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Air pollution during pregnancy and lung function in newborns: a birth cohort study

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ABSTRACT: Post-natal exposure to air pollution is associated with diminished lung growth during school age. The current authors aimed to determine whether pre-natal exposure to air pollution is associated with lung function changes in the newborn.

In a prospective birth cohort of 241 healthy term-born neonates, tidal breathing, lung volume, ventilation inhomogeneity and exhaled nitric oxide (eNO) were measured during unsedated sleep at age 5 weeks. Maternal exposure to particles with a 50% cut-off aerodynamic diameter of 10 μm (PM_{10}), nitrogen dioxide (NO_2) and ozone (O_3), and distance to major roads were estimated during pregnancy. The association between these exposures and lung function was assessed using linear regression.

Minute ventilation was higher in infants with higher pre-natal PM_{10} exposure (24.9 $mL \cdot min^{-1}$ per $\mu g \cdot m^{-3}$ PM_{10}). The eNO was increased in infants with higher pre-natal NO_2 exposure (0.98 ppb per $\mu g \cdot m^{-3}$ NO_2). Post-natal exposure to air pollution did not modify these findings. No association was found for pre-natal exposure to O_3 and lung function parameters.

The present results suggest that pre-natal exposure to air pollution might be associated with higher respiratory need and airway inflammation in newborns. Such alterations during early lung development may be important regarding long-term respiratory morbidity.

KEYWORDS: Air pollution, exhaled nitric oxide, infant, lung function, lung growth

There is growing evidence that air pollution has adverse effects on lung function and development [1]. Both cross-sectional as well as longitudinal studies have clearly shown diminished lung function in children exposed to higher levels of air pollution [1–4]. A causative association has been suggested in the observation of decreased age-related lung function decline in adults after reduced exposure to air pollution [5]. Moreover, short- and long-term exposure to pollutants has been associated with airway inflammation [6, 7].

Growth and development of the respiratory system take place mainly during the pre-natal and early post-natal periods [8, 9] and adverse effects of pre-natal exposures, such as tobacco smoking of the mother, on lung development are well documented [10]. Although air pollution might possibly lead to comparable developmental changes, no epidemiological studies have examined potential associations between pre-natal air pollution and lung functional development and inflammation or oxidative stress in the newborn [8, 11]. This early developmental phase is thought to be very important in determining long-term lung growth [9]. So-called ''tracking'' of lung function was found in retrospective [12] and prospective cohort studies [13]. Therefore, early changes in lung function may have a considerable impact upon long-term respiratory morbidity and even mortality.

The aim of the present study was, thus, to assess in a prospective birth cohort whether increased maternal exposure to air pollution during pregnancy is associated with changes in tidal breathing, lung volume or airway inflammation, measured during natural sleep in 5-week-old infants.

METHODS

Study design

The current prospective birth cohort study comprised a group of unselected, healthy neonates recruited antenatally since 1999 in the region of Bern, Switzerland [14]. Exclusion criteria for the study were pre-term delivery (<37 weeks) and significant perinatal disease, including respiratory distress and later diagnosis of chronic respiratory disease. Potential risk factors (sociodemographic status, smoke exposure and parental atopic disease) were assessed by interviews using standardised questionnaires. The Ethics Committee of the Region of Bern

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approved the study and written consent was obtained at enrolment.

Lung function

Lung function measurements were performed in unsedated neonates during quiet natural sleep in the supine position with the head mid-aligned, *via* an infant mask (Size 1; Homedica AG, Hünenberg, Switzerland), according to the European Respiratory Society (ERS)/American Thoracic Society (ATS) standards of infant lung function testing [15]. Flow was measured using an ultrasonic flowmeter (Spiroson®; EcoMedics AG, Duernten, Switzerland).

Tidal breathing

For analysis, the first 100 regular breaths of tidal breathing during non-rapid eye movement sleep from the total 10-min recording were used, and sighs and 10 breaths before and after a sigh were excluded. From these, mean tidal breathing parameters of flow, volume and flow–volume loop were calculated according to the ERS/ATS standards for infant lung function testing [15]. The main outcome parameters were minute ventilation (tidal volume multiplied with respiratory rate) and mean tidal inspiratory and expiratory flow [15].

Multiple-breath washout

Lung volume and ventilation inhomogeneity were determined using the multiple-breath washout (MBW) technique by ultrasonic flowmeter as previously described [16]. The main outcomes were functional residual capacity (FRC) at airway opening, FRC per body weight, and lung clearance index.

Nitric oxide

Exhaled nitric oxide (eNO) was measured online with a rapid-response chemiluminescence analyser (CLD 77 AM; EcoMedics AG) in the range of 0–100 ppb. Contamination of eNO by ambient NO was avoided using NO-free air for inspiration. The eNO was measured breath by breath, and mean eNO was calculated over 100 breaths [14]. As eNO is flow dependent, eNO was adjusted for minute ventilation in the multivariable analysis and results are presented for both eNO concentration and NO output (eNO concentration multiplied with corresponding expiratory flow) [14].

Exposure assessment

Air pollution data included daily mean levels of particles with a 50% cut-off aerodynamic diameter of 10 μm (PM_{10}) and nitrogen dioxide (NO_2), as well as the daily maximum of mean hourly levels of ozone (O_3) for the period from January 1999 to July 2007. Air pollution was measured at the monitoring station of Payerne (part of the Swiss National Air Pollution Monitoring Network), which lies within the study area and reflects temporal variability of air pollutants during the study period.

These regional data were used to calculate the mean exposure to each pollutant for each subject during pregnancy, between date of conception and birth date as well as separately for each trimester. In addition, post-natal exposure to air pollution between birth and date of lung function test was calculated. As a proxy for traffic-related air pollution exposure, the distances from the mother's home coordinates to the closest major road of at least 6-m width (first class road) and to that of at least 4-m width (second class road) were computed. This was performed analogously to another Swiss cohort study [17]. Calculations were performed with a geographic information system (GIS; ArcGIS, version 9; Environmental Systems Research Institute, Redlands, CA, USA). Addresses were geo-coded using the building registry of the Swiss Federal Statistical Office (Neuchâtel), and street information was obtained from the VECTOR25 map of the Swiss Federal Office of Topography (Wabern).

Statistical analysis

The association between mean exposure levels of each pollutant during pregnancy and during the post-natal period, as well as distance to 4- and 6-m roads and pulmonary function data, was assessed by linear regression analysis. First, univariable regression analysis was performed for each exposure variable and, secondly, a multivariable model was used, in which sex, post-natal age, season of birth, outdoor temperature on the measurement day and maternal smoking during pregnancy were adjusted for. Season of birth was parameterised using a cosine function that assumed a value of 0 at July 1 and a value of 1 at January 1. Sensitivity analyses that included length and weight at study date, year and month of birth, infectious season, exposure to air pollution during the last 2 days before lung function testing, paternal educational status and maternal asthmatic disease were performed.

RESULTS

Between 1999 and 2007, the study enrolled 241 infants, with data from 221 (87%) used for tidal breathing analysis, 205 (81%) for eNO analysis and 181 (72%) for MBW analysis. Reasons for exclusion were insufficient duration of quiet sleep during lung function testing (n=16), lower respiratory tract infection before the measurement (n=3), technical problems (n=1 for tidal breathing, n=17 for eNO) and strict quality control criteria of MBW (n=41). Anthropometric and lung function data, as well as air pollution exposure data, are given in table 1, and the distribution of possible risk factors is given in table 2. Daily mean values of PM_{10} and smoothed temporal trends are shown in figure 1.

The association between pre-natal exposure to air pollution and lung function at 5 weeks of age is given in table 3 for both the univariable and the adjusted model. Mean PM_{10} exposure during pregnancy was associated with changes in tidal breathing parameters. Each increase of 1 $\mu g \cdot m^{-3}$ PM_{10} was associated with an increase in minute ventilation of 24.9 (95% confidence interval (CI) 9.3–40.5) $mL \cdot min^{-1}$ ($p=0.002$). Similar associations were found between PM_{10} and respiratory rate (fig. 2) and tidal breathing flows, especially the inspiratory flow (table 3). No association was found between air pollution and lung volume or ventilation inhomogeneity assessed by MBW (table 3). The eNO was associated with mean NO_2 exposure (fig. 3), increasing by 0.98 (95% CI 0.45–1.51) ppb per $\mu g \cdot m^{-3}$ higher mean pre-natal NO_2 exposure ($p<0.001$; table 3).

The association between post-natal exposure to air pollution and lung function is given in table 4. There was no consistent association in either the univariable or adjusted model for any of the examined pollutants. Both the associations between PM_{10} and minute ventilation, as well as between NO_2 and eNO, were strongest for exposure during the third trimester of

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TABLE 1 Demographics, lung function data and exposure to air pollution of the study infants

	Median	Interquartile range	Range
Anthropometric data[#]			
Age at study date days	34	32–37	25–55
Weight at study date kg	4.3	4.0–4.8	2.9–6.3
Length at study date cm	55.0	53.3–56.8	48.0–61.5
Gestational age at birth weeks	40.0	39.0–40.9	37.0–42.3
Birthweight kg	3.4	3.1–3.7	2.2–4.9
Lung function data at 5 weeks of age			
Tidal breathing parameters[¶,+]			
Minute ventilation mL·min^{-1}	1401	1239–1566	733–2334
Respiratory rate breaths·min^{-1}	44.4	37.8–51.2	24.1–79.3
Tidal volume mL	32.3	28.1–36.6	21.0–51.1
Tidal expiratory flow mL·s^{-1}	41.5	35.6–49.1	20.0–79.6
Tidal inspiratory flow mL·s^{-1}	53.9	47.9–59.0	30.0–82.0
Ratio *t*PTEF/*t*E	34.4	27.9–41.7	14.8–73.3
Multiple-breath washout[§,ƒ]			
FRC_{ao} mL	97.2	85.1–108.0	56.3–145.4
FRC_{ao} per weight mL·kg^{-1}	22.2	19.7–25.2	12.3–35.8
LCI	6.9	6.4–7.4	5.5–10.1
Inflammatory markers[¶,##]			
eNO[¶¶] ppb	13.4	10.2–16.6	1.8–34.9
NO output[¶¶,++] nL·s^{-1}	0.582	0.458–0.721	0.068–1.422
Outdoor temperature °C	10	3–15	-6–27
Distance to measurement station[§§] km	42.1	39.6–47.4	15.9–136.4
Distance to 4-m road[§§] m	79	38–155	3–747
Distance to 6-m road[§§] m	190	83–401	3–3794
Mean daily pre-natal exposure to air pollution			
PM_{10} μg·m^{-3}	22.1	20.2–23.8	17.1–25.7
NO_2 μg·m^{-3}	15.8	14.7–17.0	11.8–19.6
O_3[ƒƒ] μg·m^{-3}	86.7	79.6–94.9	74.1–111.7
Mean daily post-natal exposure to air pollution			
PM_{10} μg·m^{-3}	20.0	16.6–23.4	10.6–49.6
NO_2 μg·m^{-3}	15.1	10.9–19.7	7.0–31.2
O_3[ƒƒ] μg·m^{-3}	87.5	56.3–109.9	25.0–156.2

*t*PTEF: time to peak tidal expiratory flow; *t*E: expiratory time; FRC_{ao}: functional residual capacity at airway opening; LCI: lung clearance index; eNO: exhaled nitric oxide; PM_{10}: particles with a 50% cut-off aerodynamic diameter of 10 μm; NO_2: nitrogen dioxide; O_3: ozone. [#]: n=222; [¶]: calculated as the mean value of 100 breaths without sighs; [+]: n=221; [§]: calculated as the mean value of all technically acceptable washout measurement traces; [ƒ]: n=181; [##]: n=205; [¶¶]: measured in the third quartile of the expiratory cycle; [++]: calculated as eNO × expiratory flow; [§§]: for one subject, this could not be determined, as no geographic information system data were available; [ƒƒ]: based on maximum hourly value of each day.

the pregnancy (fig. 4). Values (95% CI) for the association between increase in eNO per μg·m^{-3} increase in NO_2 concentration were 0.09 (-0.08–0.25) ppb for the first trimester, 0.18 (-0.09–0.45) ppb for the second trimester, 0.20 (-0.0–0.41) ppb for the third trimester and 0.06 (-0.23–0.34) ppb for the post-natal time period.

No association was found for distance to 4- or 6-m roads and lung function parameters (table 5). However, a trend towards a stronger association of pre-natal PM_{10} exposure with minute ventilation was found for newborns of mothers living close to major roads. For example, in infants of mothers who lived within 150 m of a 6-m road, minute ventilation was 39.2 (95% CI 17.2–61.1) mL·min^{-1} higher per μg·m^{-3} higher pre-natal PM_{10} exposure, compared with 12.6 (-10.0–35.3) mL·min^{-1} higher minute ventilation in infants of mothers who lived further than 150 m away (p for interaction 0.06). A comparably weak trend towards a stronger association was found in infants of smoking mothers.

The observed associations remained stable in the sensitivity analyses, as shown in table 6, including when assessing the relationships without adjustment for outdoor temperature or season and with adjustment for paternal education, month or year of birth and exposure to air pollution on the 2 days before the lung function measurement.

DISCUSSION

Summary

To the current authors' knowledge, the present study is the first to examine air pollution during pregnancy and subse-

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TABLE 2 Potential risk factors of the study infants

Male sex	122 (55)
1 older sibling	71 (32)
⩾2 older siblings	44 (20)
Maternal asthma[#]	22 (10)
Maternal atopy[¶]	85 (38)
Maternal smoking in pregnancy	26 (12)
Low maternal education[+]	74 (34)
High maternal education[+]	68 (31)
Low paternal education[+]	42 (20)
High paternal education[+]	113 (53)
Living <150 m from a 4-m road	162 (72)
Living <150 m from a 6-m road	98 (44)
Living <75 m from a 4-m road	108 (48)
Living <75 m from a 6-m road	50 (22)

Data are presented as n (%). [#]: defined as self-reported, doctor-diagnosed asthma; [¶]: defined as self-reported, doctor-diagnosed asthma, hay fever or eczema; [+]: parental education was categorised into low (<4 yrs of apprenticeship), middle (⩾4 yrs of apprenticeship) and high (tertiary education).

quent lung growth and development in early life. In the present study, exposure to PM10 during pregnancy was associated with higher respiratory need in newborns as reflected by higher minute ventilation and tidal flows. Higher levels of NO_2 during pregnancy were found to be related to elevated eNO, indicative of the induction of inflammatory processes.

Methodological aspects

In light of the cost- and time-consuming nature of infant lung function testing, which has to date hampered such research in healthy infants, the present study has several methodological strengths. Lung function was measured in a standardised way, based on ERS/ATS standards, and used the latest recommendations for analysis [15, 16]. The same equipment, the same masks and the same measurement order were used throughout the whole study period, to ensure comparability. The current authors analysed 100 tidal breaths, giving more robust estimates than the 30 breaths recommended by the standards [15]. Since all infants were healthy, breast-fed, of a narrow age range and measured during natural sleep, the contributions of these other possible influencing factors were comparable within the cohort. Causes of lung function changes in older children, such as physical activity, obesity or hypoxia, were negligible in the present study [18]. Selection bias was not an issue, as participants were recruited pre-natally without knowledge of exposure to air pollution or lung function after birth. Furthermore, both exposure and outcome variables were objective measures and were independently assessed and analysed. It was possible to adjust for known biological and time-variant confounders, such as smoking during pregnancy, season or outdoor temperature. Further possible confounding factors, such as socio-economic status, number of siblings, post-natal exposure to air pollution and seasonal fluctuations (*e.g.* month and year of birth, or infectious season) were considered in sensitivity analyses and did not affect the present results.

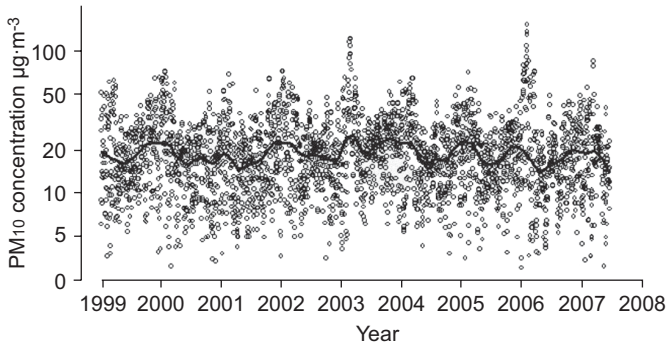

FIGURE 1. Daily mean levels of particles with a 50% cut-off aerodynamic diameter of 10 μm (PM10) concentration (○) at the fixed monitoring station in Payerne (Switzerland). The trend line was computed by a LOWESS smoother using locally weighted polynomial regression with a smoother span of 0.05. Note the logarithmic scale of the y-axis.

A limitation of the present study was that individual exposure to air pollution was not sampled, which, while possible after recruitment, is expensive and difficult to do. This is a limit of most studies assessing long-term exposure to air pollution. Therefore, in addition to using mean air pollutant levels during pregnancy as a proxy for temporal variability, the current authors also used distance of homes to major roads as a proxy for spatial variability in exposure to traffic-related air pollutants [17]. One limitation of the present study in this regard is the fact that only road proximity was used, without information about traffic density.

In contrast to studies in cooperative subjects, measurements of forced expiratory volumes and flows need sedation in infants and are, thus, not well accepted by parents of healthy subjects.

Although clear associations, robust towards a range of sensitivity analyses, may indicate a true association, causality cannot be proved. Furthermore, multiple comparisons were performed due to the nature of the study design, with different exposures and several outcome parameters. It cannot be totally excluded that positive associations may have occurred due to chance and, thus, the current authors recommend replication of the present results, preferably in a different cohort with varying pollution levels.

Comparison with other studies

Studies in cooperative school children showed a reduction of lung growth upon exposure to particulate matter [1, 3]. The present authors found no association between PM10 and lung volume in unsedated sleep, possibly because FRC in infants is dynamically regulated to maintain end-expiratory lung volume above airway closure [19]. Thus, the increased minute ventilation in infants with higher pre-natal PM10 exposure may be a compensatory mechanism for lower lung volumes. In line with these observations, no study has so far demonstrated an effect of pre-natal smoke exposure on lung volume in infants [10].

The present study found a clear association between NO_2 exposure during pregnancy and post-natal eNO levels. Such a causal association seems plausible, as NO_2 is known to induce inflammatory processes [20]. Nevertheless it cannot be

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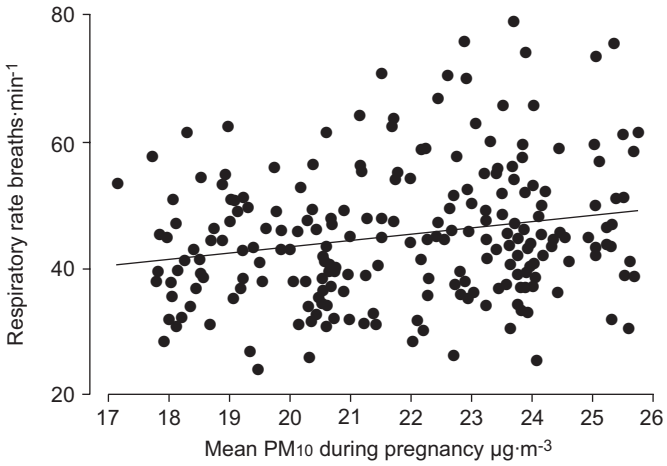

FIGURE 2. Individual respiratory rate at 5 weeks of age plotted against mean concentration of particles with a 50% cut-off aerodynamic diameter of 10 µm (PM_{10}) during pregnancy. An increase from the lowest to the highest quartile of pre-natal PM_{10} exposure was associated with a change in respiratory rate from 42 to 48 breaths·min^{-1}.

excluded that NO_2 acts as a proxy for a complex mixture of combustion pollutants that originates primarily from vehicular traffic [21]. The role of upregulated inducible nitric oxide synthase in this process is unknown, but may provide a link between environmental air pollution and the evolution of asthma.

No association between O_3 and lung function was found. Results from other studies are contradictory, with an association between O_3 and lung volume and inflammation shown by some groups [1, 6], but not by others, *e.g.* one study from Switzerland with summer daytime O_3 exposure levels comparable to those of the present study [22].

Several studies showed that living closer to major roads has adverse effects on lung function and respiratory symptoms [2, 17]. However, there is still an ongoing debate about a possible threshold regarding traffic-related pollution, as well as the boundary between traffic-related exposure and background pollution levels [23]. In the present study, in contrast to temporal variation (mean pollutant levels), spatial variation during pregnancy alone (distance to roads) was not associated with lung function changes. In this regard, important factors special to the present study have to be mentioned. First, exposure was calculated during a time period of 9 months (pregnancy) for each subject individually, whereas most other studies dealt with mean annual exposure levels. Secondly, females maintain a mobile lifestyle during pregnancy. As such, exposure at home (usually during times with lower pollution levels) does not necessarily contribute to overall exposure as much as, for example, in the less mobile elderly population. Thirdly, the particular environmental situation in urban Switzerland with high population and road network density leads to homogenous distribution of $PM_{2.5}$ and PM_{10} [24]. Taken together, these points may explain why, for exposure assessment over a limited time period (such as pregnancy) in mobile subjects living in areas with homogenously distributed PM_{10} levels, temporal variation of regional pollution levels may be more important than small-scale spatial variations of air pollutants. However, the relative contribution of background exposure and road proximity to health effects remains controversial [23].

Mechanisms

The changes in lung function seen in the present study infants with increased pre-natal exposure to particulate matter are similar to those observed in premature infants with bronchopulmonary dysplasia [25], in infants of smoking mothers [26] and in animal models of pre-natal nicotine exposure [27]. Thus, the current findings indicate considerable impact of air pollution on lung development already present at an early developmental stage, and are suggestive of either increased respiratory need due to increased resistance (smaller airways), decreased compliance (smaller or stiffer airways) and/or factors influencing control of breathing (*e.g.* hypoxia).

The exact mechanisms for these changes are unknown. Some hypotheses have been proposed in the literature [28]. Oxidative stress and inflammation of the airways in the mother after exposure to air pollution may affect the blood–air barrier [29], potentially leading to reduced foetal breathing movements and decreased alveolarisation [30]. The effect could also be mediated *via* systemic inflammation in the mother upon air pollution leading to decreased placental blood flow with reduced transfer of nutrients to the foetus [30]. Although entirely unknown, the increasing role thought to be played by nanoparticles could also be involved in either process [31]. Furthermore, evidence for decreased birthweight upon higher exposure to air pollution suggests that growth factors may be involved [32], which may also be applicable to lung growth. In the current study cohort, all associations were unaltered by birthweight, suggesting that the effect is even independent of birthweight.

Relevance

Due to the need for an outcome parameter assessable early after birth, the current authors measured well-defined physiological surrogates for lung growth and development in this age group. Early changes in lung function track into later life [13] and are believed to have a huge impact upon long-term respiratory morbidity, *e.g.* asthma occurrence [33].

The higher respiratory need, as reflected by increased minute ventilation upon higher pre-natal exposure to particulate matter, might be clinically important, especially for pre-morbid infants with an already reduced breathing capacity or infants that are acutely sick.

From a public health point of view, the present results are particularly crucial in areas with higher outdoor pollution, or considerable indoor pollution from biomass fuels where it is practically impossible for an individual to avoid exposure. Especially in these areas, altered lung function may be one mechanism responsible for the association between PM_{10} and infant mortality, particularly since a stronger association for respiratory rather than nonrespiratory mortality has been observed after PM_{10} exposure [34]. Therefore, it seems worthwhile to investigate a possible effect of activities during pregnancy associated with exposure to pollution (*e.g.* cooking at open fireplace) on infant morbidity, because exposure to indoor pollution could be substantially reduced by using

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TABLE 3 Associations between pre-natal pollutant levels and lung function at age 5 weeks

	Univariable model			Adjusted model#		
	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value
Tidal breathing						
Minute ventilation¶ mL·min^{-1}						
PM_{10}	19.8	4.9–34.7	0.010	24.9	9.3–40.5	0.002
NO_2	0.6	-18.3–19.5	0.948	12.5	-12.4–37.5	0.324
O_3	0.7	-2.8–4.2	0.696	-0.2	-5.0–4.6	0.929
Mean tidal expiratory flow mL·s^{-1}						
PM_{10}	0.59	0.02–1.16	0.043	0.81	0.22–1.40	0.007
NO_2	-0.13	-0.84–0.59	0.730	0.61	-0.33–1.54	0.202
O_3	0.05	-0.08–0.19	0.448	-0.04	-0.22–0.14	0.666
Mean tidal inspiratory flow mL·s^{-1}						
PM_{10}	0.71	0.18–1.23	0.009	0.81	0.26–1.36	0.004
NO_2	0.17	-0.49–0.84	0.610	0.07	-0.81–0.95	0.871
O_3	-0.007	-0.13–0.12	0.909	0.05	-0.12–0.22	0.560
Respiratory rate breaths·min^{-1}						
PM_{10}	0.97	0.36–1.58	0.002	1.15	0.52–1.77	<0.001
NO_2	0.32	-0.46–1.10	0.419	1.50	0.52–2.49	0.003
O_3	0.08	-0.07–0.22	0.297	-0.005	-0.20–0.19	0.961
Tidal volume¶ mL						
PM_{10}	-0.23	-0.56–0.09	0.158	-0.23	-0.56–0.10	0.177
NO_2	-0.28	-0.69–0.12	0.173	-0.78	-1.30– -0.27	0.003
O_3	-0.02	-0.09–0.06	0.629	0.01	-0.09–0.11	0.830
Multiple-breath washout						
FRC_{ao} mL·kg^{-1}						
PM_{10}	0.09	-0.19–0.37	0.525	0.09	-0.22–0.39	0.573
NO_2	0.06	-0.26–0.39	0.713	0.09	-0.38–0.56	0.706
O_3	0.01	-0.04–0.07	0.635	0.03	-0.06–0.11	0.537
LCI						
PM_{10}	-0.02	-0.07–0.03	0.425	-0.02	-0.07–0.04	0.565
NO_2	-0.04	-0.09–0.02	0.170	-0.05	-0.13–0.03	0.247
O_3	-0.001	-0.01–0.01	0.825	-0.006	-0.02–0.01	0.368
Inflammatory markers						
eNO^+ ppb						
PM_{10}	0.17	-0.20–0.53	0.369	0.44	-0.08–0.80	0.016
NO_2	0.66	0.22–1.10	0.004	0.98	0.45–1.51	<0.001
O_3	-0.02	-0.11–0.06	0.608	0.01	-0.09–0.11	0.856
NO output pL·s^{-1}						
PM_{10}	16.5	1.5–31.5	0.031	15.6	-0.2–31.3	0.053
NO_2	23.6	5.2–42.1	0.012	41.0	17.8–64.2	0.001
O_3	-0.21	-3.7–3.3	0.905	-0.13	-4.7–4.5	0.956

Data are given as unit change in lung function value per μg·m^{-3} increase in the mean pre-natal exposure of the respective pollutant. CI: confidence interval; PM_{10}: particles with a 50% cut-off aerodynamic diameter of 10 μm; NO_2: nitrogen dioxide; O_3: ozone; FRC_{ao}: functional residual capacity at airway opening; LCI: lung clearance index; eNO: exhaled nitric oxide. #: this model was adjusted for sex, post-natal age, season of birth, outdoor temperature on the measurement day and maternal smoking in pregnancy; ¶: similar results were obtained with minute ventilation and tidal volume per body weight; +: in adjusted model, this was further adjusted for minute ventilation.

cleaner fuels and improved stove constructions for cooking [35].

Conclusion

The present study is the first prospective birth cohort study suggesting a relationship between pre-natal levels of particles with a 50% cut-off aerodynamic diameter of 10 μm and nitrogen dioxide exposure with lung function and inflammation after birth. The current findings involving airway mechanics provide additional evidence to epidemiological studies, since they suggest potential mechanisms behind adverse outcomes of pollution. Influences during the vulnerable phase of pregnancy are known to affect lung development and growth [9, 13] and the evolution of asthma and allergy. If

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TABLE 4 Associations between post-natal pollutant levels and lung function at age 5 weeks

	Univariable model			Adjusted model#		
	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value
Tidal breathing						
Minute ventilation¶ mL·min^{-1}						
PM_{10}	5.5	-0.9–11.9	0.090	4.3	-3.6–12.2	0.280
NO_2	0.9	-5.2–7.0	0.770	-11.3	-24.2–1.6	0.087
O_3	0.2	-0.9–1.3	0.749	2.4	0.27–4.6	0.028
Mean tidal expiratory flow mL·s^{-1}						
PM_{10}	0.27	0.02–0.50	0.031	0.14	-0.16–0.43	0.361
NO_2	0.13	-0.10–0.36	0.260	-0.41	-0.90–0.08	0.097
O_3	-0.01	-0.05–0.03	0.601	0.09	0.005–0.17	0.038
Mean tidal inspiratory flow mL·s^{-1}						
PM_{10}	0.05	-0.18–0.27	0.678	0.12	-0.15–0.40	0.377
NO_2	-0.11	-0.33–0.10	0.312	-0.33	-0.78–0.13	0.161
O_3	0.03	-0.01–0.07	0.145	0.07	-0.004–0.15	0.063
Respiratory rate breaths·min^{-1}						
PM_{10}	0.30	0.04–0.56	0.026	0.08	-0.23–0.40	0.599
NO_2	0.18	-0.07–0.43	0.419	-0.31	-0.84–0.21	0.238
O_3	-0.01	-0.06–0.04	0.629	0.09	0.007–0.18	0.035
Tidal volume¶ mL						
PM_{10}	-0.08	-0.22–0.06	0.242	0.01	-0.15–0.18	0.886
NO_2	-0.08	-0.22–0.05	0.204	-0.07	-0.35–0.20	0.595
O_3	0.01	-0.02–0.03	0.611	-0.01	-0.06–0.03	0.573
Multiple-breath washout						
FRC_{ao} mL·kg^{-1}						
PM_{10}	-0.06	-0.16–0.05	0.290	-0.09	-0.22–0.04	0.174
NO_2	-0.05	-0.15–0.06	0.379	-0.23	-0.46– -0.01	0.042
O_3	-0.002	-0.02–0.02	0.780	-0.008	-0.05–0.03	0.649
LCI						
PM_{10}	0.01	-0.008–0.03	0.265	0.007	-0.02–0.03	0.527
NO_2	0.01	-0.006–0.03	0.212	0.02	-0.02–0.06	0.407
O_3	-0.001	-0.01–0.002	0.555	0.001	-0.006–0.01	0.819
Inflammatory markers						
eNO^+ ppb						
PM_{10}	-0.04	-0.20–0.11	0.614	0.05	-0.14–0.23	0.614
NO_2	-0.003	-0.15–0.14	0.968	0.06	-0.22–0.35	0.666
O_3	0.01	-0.02–0.04	0.418	0.02	-0.02–0.07	0.302
NO output pL·s^{-1}						
PM_{10}	3.9	-2.8–10.6	0.254	1.9	-6.1–10.0	0.635
NO_2	2.4	-3.6–8.4	0.438	0.04	-12.5–12.6	0.994
O_3	0.17	-0.93–1.28	0.758	0.97	-1.09–3.03	0.356

Data are given as unit change in lung function value per μg·m^{-3} increase in the mean post-natal exposure of the respective pollutant. CI: confidence interval; PM_{10}: particles with a 50% cut-off aerodynamic diameter of 10 μm; NO_2: nitrogen dioxide; O_3: ozone; FRC_{ao}: functional residual capacity at airway opening; LCI: lung clearance index; eNO: exhaled nitric oxide. #: this model was adjusted for sex, post-natal age, season of birth, outdoor temperature on the measurement day and maternal smoking in pregnancy; ¶: similar results were obtained with minute ventilation and tidal volume per body weight; +: in adjusted model, this was further adjusted for minute ventilation.

the hypothesis of BARKER *et al*. [12] is correct, these early influences on the respiratory system result in a higher burden of respiratory disease in older people and shortened life expectancy. The present results thus provide further rationale for more stringent measures to reduce air pollution.

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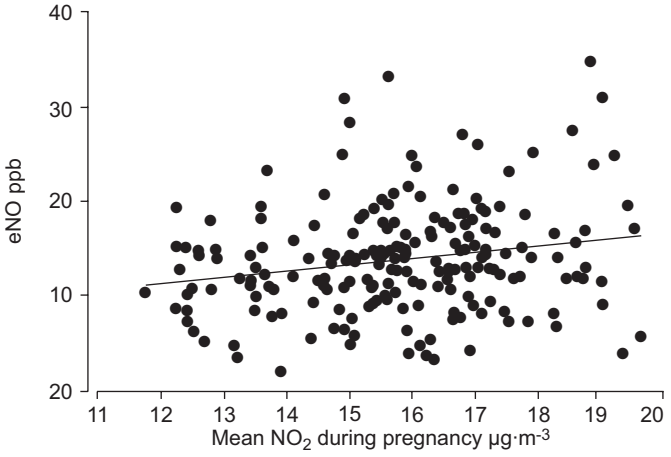

FIGURE 3. Individual concentration of exhaled nitric oxide (eNO) at 5 weeks of age plotted against mean concentration of nitric dioxide (NO_2) levels during pregnancy. An increase from the lowest to the highest quartile of pre-natal NO_2 exposure was associated with a change in eNO levels from 12.1 to 15.2 ppb.

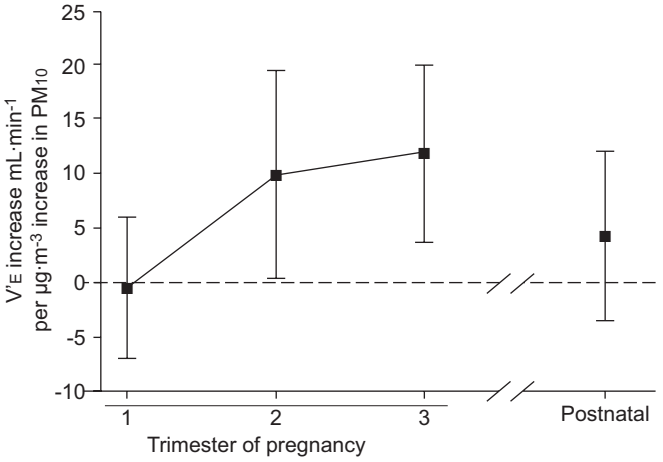

FIGURE 4. Association between lung function and pollution levels during the three trimesters of pregnancy and the time between birth and lung function (post-natal). The increase (with 95% confidence interval) in minute ventilation (V'_E), in mL·min^{-1} per µg·m^{-3} of particles with a 50% cut-off aerodynamic diameter of 10 µm (PM_{10}) for the respective trimester of pregnancy or the post-natal time period, is indicated.

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TABLE 5 Associations between distance to roads and lung function at age 5 weeks

	Univariable model			Adjusted model#		
	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value
Tidal breathing						
Minute ventilation¶ mL·min^{-1}						
Distance 6-m road	0.06	-0.57–0.69	0.853	-0.02	-0.66–0.62	0.957
Distance 4-m road	1.12	-1.54–3.79	0.407	1.13	-1.54–3.79	0.407
Mean tidal expiratory flow mL·s^{-1}						
Distance 6-m road	0.005	-0.02–0.03	0.679	0.003	-0.02–0.03	0.809
Distance 4-m road	0.05	-0.06–0.15	0.375	0.05	-0.05–0.15	0.349
Mean tidal inspiratory flow mL·s^{-1}						
Distance 6-m road	-0.006	-0.03–0.02	0.618	-0.006	-0.03–0.02	0.585
Distance 4-m road	0.03	-0.06–0.13	0.535	0.03	-0.07–0.12	0.583
Respiratory rate breaths·min^{-1}						
Distance 6-m road	0.0007	-0.03–0.03	0.955	0.001	-0.03–0.02	0.931
Distance 4-m road	0.02	-0.09–0.13	0.717	0.02	-0.08–0.13	0.657
Tidal volume¶ mL						
Distance 6-m road	-0.0007	-0.01–0.01	0.922	-0.0007	-0.01–0.01	0.921
Distance 4-m road	0.004	-0.05–0.06	0.886	0.003	-0.05–0.06	0.923
Multiple-breath washout						
FRC_{ao} mL·kg^{-1}						
Distance 6-m road	0.003	-0.01–0.01	0.575	0.005	-0.01–0.02	0.356
Distance 4-m road	0.02	-0.02–0.07	0.296	0.02	-0.02–0.07	0.303
LCI						
Distance 6-m road	0.0015	-0.001–0.004	0.162	0.001	-0.001–0.003	0.325
Distance 4-m road	-0.003	-0.01–0.006	0.530	-0.002	-0.01–0.006	0.663
Inflammatory markers						
eNO^{+} ppb						
Distance 6-m road	-0.002	-0.02–0.01	0.784	-0.004	-0.02–0.01	0.608
Distance 4-m road	-0.006	-0.07–0.05	0.845	-0.005	-0.07–0.05	0.860
NO output pL·s^{-1}						
Distance 6-m road	0.0001	-0.001–0.001	0.778	0.0001	-0.001–0.001	0.857
Distance 4-m road	0.0001	-0.002–0.003	0.695	0.0001	-0.002–0.003	0.608

Data are given as unit change in lung function value per living 10 m further away from the next road. CI: confidence interval; FRC_{ao}: functional residual capacity at airway opening; LCI: lung clearance index; eNO: exhaled nitric oxide. #: this model was adjusted for sex, post-natal age, season of birth, outdoor temperature on the measurement day and maternal smoking in pregnancy; ¶: similar results were obtained with minute ventilation and tidal volume per body weight; +: in adjusted model, this was further adjusted for minute ventilation.

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TABLE 6 Sensitivity analyses of the association between PM_{10} and minute ventilation, and between nitrogen dioxide and exhaled nitric oxide (eNO)

	PM_{10} and minute ventilation[#]		Nitrogen dioxide and eNO[¶]	
	Coefficient	95% CI	Coefficient	95% CI
Main model[+]	24.9	9.3–40.5	0.98	0.45–1.51
Without adjustment for age	25.3	9.7–40.9	1.03	0.50–1.55
Without adjustment for sex	22.9	7.3–38.5	0.98	0.45–1.51
Without outdoor temperature	24.8	9.4–40.2	1.01	0.52–1.50
Without seasonal adjustment	25.0	9.4–40.7	0.93	0.40–1.46
Without smoking during pregnancy	24.7	9.0–40.4	0.98	0.46–1.51
With distance to 6-m roads	24.7	8.9–40.5	0.96	0.44–1.48
With gestational age	24.7	9.0–40.4	0.99	0.47–1.52
With height and weight at study date	23.6	9.0–38.2	0.93	0.41–1.46
With paternal education	24.3	8.6–40.1	0.93	0.39–1.47
With maternal asthmatic disease	24.8	9.1–40.5	0.97	0.45–1.50
With year of birth	28.0	7.0–49.0	0.94	0.28–1.60
With month of birth	22.5	5.6–39.4	1.20	0.58–1.83
With infectious season[§]	23.8	7.2–40.4	0.85	0.20–1.51
With number of siblings[ƒ]	24.5	9.0–40.0	1.02	0.49–1.56
With air pollution 2 days before lung function[##]	24.1	8.3–39.9	1.07	0.53–1.61

PM_{10}: particles with a 50% cut-off aerodynamic diameter of 10 μm; CI: confidence interval. [#]: Values refer to changes in minute ventilation in $mL \cdot min^{-1}$ per $\mu g \cdot m^{-3}$ increase in mean pre-natal PM_{10} exposure. [¶]: Values refer to changes in eNO in ppb per $\mu g \cdot m^{-3}$ increase in mean pre-natal nitrogen dioxide exposure. [+]: The main model is equivalent to the adjusted model in table 2. This model was adjusted for sex, post-natal age, season of birth, outdoor temperature on the measurement day and maternal smoking in pregnancy. Additionally, eNO was also adjusted for minute ventilation. [§]: Infectious season was divided into four categories, based on month of birth (November–April for winter season and May–October for summer season) and on a known 2-yr periodicity of respiratory virus epidemics. [ƒ]: Number of siblings was categorised into no older siblings, one older sibling or two and more older siblings. [##]: mean exposure to PM_{10} on the 2 days before lung function testing was included in the model for minute ventilation and mean exposure to nitrogen dioxide was included in the model for eNO.

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