FEV_1 (mL)			PEF (mL/s)			$FEF_{25–75}$ (mL/s)		
Exposure	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**
Base model*												
Benzene												
Entire pregnancy (n=605)	−13.4	(−31.7 to 4.8)	0.149	−14.9	(−30.9 to 1.1)	0.069	−26.1	(−71.6 to 19.3)	0.259	−30.1	(−65.9 to 5.7)	0.100
First trimester	−6.8	(−24.6 to 11.0)	0.454	−10.6	(−26.3 to 5.0)	0.182	−27.1	(−71.4 to 17.3)	0.231	−19.9	(−54.9 to 15.1)	0.264
Second trimester	−16.7	(−34.8 to 1.30)	0.069	−18.1	(−33.9 to −2.3)	0.025	−51.1	(−95.9 to −6.2)	0.026	−30.4	(−65.9 to 5.1)	0.093
Third trimester	−13.3	(−31.3 to 4.8)	0.149	−13.2	(−29.1 to 2.6)	0.101	−22.6	(−67.6 to 22.4)	0.324	−24.4	(−59.9 to 11.1)	0.177
First year of life (n=599)	−1.9	(−19.8 to 15.9)	0.832	−7.5	(−23.2 to 8.2)	0.347	−13.5	(−57.9 to 30.9)	0.550	−22.3	(−57.4 to 12.9)	0.214
NO_2												
Entire pregnancy (n=607)	−24.4	(−52.4 to 3.5)	0.087	−21.5	(−46.1 to 3.0)	0.086	−32.6	(−102.3 to 37.2)	0.360	−34.0	(−89.0 to 21.0)	0.225
First trimester	−12.9	(−40.4 to 14.6)	0.358	−13.9	(−38.2 to 10.2)	0.256	−24.9	(−93.5 to 43.7)	0.476	−20.8	(−74.9 to 33.4)	0.452
Second trimester	−29.4	(−56.4 to −2.4)	0.033	−25.7	(−49.4 to −2.0)	0.033	−68.0	(−135.2 to −0.8)	0.047	−32.6	(−85.7 to 20.6)	0.230
Third trimester	−23.1	(−50.8 to 4.6)	0.102	−19.2	(−43.5 to 5.1)	0.120	−26.6	(−95.7 to 42.4)	0.449	−29.1	(−83.6 to 25.3)	0.294
First year of life (n=601)	−10.1	(−36.3 to 16.1)	0.449	−13.7	(−36.7 to 9.3)	0.244	−25.7	(−91.0 to 39.6)	0.440	−32.1	(−83.8 to 19.5)	0.222
Adjusted model†												
Benzene												
Entire pregnancy (n=566)	−15.0	(−33.9 to 4.0)	0.123	−16.3	(−32.9 to 0.2)	0.054	−21.6	(−68.6 to 25.5)	0.368	−32.6	(−70.0 to 4.7)	0.087
First trimester	−10.2	(−28.6 to 8.3)	0.280	−13.4	(−29.5 to 2.7)	0.103	−26.5	(−72.1 to 19.1)	0.254	−22.3	(−58.6 to 13.9)	0.227
Second trimester	−18.0	(−36.7 to 0.7)	0.060	−18.4	(−34.8 to −2.1)	0.027	−45.2	(−91.5 to 1.0)	0.055	−28.8	(−65.7 to 8.1)	0.125
Third trimester	−13.9	(−32.6 to 4.9)	0.147	−13.8	(−30.2 to 2.6)	0.099	−16.1	(−62.5 to 30.4)	0.497	−24.7	(−61.7 to 12.2)	0.188
First year of life (n=560)	−3.2	(−21.7 to 15.2)	0.733	−8.8	(−24.9 to 7.3)	0.283	−8.6	(−54.2 to 36.9)	0.710	−25.0	(−61.3 to 11.3)	0.176
NO_2												
Entire pregnancy (n=567)	−28.9	(−58.5 to 0.6)	0.055	−26.1	(−51.9 to −0.2)	0.048	−26.9	(−100.2 to 46.4)	0.471	−44.8	(−103.0 to 13.5)	0.132
First trimester	−19.8	(−48.7 to 9.0)	0.177	−20.4	(−45.6 to 4.8)	0.113	−25.4	(−96.9 to 46.1)	0.486	−31.3	(−88.1 to 25.6)	0.280
Second trimester	−32.8	(−61.2 to −4.4)	0.024	−28.0	(−52.9 to −3.2)	0.027	−61.6	(−132.0 to 8.8)	0.086	−36.4	(−92.5 to 19.7)	0.203
Third trimester	−25.5	(−54.4 to 3.5)	0.085	−21.9	(−47.2 to 3.4)	0.090	−17.8	(−89.6 to 53.9)	0.626	−35.0	(−92.1 to 22.0)	0.228
First year of life (n=561)	−13.5	(−41.3 to 14.3)	0.342	−18.1	(−42.4 to 6.2)	0.145	−19.7	(−88.5 to 49.1)	0.574	−44.5	(−99.3 to 10.2)	0.111

*Base model adjusted for area of study, child's sex, and child`s age, height and weight at assessment and ethnic background.
†Base model further adjusted for birth weight, maternal social class, maternal education level, maternal smoking in pregnancy and paternal smoking in pregnancy, environmental tobacco smoke 0–14 months, and lower respiratory tract infections 0–14 months.
Coef (95% CI), Coefficient and 95% CI for an IQR increase in exposure estimated by linear regression models.
NO_2, nitrogen dioxide; PEF, peak expiratory flow.

Table 5 Associations of lung function parameters in preschoolers aged 4.5 years with recent and current residential exposure levels of air pollutants

	FVC (mL)			FEV_1 (mL)			PEF (mL/s)			$FEF_{25–75}$ (mLl/s)		
Exposure	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**	**Coef**	**(95% CI)**	**p Value**
Base model*												
NO_2												
Recent exposure† (n=607)	−29.1	(−71.8 to 13.4)	0.179	−27.7	(−65.1 to 9.7)	0.146	11.7	(−94.5 to 117.9)	0.829	−48.0	(−131.8 to 35.7)	0.260
Current exposure‡ (n=561)	−14.4	(−43.7 to 14.9)	0.335	−5.6	(−31.3 to 20.0)	0.666	−8.1	(−80.2 to 64.1)	0.826	4.2	(−53.1 to 61.6)	0.885
NO_x												
Recent exposure† (n=607)	−19.7	(−48.3 to 8.8)	0.175	−16.2	(−41.3 to 8.8)	0.204	14.3	(−57.0 to 85.5)	0.694	−26.9	(−83.2 to 29.2)	0.347
Current exposure‡ (n=561)	−6.4	(−21.3 to 8.5)	0.398	−1.0	(−14.0 to 12.1)	0.885	−12.3	(−48.9 to 24.4)	0.512	1.0	(−28.1 to 30.2)	0.944
Adjusted model§												
NO_2												
Recent exposure† (n=567)	−36.1	(−80.7 to 8.5)	0.113	−32.3	(−71.3 to 6.6)	0.104	−2.6	(−113.1 to 107.9)	0.963	−63.1	(−150.8 to 24.7)	0.159
Current exposure‡ (n=524)	−20.8	(−51.7 to 10.0)	0.186	−9.2	(−36.1 to 17.7)	0.504	−22.7	(−97.9 to 52.4)	0.553	−6.4	(−66.6 to 53.8)	0.835
NO_x												
Recent exposure† (n=567)	−23.9	(−53.6 to 5.7)	0.113	−19.1	(−45.0 to 6.8)	0.148	5.9	(−67.5 to 79.4)	0.874	−35.9	(−94.3 to 22.5)	0.228
Current exposure‡ (n=524)	−8.3	(−23.8 to 7.1)	0.289	−1.8	(−15.3 to 11.6)	0.790	−17.8	(−55.3 to 19.8)	0.353	−2.9	(−33.0 to 27.2)	0.849

*Base model adjusted for area of study, child's sex, and child's age, height and weight at assessment and ethnic background.
†One-year average before lung function testing.
‡One-week average before lung function testing.
§Base model further adjusted for birth weight, maternal social class, maternal education level, maternal smoking in pregnancy and paternal smoking in pregnancy, environmental tobacco smoke 0–14 months, and lower respiratory tract infections 0–14 months.
Coef (95% CI), Coefficient and 95% CI for an IQR increase in exposure estimated by linear regression models.
NO_2, nitrogen dioxide; NO_x, nitrogen oxides; PEF, peak expiratory flow.

Table 6 Risk of low lung function (FEV_1 <80% predicted) in preschoolers aged 4.5 years in relation to exposure levels of air pollutants

Exposure	N low/normal lung function	RR	(95% CI)	p Value
Benzene				
Entire pregnancy	112/453	1.13	(0.93 to 1.36)	0.208
First trimester	112/453	1.10	(0.92 to 1.33)	0.270
Second trimester	112/453	1.22	(1.02 to 1.46)	0.027
Third trimester	112/453	1.15	(0.96 to 1.37)	0.134
First year of life	111/448	1.03	(0.86 to 1.23)	0.768
NO_2				
Entire pregnancy	112/454	1.18	(0.85 to 1.63)	0.324
First trimester	112/454	1.10	(0.81 to 1.48)	0.545
Second trimester	112/454	1.30	(0.97 to 1.76)	0.080
Third trimester	112/454	1.21	(0.89 to 1.64)	0.222
First year of life	111/449	1.01	(0.74 to 1.36)	0.962
Recent exposure*	112/454	1.31	(0.87 to 1.97)	0.193
Current exposure†	108/415	1.13	(0.84 to 1.51)	0.410
NO_x				
Recent exposure*	112/454	1.16	(0.91 to 1.49)	0.230
Current exposure†	108/415	1.05	(0.90 to 1.22)	0.553

All models adjusted for area of study, birth weight, maternal social class, maternal education level, maternal smoking in pregnancy and paternal smoking in pregnancy, environmental tobacco smoke 0–14 months, and lower respiratory tract infections 0–14 months.
*One-year average before lung function testing.
†One-week average before lung function testing.
NO_2, nitrogen dioxide; NO_x, nitrogen oxides; RR (95% CI), Relative Risk and 95% CIs for anIQR increase in exposure estimated by log-binomial regression.

from mutually adjusted models. By contrast with studies that characterise exposures based on measurements from the nearest fixed monitoring stations,[33] our exposure assessment approach emphasised spatial over temporal variation, which may have contributed to the very high correlations between prenatal and early postnatal exposures in our study. A different LUR model was used for the more recent (ESCAPE model) exposures than for the pregnancy and early life exposures (INMA model), which may be difficult for direct comparisons. However, for NO_2, the ESCAPE model performed well at the ESCAPE sites in Sabadell (R^2=0.69), and ESCAPE and INMA-Sabadell model predictions at INMA-Sabadell cohort addresses were relatively well correlated (R^2=0.56).[34] Not all participants were able to perform spirometry testing; although preschool children are able to perform these manoeuvres.[35] Nevertheless, reproducibility rate was nearly 50% in our study, and estimates were essentially the same among participants with reproducible tests. Lack of information on respiratory infection at the current time of lung function testing could have resulted in some residual confounding. Additionally, we cannot exclude potential residual confounding by unmeasured factors including effects of acute recent temperature and maternal occupation exposure to gas, dust or fumes during pregnancy.

In summary, we found that exposure to higher levels of benzene and NO_2 during pregnancy was associated with clinically relevant deficits in lung function at preschool age. Results suggest that exposure to traffic-related air pollutants acting during the prenatal period could adversely impact the developing lung. Public policies to reduce exposure to traffic-related air pollution may avoid harmful effects on lung development and function with substantial public health benefits.

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Contributors JS had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. EM, OAdlC, MB, JS contributed to the study conception and design. RG-E, OAdlC, MB, AL, MDMLdD, CZ acquired the data. EM analysed the data. EM drafted the manuscript. All authors discussed and interpreted the results, and revised the paper.

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