Increased Impact of Air Pollution on Lung Function in Preterm vs. Term Infants: The BILD Study

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Contributions UF, MR, PL conceived the study. FD, UF performed data analysis and manuscript writing. Measurements were recruited, recorded or analyzed by SS, FD, OG, JU, IK and YS. Statistical analyses were done by FD, OG and MR. DV and KdH modeled the air pollution data. PS provided biochemical expertise. All coauthors have critically reviewed the manuscript and have seen and approved the final manuscript.

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Air pollution and postnatal lung function in preterm infants

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Today, approximately 10% of all infants are born prematurely, pre-existing vulnerability means that their lungs are potentially more susceptible to ambient air pollution. This is the first study showing that the effect size of low-to-moderate levels of air pollution exposure during pregnancy on postnatal lung function is significantly higher in preterm than in healthy term infants of similar postconceptional age of 44 weeks. The effect was best detectable in moderate to late preterm infants and these findings are highly relevant, since recent evidence indicates

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that early-life lung functional impairment and its lifespan developmental tracking may be an important early-life risk factor for chronic obstructive lung disease in adulthood.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

At a Glance

What is the current scientific knowledge on this subject?

Today, approximately 10% of all infants are born prematurely, pre-existing vulnerability means that their lungs are potentially more susceptible to ambient air pollution. However, upto date it is unkown whether these infants show impaired lung function after pre- and postnatal air pollution exposure in comparison to their term peers.

What does this study add to the field?

This is the first study showing that the effect size of low-to-moderate levels of air pollution exposure during pregnancy on postnatal lung function is significantly higher in preterm than in healthy term infants of similar postconceptional age of 44 weeks. The effect was best detectable in moderate to late preterm infants and these findings are highly relevant, since recent evidence indicates that early-life lung functional impairment and its lifespan developmental tracking may be an important early-life risk factor for chronic obstructive lung disease in adulthood.

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ABSTRACT

Rationale Infants born prematurely have impaired capacity to deal with oxidative stress shortly after birth.

Objectives We hypothesize that the relative impact of exposure to air pollution on lung function is higher in preterm than in term infants.

Methods In the prospective BILD-birth-cohort of 254 preterm and 517 term infants, we investigated associations of particulate matter (PM_{10}) and nitrogen dioxide with lung function at 44 weeks postconceptional age and exhaled markers of inflammation and oxidative stress response (fraction of exhaled nitric oxide (FeNO)) in an explorative hypothesis-driven study design. Multilevel mixed-effects models were used and adjusted for known confounders.

Measurements and Main Results Significant associations of PM_{10} during the second trimester of pregnancy with lung function and FeNO were found in term and preterm infants. Importantly, we observed stronger positive associations in preterm infants (born 32 – 36 weeks), with an increase of [184.9 (79.1, 290.7) mL/min] minute ventilation per 10 $\mu g/m^3$ increase in PM_{10} than in term infants [75.3 (19.7, 130.8) mL/min] ($p_{prematurity \times PM10\ interaction}$ = 0.04, after multiple comparison adjustment p_{adj} = 0.09). Associations of PM_{10} and FeNO differed between moderate to late preterm [3.4 (-0.1, 6.8) ppb] and term [-0.3 (-1.5, 0.9) ppb] infants, the interaction with prematurity was significant ($p_{prematurity \times PM10\ interaction}$ = 0.006, p_{adj} = 0.036).

Conclusion Preterm infants showed significant higher susceptibility even to low-to-moderate prenatal air pollution exposure than term infants, leading to increased impairment of postnatal lung function. FeNO results further elucidate differences in inflammatory/oxidative stress response comparing preterms to terms.

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INTRODUCTION

Adverse effects of high air pollution levels on respiratory health in children and adults have been shown (1-3). Similarly, low-to-moderate air pollution levels and their impact on lung function and respiratory symptoms in infants and children have been subject to recent research (4-8). Most recent studies indicate the necessity of further air pollution reductions, since clear associations between low-to-moderate air pollution and impaired lung function in preschool and school-aged children have been found (4-6). These studies further indicate the need for precise analysis and large prospective cohort studies to assess the impact on more vulnerable subgroups, such as asthmatics, preterm born children or boys (9, 10). Children starting with impaired lung function are especially at risk for later respiratory morbidity and mortality (11, 12). Further, specific exposure windows, especially the second and third trimester of pregnancy, have been shown to be vulnerable phases for air pollution exposure in terms of association with lung function in the offspring (5, 13).

Survival rates of preterm infants are increasing throughout high-income countries (14) due to substantial progress in perinatal care. However, preterm infants are known to have impaired capacity to deal with oxidative stress shortly after birth, which reflects in higher levels of inflammatory and oxidative stress markers, such as FeNO (15-18). Air pollution, a known oxidative stressor to the airways and lungs (18) and its effects on lung function in this vulnerable subgroup, should therefore be examined. We have previously shown that prenatal air pollution exposure affects lung function in healthy term born infants (19). We hypothesize that exposure to pre- and postnatal air pollution has a stronger effect on lung function in preterm infants due to increased vulnerability. Our main aim was therefore to investigate whether moderate to late preterm infants show stronger impairment of lung function (ventilatory need) and airway inflammation (FeNO) in infancy following pre- and postnatal air pollution exposure.

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In an explorative-multivariable statistical approach after adjustment for known covariates of prematurity, we aimed to determine whether pre- and postnatal exposure to particulate matter with an aerodynamic diameter of <10 µm (PM_{10}) and nitrogen dioxide (NO_2) affects lung function at 44 weeks of postconceptional age (PCA, defined as the time between the date of conception and the date of lung function (20)) in preterm more prominently than in term infants. Following our main hypothesis, we stratified the analysis in groups of preterm infants born prior to or after 32 weeks of gestational age (GA, defined as the time between the first day of the last menstrual period and the date of birth (20)), knowing that in the earlier group lung functional abnormalities may be more affected by mechanical ventilation and chronic lung disease (CLD) of infancy (21). Some of the study participants (207 healthy term infants) from this study have been previously included in a study by Latzin et al. (19).

METHODS

Study design

The prospective Basel-Bern Infant Lung Development (BILD) cohort (https://www.bild-cohort.ch/) comprises unselected neonates recruited since 1999 in the region of Bern and since 2012 in Basel, Switzerland (22). Prematurity was defined as GA at birth <37 weeks (23). The ethics committees from Basel and Bern approved the study and written consent was obtained. We performed an explorative hypothesis-driven analysis in accordance with previous study designs (5, 9, 19) on air pollution effects in small children.

Air pollution exposure

For the calculation of individual mean exposure to NO_2 and PM_{10} time-space hybrid models were used. Background air pollution was measured at the Payerne monitoring station. For the NO_2 model high-quality information on land use, population density, traffic, dispersion

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models, meteorological data and air quality from Payerne was integrated with 28,849 NO_2 biweekly and monthly passive sampler measurements. Observations were collected consecutively over more than ten years at 146 locations, for external validation of the model (24). Air pollution models are described in more detail in previously published articles (4, 24) and in the online supplement. Due to best comparability and highest expected effect size (19), mean exposure for the 2nd trimester was calculated, for sensitivity analysis exposure of the 1st and 3rd trimester and postnatal time period were compared.

Lung function outcomes

Pulmonary function was performed at 44 weeks PCA using Exhalyzer D (EcoMedics, Duernten, Switzerland) according to current ERS/ATS guidelines (25) and procedures for analysis as described previously (19).

We investigated minute ventilation (V_E = respiratory rate * tidal volume) and ratio of time to peak tidal expiratory flow (PTEF) and expiratory time (t_{PTEF}/t_E) was used to describe TBFVL shapes. Further, fraction of exhaled nitric oxide (FeNO) was measured as a marker for airway inflammatory response and oxidative stress (18).

Covariates

After inclusion of *a priori* selected covariates from previous literature (3, 19, 21), we performed a stepwise backward selection and removed one covariate at a time with the highest p-value until only significantly associated variables were left, and built one final model including all the variables that were significantly associated (p-value <0.05). For FeNO analyses we additionally adjusted for V_E (19).

Statistical analysis

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We performed multilevel mixed-effects linear regression, adjusted for the covariates from the stepwise backward selection and corrected for clustering on center level (Basel vs. Bern).

Since we previously showed that lung functional abnormalities can be the result of prematurity and perinatal insults, particularly in extremely and very early preterm infants (21), we conducted a stratified analysis according to GA at birth into four clinically relevant groups: extremely (<28 weeks GA), very early (29 – 31 weeks GA) or moderate to late (32 – 36 weeks GA) preterm and term infants. We tested the presence of interaction within term and moderate to late preterm infants adding the interaction terms between exposure to air pollution and prematurity.

In a sensitivity analysis, we performed multiple comparison adjustments for our main hypothesis according to Benjamini-Hochberg (26). More detailed information on method, sensitivity analyses and power calculation is given in the online supplement.

RESULTS

From 1999 to 2017, 1100 infants were recruited. We had complete data available for 890 (81%) children on pulmonary function testing at 44 weeks of PCA, modeled air pollution exposure and potential risk factors (***Figure E1***). Of these, data from 775 infants (87%) passed quality control of lung function measurements according to ERS/ATS guidelines (25), and after excluding outliers (see online supplement) in the moderate to late preterm infants with severe asphyxia a total of 771 infants were included for the main analyses. Anthropometric information and potential confounders are depicted in ***Table 1*** and for extremely and very early preterm infants in ***Table E1***. Of the 771 infants, 517 (67%) were term and 254 (33%) were preterm infants. The preterm population consisted of 65 (26%) extremely, 90 (35%) very early and 99 (39%) moderate to late preterm infants. Overall, 60 infants (8%) were children from actively smoking mothers, 85 (11%) had asthmatic mothers and 108 (14%) had a diagnosis of

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CLD. Comparison between participants lost to follow-up and included participants is given in ***Table E2***. As most infants lost to follow-up or excluded were preterm infants, the excluded population differed significantly from the population included in the study. Main differences were in GA at birth, maternal smoking during pregnancy, diagnosis of maternal asthma and duration of oxygen supplementation.

Lung function data at 44 weeks of PCA, completed between 16th March 2000 and 12th January 2018 are shown in ***Table 2***. Lung function values differed between subgroups with the highest values of V_E, V_T and FeNO in moderate to late (32 – 36 weeks GA) preterm infants, when not adjusted for weight at lung function measurement. Distribution of duration of mechanical ventilation and of supplementary oxygen is shown for preterm infants in ***Figure E2.***

The distribution of air pollution in the study region over the 18-year study period is depicted in ***Figure 1***. Air pollution concentrations for each pollutant during the second trimester of pregnancy and postnatal time period are shown in ***Table 3*** and for all exposure windows in ***Table E4***.

Association of air pollution with lung function at 44 weeks of postconceptional age

(tidal breathing flow volume loops)

Due to the explorative nature of the analysis, we report the findings related to the main exposure (2nd trimester) and main outcomes (V_E and its component V_T and FeNO) in ***Table 4***, and provide details of potential multiple comparison effects in the sensitivity analysis and in the online supplement. The association between prenatal air pollution with lung function in early infancy is given in ***Table 4*** and for all exposure windows in ***Table E5***. Associations of exposure to air pollution on lung function were observed during the second trimester of pregnancy.

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Pollution exposures during other trimesters and the postnatal time period showed no differences (online supplement).

Moreover, when comparing preterm children, divided into three clinically relevant groups, to term born children, a clear differentiation was seen between moderate to late preterm (32 – 36 weeks GA) and term infants; also, within these two groups an interaction of prematurity and air pollution was found (p-value for interaction = 0.040, p_{adj} = 0.09). However, no association of air pollutants on lung function was seen in the subgroup of extremely (<28 weeks GA) and very early (29 – 31 weeks GA) preterm infants (data shown in the online supplement and ***Figure E3***).

Within the term group V_E increased by [coefficient (β) (95% confidence intervals (CIs))] [75.3 (19.7 to 130.8) mL/min] per 10 µg/m^3 increase in PM_{10} during the second trimester, whereas in all preterm infants, V_E increased by [88.6 (18.6 to 158.7) mL/min] and within the moderate to late preterm infants by [184.9 (79.1 to 290.7) mL/min] (***Figure 2***). Interactions of prematurity and PM_{10} during the second trimester of pregnancy for V_E within the term and moderate to late preterm infants were significant (p = 0.040, p_{adj} = 0.09).

Fraction of exhaled nitric oxide (FeNO)

In the preterm group, FeNO increased significantly by [2.8 (0.8 to 4.8) ppb] and in the moderate to late preterm infants by [3.4 (-0.1 to 6.8) ppb] per 10 µg/m^3 increase in PM_{10} during the second trimester. However, in term infants, no clear associations between prenatal exposure to air pollution and FeNO were found. Associations of PM_{10} and FeNO differed between moderate to late preterm and term infants ($p_{prematurity \times PM10\ interaction}$ = 0.006, p_{adj} = 0.036).

In several sensitivity analyses we checked whether known risk factors, such as CLD classification (27), education status of the mother (10, 28) or exposure to caffeine citrate (29,

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30) were a confounder to the main model, and stratified the main model by sex (8-10). The inclusion of these additional confounders or mediators did not lead to substantial changes in the effect sizes and significance levels, which remained comparable to results reported from the main model.

Sensitivity analysis

After multiple comparison adjustment according to Benjamini-Hochberg for our main hypothesis (6 tests = 2 exposures [PM_{10} and NO_2 during the second trimester] * 3 outcomes [V_E, t_{PTEF}/t_E and FeNO]), the interaction of prematurity and air pollution on V_E in term and moderate to late preterm infants reached a non-significant p-value ($p_{prematurity\ x\ PM10} = 0.09$). However, the interaction between PM_{10} and prematurity on FeNO remained significant ($p_{prematurity\ x\ PM10} = 0.036$). Additional explorative analyses were further adjusted for multiple testing (176 tests) in a second step and non-adjusted and adjusted results are reported ***Table 4***, ***Table E5***, ***Table E6***. Further results of sensitivity analyses are described in the online supplement.

DISCUSSION

We showed significantly impaired lung function values with increasing air pollution exposure during the second trimester of pregnancy in term and preterm infants. However, in comparison to the age-matched term infants, the effect of air pollution on alterations of postnatal lung function in preterm infants was larger, although not statistically significant after multiple comparison adjustment ($p_{adj} = 0.09$). The effect was best detectable in the subgroup of moderate to late preterm infants (32 – 36 weeks GA). The stratified analysis was supported by complementary statistical analysis showing significant interaction of prematurity and PM_{10} exposure in term and moderate to late preterm infants also after multiple comparison adjustment ($p_{adj} = 0.036$). Statistical findings remained robust after extensive sensitivity

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analysis accounting for concomitant perinatal and biometric factors. This supports the suspected hypothesis of increased vulnerability of premature born infants due to pre-existing conditions (31). However, literature on the effects of outdoor air pollution on lung function in infancy is scarce. Latzin et al. (19) showed significant associations between prenatal PM_{10} exposure and increases in V_E at 44 weeks GA in around 240 healthy term children (207 infants are part of the healthy control group in this study). These effects are qualitatively similar but around three-times higher than the effect sizes seen in the term population in this current extended study. We assume that this was due to the larger sample size of 517 term infants, more precise exposure modeling (24), and decreasing air pollution levels in Switzerland over the observation time (1999–2016) by around 30%. The relatively low air pollution levels were potentially the reason why no associations of prenatal NO_2 exposure with infant lung function was seen in the current study (NO_2 levels were on average half of the World Health Organization annual guideline limits of 40 $\mu g/m^3$, but PM_{10} levels were around the WHO threshold levels of 20 $\mu g/m^3$ (32)). As a novel finding, effect sizes of exposure to PM_{10} were significantly larger in preterm vs. term infants, particularly in moderate to late preterm infants. The GRAPHS trial, however, focused on the effects that indoor air pollution, in particular carbon monoxide (CO), might have on infant lung function in nearly 400 infants at 30-days of age (9). Within their cohort V_E and RR increased significantly with increasing levels of CO. V_E has been shown to be a parameter of ventilatory need (33), with preterm infants and infants with wheeze increasing their V_T and lowering their respiratory rate in order to maintain or elevate V_E.

Air pollution also has an impact on biomarkers associated with airway inflammation and/or oxidative stress. In healthy term infants, associations of air pollution and increasing FeNO levels have been found (19). In the current study, we found associations between PM_{10} and NO_2 levels during the second trimester, increasing FeNO values in preterm and moderate to

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late preterm infants and no clear association in term infants. These findings reflect a different inflammatory context or oxidative stress response to pollutants in preterm infants in comparison to term infants. We hypothesize that in preterm infants there is a persisting underlying process, consistent with the observations of Filippone et al. (34) or Teig et al. (35), who show that even in adolescent children after prematurity, oxidative stress response is different. Alternatively, one could speculate differences in underlying inflammatory response in accordance with other observations e.g., of Paunescu et al. (36) who show increased FeNO values after higher black carbon exposures only in children with persistent respiratory symptoms. On a cellular level, inflammation in preterm infants after birth could be related to persistent neutrophil activity and oxidative stress, which have both previously been identified as affecting FeNO levels (18, 37). Both promote the oxygenation of nitrogen oxide (NO) to soluble NO metabolites, which have been shown in increased concentrations in plasma and bronchoalveolar lavage (BAL) fluids during the first month of life (38, 39). However, after peaking during this time window, the neutrophil activity seems to decrease (37). It is unclear how long this process persists after term.

We and others have shown that infant lung function is most severely altered in very immature infants with chronic lung disease of infancy (21, 40, 41). Furthermore, in our cohort, extremely and very early preterm infants did not show any clear associations of air pollution on lung function. Although we carefully adjusted for known confounders and covariates, and performed extensive sensitivity analysis, in the very preterm group (GA <32 weeks), the relatively small association between air pollution and lung function may have been undetectable. We assume that the lack of association in this subgroup is due to the overwhelming effect of many other dominant developmental and perinatal therapeutic factors which may outweigh the small effects that air pollution might have on overall lung mechanics (21, 27) and ventilatory needs.

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Nevertheless, to the best of our knowledge this is the first study examining the effects of low-to-moderate air pollution levels during pregnancy and early infancy on infant lung function in preterm infants. Across all infants, air pollution showed the strongest association with lung function impairment when occurring during the second trimester of pregnancy. These findings are supported by the physiological development of the lung, where the second trimester is the most important and most susceptible phase (42). However, exact mechanisms underlying the associations of air pollution exposure in-utero with impaired lung function in offspring are still mostly unclear. There is evidence that the interplay of environmental factors and epigenetics lead to impairment of lung development, although the exact interaction is not fully understood (43, 44). It has been suggested that epigenetic mechanisms of lung gene regulation, DNA methylation, ATP-dependent complexes and noncoding RNA might play a role (43). The basic principle seems to be that the pregnant mother inhales pollutants, which either cross the alveoli and the placental barrier and act directly on the fetus or lead to systemic immune or inflammatory reactions in the mother, which ultimately decrease fetal supply of nutrients and/or oxygen (45).

Clinical relevance

Harmful effects of air pollution on the developing lung, especially during time windows of fast growth and when exposed to high air pollution levels have been explored widely (5, 46, 47). As a novelty and proof of principle, we demonstrated that groups of infants with pre-existing vulnerability, such as preterm infants, are more susceptible to detrimental effects of air pollution. Furthermore, the effect of air pollution may depend on the window of exposure during rapid lung development (42, 47), e.g., during the second trimester of pregnancy. The results additionally support the hypothesis of susceptibility of preterm infants to oxidative stress and therefore the necessity to protect them. The findings also have a highly relevant impact on population health. Recent evidence has shown that early-life lung function

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impairment may track through the lifespan, and poor lung functional trajectories from childhood to late senescence are important early-life risk factors for chronic respiratory disease in adults (2, 48-50). Prevention in early-life is therefore one of the most important factors to avert development of chronic respiratory disease (e.g., COPD) and long-term negative health effects in adulthood, and subsequently senescence (2, 12, 48-52). Even though the effect size on an individual level might be small, the effect on a population basis (attributable risk) is of major concern (53). Today, approximately 10% of all infants are born prematurely (14). We best see pollution effects in the epidemiologically largest group of moderate to late preterm infants (32 – 36 weeks GA) and, in some areas of the world, air pollution levels might be even higher (32).

Strengths and limitations

A challenge in this study is the comparison of the exposure windows. The second trimester (*a priori* chosen main exposure window) offered the best comparability and is known for its importance in respect to lung development (42, 47). In our cohort, mothers of preterm infants were often hospitalized on prenatal obstetrics wards several days before giving birth. Term infants left hospital after days, whereas preterm infants were typically hospitalized for weeks after birth. Thus, the calculated and weighted outdoor air pollution exposures for the perinatal (mainly third trimester and postnatal) time periods probably overestimate the true level of air pollution these children were exposed to inside the hospital. This may explain why we did not find any associations between air pollution exposure and lung function during those time periods in the preterm infants.

One clear strength of our study was the assessment of air pollution during pregnancy and after birth, enabling us to study different exposure windows in a sensitivity analysis. Due to the prospective design of the BILD cohort, we additionally had detailed data on potential

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confounders and effect modifiers, gathered from questionnaires and medical records. Additionally, throughout the whole study period lung function measurements were performed using the same equipment and following ERS/ATS guidelines (25). As major improvements in perinatal care were already present at the beginning of this study and pertain predominantly to the care of extremely preterm infants, the main message of the paper is unaffected by changes in perinatal care over the study period.

Although $PM_{2.5}$ effects may biologically be more detrimental, satellite models measuring $PM_{2.5}$ were not available in the early phase of the study period. There is a high collinearity between $PM_{2.5}$ and PM_{10} exposure in Switzerland (54).

Multiple testing adjustment procedures naturally reduce significance levels. Thus, since this is mainly an explorative hypothesis-driven study, further very large studies with higher numbers of subjects are needed to confirm our findings or to perform subgroup analyses as reported by others (6, 46).

Children lost to follow-up were significantly more often exposed to tobacco during pregnancy compared to those followed up. A lower rate of maternal smoking during pregnancy in those children investigated may have led to a selection of children with potentially better lung function.

Conclusion

We demonstrated that prenatal exposure to low-to-moderate air pollution levels as observed in Switzerland was associated with impaired postnatal lung function in term and preterm infants. Significantly increased effects were shown in the moderate to late preterm infants, suggestive of an amplified susceptibility to environmental stimuli such as air pollution. A broad body of literature provides evidence that impaired early-life lung function is associated with later

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respiratory morbidity in adulthood (e.g., COPD). This proof of principle study highlights that some parts of the population, in our situation 10% of infants born prematurely, may have differences in susceptibility to air pollution. This will impact future health and prevention policies in respect to our newborns.

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Figure 1 Decreasing air pollution levels over the 18 years of observation. Depicted are the individual mean levels of PM_{10} ($\mu g/m^3$) exposure for the whole observation period, consisting of pregnancy and postnatal time (defined time between birth and lung function measurement).

Figure 2 Adjusted effect of PM_{10} during the second trimester of pregnancy on lung function parameters in term, preterm and moderate to late preterm infants. Effect estimates of (**A**) minute ventilation (mL/min), (**B**) FeNO (ppb) are presented as coefficient (β) and 95% confidence intervals (CIs) for each 10 $\mu g/m^3$ increase in PM_{10} during second trimester of pregnancy for term, preterm and moderate to late preterm infants. The model was adjusted for sex, weight at lung function measurement, season and postconceptional age at lung function measurement, gestational age at birth, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP and intubation). Analyses for FeNO were additionally adjusted for minute ventilation (mL/min).

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Table 1 Anthropometric data and considered risk factors

	Overall	**Term**	**Preterm**	**Moderate to late preterm (32 – 36 weeks)**
Study participants, n	771	517	254	99
Gestational age at birth, w	36.7 (4.8)	39.8 (3.3)	30.6 (3.2)	33.9 (1.4)
Postconceptional age at lung function measurement, w	44.8 (1.1)	44.8 (1.1)	44.7 (1.2)	44.5 (1.0)
Weight at lung function measurement, g	4327.8 (631.4)	4394.5 (548.1)	4192.0 (757.0)	4438.2 (711.0)
Length at lung function measurement, cm	54.2 (2.7)	54.7 (2.2)	53.2 (3.2)	54.5 (2.8)
Male sex	413 (54)	268 (52)	144 (57)	56 (57)
Maternal smoking during pregnancy	59 (8)	33 (6)	26 (10)	4 (4)
Maternal asthma *	85 (11.0)	65 (13)	20 (8)	9 (9)
Duration of oxygen supplementation, d	10.2 (30.7)	0	30.9 (47.1)	2.6 (5.8)
Duration of mechanical ventilation †, d	5.9 (15.1)	0	18.0 (21.7)	1.5 (2.7)

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Values are mean (standard deviation) or number (percentage). * Asthma was defined as self-reported or doctor-diagnosed asthma. † mechanical ventilation was defined as cPAP and intubation

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Table 2 Lung function data at 44 weeks of postconceptional age

	Overall	**Term**	**Preterm**	**Moderate to late preterm (32 – 36 weeks)**
Tidal breathing				
Minute ventilation, mL/min	1469.3 (323.0)	1451.4 (308.3)	1505.7 (348.7)	1580.3 (339.6)
Respiratory rate, /min	46.9 (11.6)	45.0 (10.6)	50.6 (12.7)	46.9 (9.5)
Tidal volume, mL	32.4 (7.0)	33.1 (5.9)	31.0 (8.6)	34.7 (8.2)
T_{PTEF}/T_E, %	34.0 (10.8)	36.6 (10.9)	28.8 (8.7)	31.9 (9.1)
FeNO, ppb *	14.1 (6.9)	13.7 (6.4)	15.0 (7.7)	17.6 (7.4)

Values are mean (standard deviation) or number (percentage). * information is missing for 71 children (26 term, 45 preterm infants)

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Table 3 Distribution of estimated residential outdoor air pollutants

	Overall	Term	Preterm	Moderate to late preterm (32 – 36 weeks)
PM_{10} µg/m³				
PM_{10} 2nd trimester	19.9 (6.1–44.7)	19.9 (7.5–37.7)	20.0 (6.1–44.7)	18.5 (6.3–38.3)
Postnatal PM_{10} *	19.1 (6.8–54.7)	18.9 (6.8–54.7)	19.5 (10.0–43.9)	17.1 (10.0–43.9)
NO_2 µg/m³				
NO_2 2nd trimester	17.6 (5.3–52.8)	17.9 (5.5–40.7)	16.9 (5.3–52.8)	16.1 (5.3–52.8)
Postnatal NO_2 *	17.4 (4.8–42.3)	16.7 (5.3–41.8)	18.8 (4.8–42.3)	15.2 (4.8–42.3)

Values are mean (range). * postnatal was defined as time between birth and lung function measurement.

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Table 4 Associations of prenatal air pollution exposure with lung function at 44 weeks of postconceptional age

Minute ventilation, mL/min	**Term n = 517**				**Preterm n = 254**				**Moderate to late preterm (32 – 36 weeks) n = 99**			
	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value
PM_{10} 2nd trimester	75.3	(19.7,130.8)	0.008	0.074	88.6	(18.6,158.7)	0.013	0.090	184.9	(79.1,290.7)	0.001	0.139
NO_2 2nd trimester	-2.5	(-43.8,38.9)	0.907	0.769	-12.7	(-71.5,46.0)	0.671	0.707	45.4	(-32.4,123.2)	0.252	0.422
t_{PTEF}/t_E												
PM_{10} 2nd trimester	0.27	(-1.74,2.28)	0.794	0.726	-0.66	(-2.59,1.27)	0.506	0.657	-0.49	(-3.71,2.74)	0.767	0.746
NO_2 2nd trimester	1.07	(-0.46,2.61)	0.17	0.369	0.82	(-0.83,2.47)	0.331	0.489	1.25	(-1.02,3.51)	0.281	0.444
FeNO, ppb												

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PM_{10} 2nd trimester	-0.3	(-1.5,0.9)	0.601	0.679	2.8	(0.8,4.8)	0.005	0.063	3.4	(-0.1,6.8)	0.056	0.229
NO_2 2nd trimester	-0.6	(-1.4,0.3)	0.226	0.413	1.5	(-0.2,3.1)	0.075	0.282	0.5	(-1.4,2.5)	0.591	0.679

Estimates derived from mixed modelling for the association per 10 μg/m^3 increase of PM_{10} and NO_2 with lung function measurements at 44 weeks of postconceptional age. Multivariable model adjusted for sex, weight at lung function measurement, season and postconceptional age at lung function measurement, gestational age at birth, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP and intubation). Analyses for FeNO were additionally adjusted for minute ventilation (mL/min). Results are presented as coefficient (Coef) and 95% confidence intervals (95% CIs) and adjusted p-values after Benjamini-Hochberg correction (for 176 tests).

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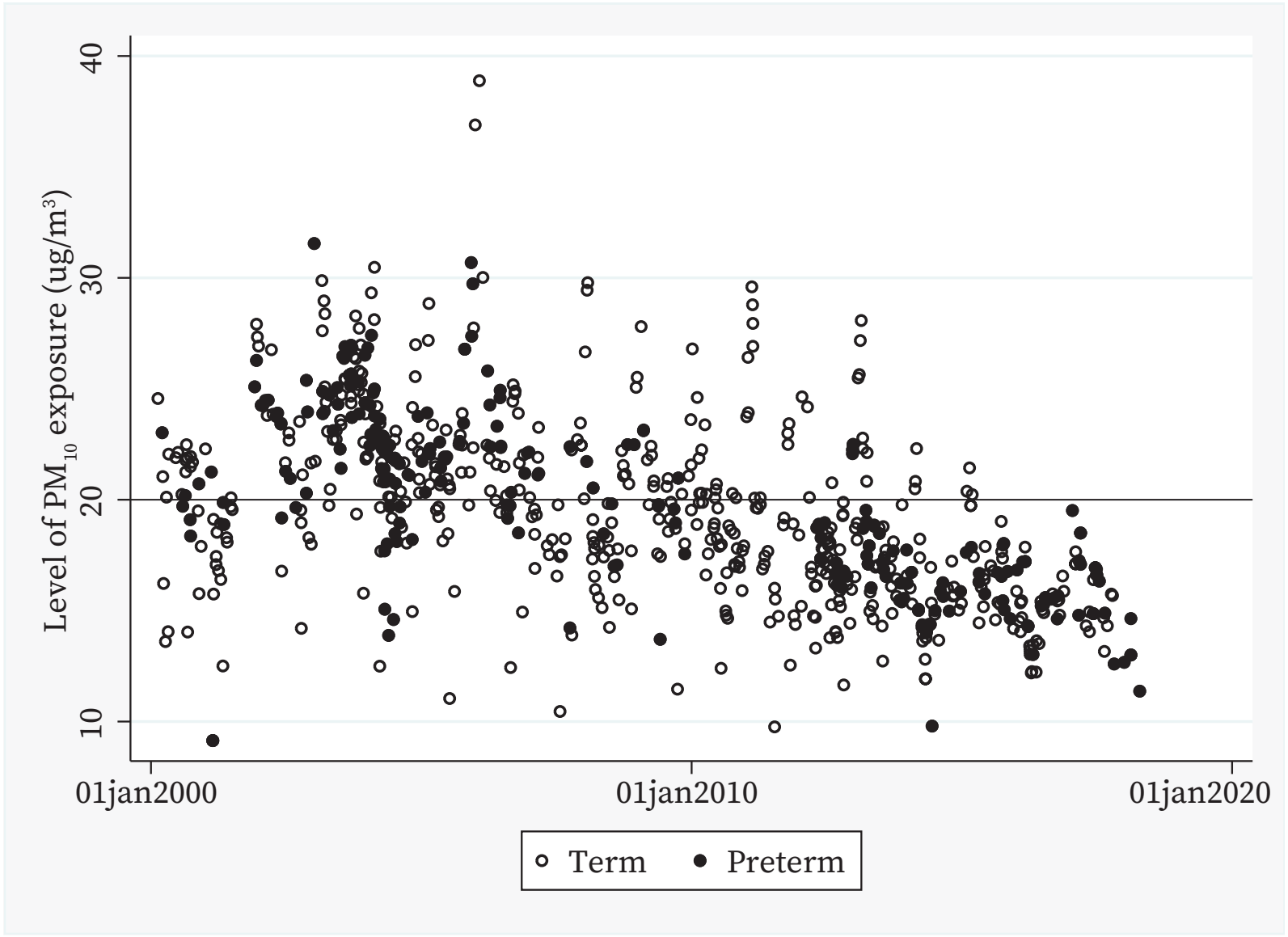

Figure 1

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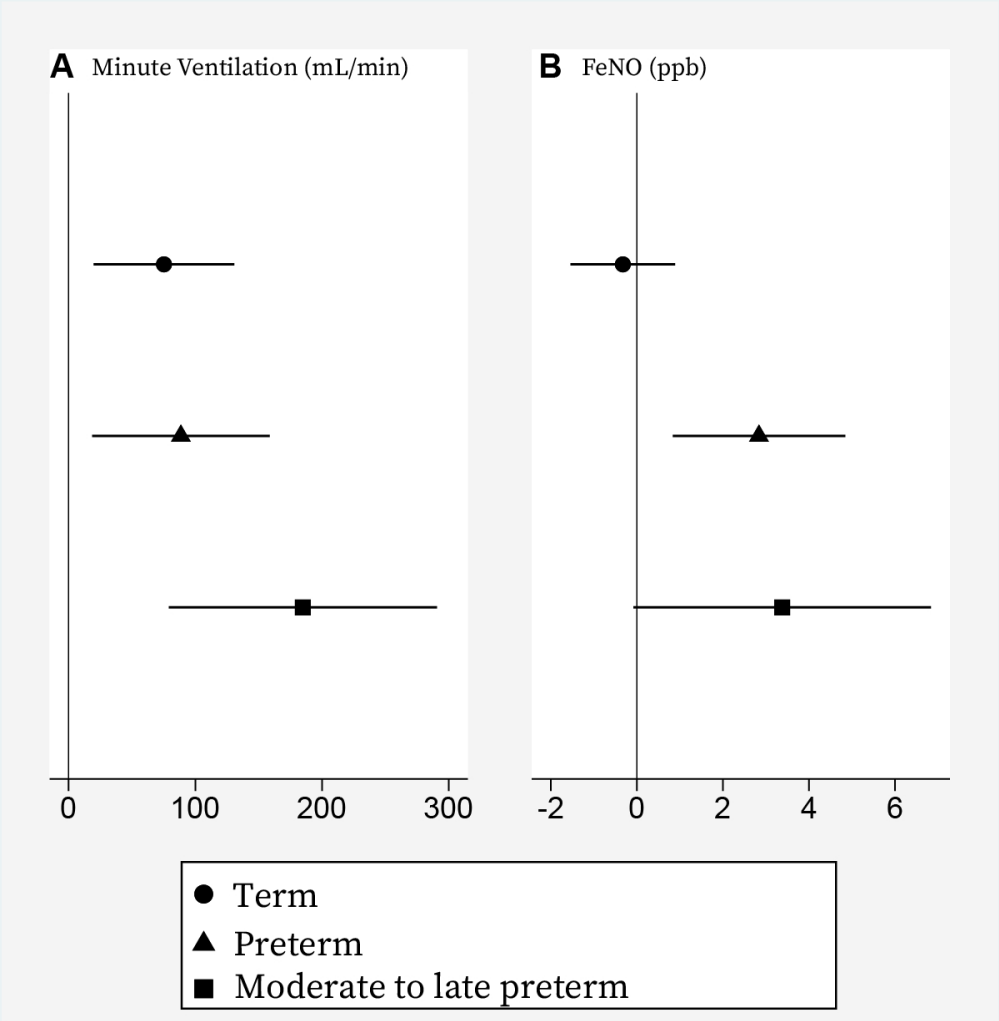

Figure 2 Adjusted effect of PM_{10} during the second trimester of pregnancy on lung function parameters in term, preterm and moderate to late preterm infants. Effect estimates of (**A**) minute ventilation (mL/min), (**B**) FeNO (ppb) are presented as coefficient (β) and 95% confidence intervals (CIs) for each 10 $\mu g/m^3$ increase in PM_{10} during second trimester of pregnancy for term, preterm and moderate to late preterm infants. The model was adjusted for sex, weight at lung function measurement, season and postconceptional age at lung function measurement, gestational age at birth, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP and intubation). Analyses for FeNO were additionally adjusted for minute ventilation (mL/min).

254x259mm (120 x 120 DPI)

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Increased Impact of Air Pollution on Lung Function in Preterm vs. Term Infants: The BILD Study

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Online data supplement

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Methods

Study design

The prospective and ongoing Basel-Bern Infant Lung Development (BILD) birth cohort (https://www.bild-cohort.ch/) comprises unselected neonates recruited antenatally since 1999 in the region of Bern and since 2012 in the region of Basel, Switzerland (E1). Potential risk factors were assessed by interviews using standardized questionnaires (E1). Children underwent lung function measurement at 44 weeks of PCA. Prematurity was defined following International Statistical Classification of Diseases and Related Health Problems (ICD 10) as GA at birth <37 weeks (E2). The ethics committees of the regions of Bern and of Basel approved the study and written consent was obtained.

Air pollution exposure

Air pollution data included daily mean levels of PM_{10}, NO_2 for the period from September 1999 to October 2017. Background air pollution was measured at the Payerne monitoring station (part of the Swiss National Air Pollution Monitoring Network). We estimated NO_2 exposure using a time-space hybrid model, in order to capture seasonal air pollution variations during the entire study period and spatial variation in different study areas. This model was based on high-quality information on land use, population density, traffic, road network, dispersion models, meteorological data and air quality from the fixed measurement station (i.e. Payerne), trained with 28,849 NO_2 biweekly and monthly passive sampler measurements. Observations were collected consecutively over more than ten years at 146 locations, for external validation of the model (E3). We estimated PM_{10} exposure using a simplified spatial-temporal model in which the temporal variation from Payerne was superimposed on the annual dispersion model from Pollumap (E4) which provided the spatial contrast in exposures. Thus, in this model, the averaged PM_{10} per time period (e.g., 1st trimester), based on daily

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measurements at the central monitoring site (i.e., Payerne), were corrected for each address using the ratio between the annual dispersion model value at the home or hospital address (i.e., Pollumap from METEOTEST, 200×200m resolution, 1998- 2015 for PM_{10} (E4)) and the annual mean from Payerne.

From these data we calculated the mean exposure by pollutant for each subject for different exposure windows: 1st trimester, 2nd trimester, 3rd trimester and postnatal. For families who changed their residential address during the study period or for preterm infants hospitalized, we calculated an average exposure estimate weighted by the time spent at each residence or hospital. Addresses were geocoded using a reference file from the Swiss Federal Statistical Office (Neuchâtel).

Lung function outcomes

Pulmonary function was performed at 44 weeks of PCA using Exhalyzer D (EcoMedics, Duernten, Switzerland) according to current ERS/ATS guidelines (E5). For analysis we used the first 100 regular breaths during non-REM (non-rapid eye movement) sleep from the total recorded breathing. We excluded sighs and ten breaths before and after a sigh. Simultaneous to tidal breathing recording, the fraction of exhaled nitric oxide (FeNO) was measured online with a chemoluminescence analyzer during the third quartile of expiration and averaged over the 100 breaths used for analysis. Following ERS/ATS guidelines for infant lung function testing, mean tidal flows, volume and flow-volume loop were calculated.

The following parameters were investigated: respiratory rate (RR), mean tidal volume (V_T), minute ventilation (V_E). V_E is a robust overall estimate of the effectiveness of lung function (ventilatory need) (E6). It does not allow for separating obstructive, or restrictive components of lung mechanics and is also affected by diffusion capacity of the lung. In preterm infants, we typically have a mixture of obstructive, restrictive and impaired diffusion functional

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abnormalities, thus V_E as a composite estimate is an optimal representation of lung function impairment. Ratio of time to peak tidal expiratory flow (PTEF) and expiratory time (t_{PTEF}/t_E) were used to describe TBFVL shapes. Online FeNO measurements were performed with a rapid-response chemoluminescence analyser (CLD 77 AM; EcoMedics AG, Duernten, Switzerland) concurrently with the TBFVL recording. We used air free of nitric oxide (NO) for respiration to prevent contamination of FeNO with ambient NO. The third quartile of each expiration was used to calculate mean FeNO over the 100 breaths recorded and was adjusted for V_E, as previously described (E7).

Covariates

Potential confounders were assessed by interviews using standardized questionnaires (E1) including sociodemographic factors, pre- and postnatal smoke exposure and, parental atopic disease. To confirm prenatal smoke exposure, the cotinine level in the infant's first urine was used.

After inclusion of *a priori* selected covariates from previous literature (E8-E10) the following covariates were included before eliminating the ones with the highest p-values until only significantly associated covariates (p-value <0.05) remained in the model:

Length at birth, sex, length and weight at lung function measurement, gestational age at birth, season and postconceptional age at lung function measurement, maternal smoking during pregnancy, days of supplementary oxygen, days of mechanical ventilation, maternal asthma and, maternal education (as a proxy of socioeconomic status).

Our final model therefore consisted of the following covariates: GA at birth, weight at lung function measurement, maternal asthma (defined as self-reported or doctor-diagnosed), sex, season and postconceptional age at lung function, maternal smoking during pregnancy, days of supplementary oxygen, days of mechanical ventilation (defined as days of continuous positive

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airway pressure (cPAP) and intubation). For FeNO analyses we additionally adjusted for V_E, as previously described (E8, E11).

Statistical analysis

We performed a multilevel mixed-effects linear regression for term and preterm infants separately, adjusted for the above-mentioned covariates and corrected for clustering on center level (Basel vs. Bern). Inspection of the outcome variables suggested normal distribution.

Since we previously showed that lung functional abnormalities can also be the result of prematurity and perinatal insults, particularly in extreme and very early premature born infants (E10), we also performed analysis in subgroups of preterm born infants. We therefore stratified the preterm group according to their gestational age at birth into three clinically relevant groups; extreme (<28 weeks of GA), very early (29 – 31 weeks of GA) and moderate to late (32 – 36 weeks of GA) preterm. We tested the presence of interaction within term and moderate to late preterm born infants adding the interaction term between exposure to air pollution and prematurity.

In a first sensitivity analysis, we performed multiple comparison adjustments for our main hypothesis according to Benjamini-Hochberg (E12). Based on physiological and developmental considerations, such as the timing of lung development in utero, we based our analysis on the second trimester. This was mainly due to the second and third trimester being the most crucial time windows for lung development, however with the third trimester being hardly comparable between term and preterm infants. The issue of multiple lung function outcomes was further to be considered, as minute ventilation is a composite parameter of respiratory rate and tidal volume. Thus, we restricted our analysis to the physiological most independent parameters: minute ventilation, t_{PTEF}/t_E and FeNO (resulting in 6 tests = 2 exposures * 3 outcomes).

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We performed a second sensitivity analysis in respect to previously investigated risk factors, modifiers and mediators, which were however not significantly associated with our outcome in the stepwise backward regression. First, we included education status of the mother in the main model, a known potential confounder for lung function of the offspring (E13, E14). We did not include this risk factor in the main analysis *a priori* as we had several missing values (around 70 children had no information on education status of the mother). We further included CLD classification to the main model, as CLD has been reported to affect lung function irrespective of gestational age (E15). We did not include CLD classification initially, as our main hypothesis was to show differences in the association of air pollution with lung function in moderate to late preterm versus term infants. In these populations only three children had a CLD I. As exposure to air pollution has been linked with poor fetal somatic growth (E16), we additionally adjusted our model for weight at birth. Further, as colleagues previously described the modifying effect of sex on the association between air pollution and lung function (E13, E17, E18), we investigated, whether such modifying effects are reproducible in our population by stratifying for sex. Exposure to caffeine has been shown to influence levels of FeNO measured (E11, E19). We therefore added exposure to caffeine to the main model. We examined whether gestational age was a mediator to the associations of air pollution exposure and lung function (E20). We therefore performed a mediation analysis for PM_{10} and NO_2 using the Structural Equation Modeling (SEM) approach.

In a third sensitivity analysis, we examined the potential effects of other exposure windows and multipollutant models. As short-term exposure to air pollution has been shown to influence lung function in adults (E21), we investigated the impact of short-term exposure to air pollutants (14-day average before lung function measurement) on infant lung function and did not find any associations.

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In a fourth sensitivity analysis, we examined the exact same recruitment periods for term and preterm infants. As no preterm infants were recruited between 2010 and 2012, we excluded term infants born during this time period for a sensitivity analysis.

Pearson's correlation was used to assess correlation between different exposure windows of air pollution and between different air pollutants among these exposure windows (***Table E7***).

Effect estimates are presented as coefficient (β) and 95% confidence intervals (CIs) per 10 $\mu g/m^3$ increase in each pollutant. Data were analysed using Stata® (Stata Statistical Software: release 16. STATA Cooperation; College Station, TX).

Power analysis

We calculated that a sample of 61 subjects would have a power of >80%, at a significance level of 0.05 to detect changes of 24.9 mL/min in V_E per 1 ug/m^3 increase in PM_{10} (E8).

Results

Technical measurements have been systematically harmonized and tested between the centers. Inter-center differences (different populations and different exposures) of the current study and the characteristics and lung function values in preterm and term infants are shown in ***Table E3***.

Association of air pollution with lung function at 44 weeks of postconceptional age

(tidal breathing flow volume loops)

Within the extremely and very early preterm infants no significant associations of prenatal exposure to air pollution on lung function parameters were seen. In the extremely preterm group V_E decreased by [-4.6 (-11.1 to 1.8) mL/min] per 10 $\mu g/m^3$ increase in PM_{10} during the second trimester, whereas in the very early preterm infants, V_E increased by [3.6 (-3.3 to 10.4) mL/min].

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The association of postnatal air pollution with pulmonary function is given in ***Table E5***. In term infants, associations of postnatal PM_{10} exposure with RR and V_T were observed [coefficient (β) (95% confidence intervals (CIs))] [2.1 (0.7 to 3.6) /min], and [-0.9 (-1.7 to -0.2) mL], respectively. Further, V_T decreased by [-1.0 (-1.8 to -0.3) mL] per increase of 10 $\mu g/m^3$ in NO_2. No significant associations between postnatal exposure to air pollutants and lung function were seen in the entire group of preterm infants, nor in the strata.

Fraction of exhaled nitric oxide (FeNO)

Within the extremely and very early preterm infants no significant associations of prenatal exposure to air pollution on FeNO were seen. In the extremely preterm group FeNO increased by [2.6 (-0.3 to 5.4) ppb] per 10 $\mu g/m^3$ increase in PM_{10} during the second trimester, whereas in the very early preterm infants, FeNO increased by [-1.6 (-3.8 to 0.6) ppb].

Postnatal exposure to PM_{10} and NO_2 showed no association with FeNO either in term or preterm infants (***Table E5***).

Sensitivity analysis

a. <u>Multiple comparison adjustment</u>

After multiple comparison adjustment according to Benjamini-Hochberg (6 tests = 2 exposures * 3 outcomes), the interaction of prematurity and air pollution on V_E in term and moderate to late preterm infants reached a non-significant p-value ($p_{prematurity \times PM10} = 0.09$). However, the interaction between PM_{10} and prematurity on FeNO remained significant ($p_{prematurity \times PM10} = 0.036$).

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b. Potential confounders

Using CLD classification as an additional confounder to the main model resulted in an increase in V_E [197.6 (92.1 to 303.2) mL/min] in moderate to late and [86.6 (16.9 to 156.4) mL/min] in preterm infants per 10 μg/m^3 increase in PM_{10} during the second trimester.

When adjusting the main model for birth weight, associations did not change substantially and remained robust.

When stratifying the main model by sex and using interaction terms of sex and air pollution, no clear differences between boys and girls were seen, nor did the effect sizes change substantially to the earlier reported findings. Interaction terms were not significant, and were therefore not included in the main model.

In the preterm group, FeNO increased by [9.0 (3.3 to 14.6) ppb] per 10 μg/m3 increase in PM_{10} and by [5.1 (-0.5 to 10.6) ppb] per 10 μg/m^3 increase in NO_2 during the second trimester when exposure to caffeine citrate was included in the main model. However, information on exposure to caffeine citrate was only available for 47 preterm infants, all infants were off drugs for > 6 weeks.

As we had many missing data on education status of the mother (70 missing), we included it as a confounder in the sensitivity analysis. Education status of the mother, categorized into low (less than four years of apprenticeship), middle (four years of apprenticeship and above) and high (tertiary education), as an additional confounder in the main model, did not have any substantial changes in effect sizes nor significance level (V_E in term infants increased by [75.1 (19.6 to 130.6) mL/min] per 10 μg/m^3 increase in PM_{10} during the second trimester).

Including length as a confounder in the main model, did not change the effect sizes, significance levels or interaction terms (V_E in term infants increased by [77.1 (21.3 to 132.8)

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mL/min] per 10 µg/m^3 increase in PM_{10} during the second trimester). However, correlation between weight and length at measurement date was high with 0.76 (Pearson correlation).

The effect of air pollution on V_E was not mediated by gestational age (***Figure E4***).

c. Potential effects of other pre- and postnatal exposure windows and multipollutant models

Additional analysis for short-term exposure to air pollutants showed no significant associations with lung function parameters (V_E in term infants increased by [16.3 (-11.5 to 44.1) mL/min], in preterm infants decreased by [-5.8 (-46.8 to 35.2) mL/min] and in moderate to late preterm infants decreased by [-51.0 (-136.3 to 34.3) mL/min] per 10 µg/m^3 increase in PM_{10}). No significant associations were seen between short-term exposure to air pollution and FeNO (FeNO in term infants decreased by [-0.4 (-1.0 to 0.3) ppb], in preterm infants increased by [0.6 (-0.6 to 1.7) ppb] and in moderate to late preterm infants increased by [0.7 (-1.4 to 2.9) ppb] per 10 µg/m^3 increase in PM_{10}). When analysing the association of exposure to air pollution in multipollutant models, effect sizes and significance levels remained comparable to the results from one-pollutant models. V_E increased significantly in term by [95.3 (33.3 to 157.2) mL/min], in preterm by [96.0 (22.4 to 169.7) mL/min] and in moderate to late preterm by [184.5 (71.8 to 297.2) mL/min] (***Table E6***). Associations of PM_{10} with V_E and PM_{10} with FeNO differed between moderate to late preterm and term infants ($p_{\text{prematurity} \times \text{PM10 interaction}} = 0.045$ and $p_{\text{prematurity} \times \text{PM10 interaction}} = 0.009$, adjusted for multiple testing $p_{\text{prematurity} \times \text{PM10 interaction}} = 0.202$ and $p_{\text{prematurity} \times \text{PM10 interaction}} = 0.074$).

d. Recruitment period

430 term infants remained for the sensitivity analysis after excluding term infants born between 2010 and 2012. In this population of term infants V_E increased by [80.6 (18.9 to 142.3)

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mL/min] and FeNO decreased by [-1.7 (-2.9 to -0.4) mL/min] per 10 μg/m^3 increase in PM_{10} during the second trimester.

Correlation between different exposure windows for air pollutants was low-to-moderate for different exposure windows of PM_{10} (***Table E7***).

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Figure E1 Flow chart of the study population. *Dropout due to missing lung function measurement at 44 weeks of postconceptional age, missing air pollution exposure values, missing information on important risk factors and dropout of the child from the study. † Quality check included lung function quality or gestational age at lung function measurement <42 or >48 weeks of postconceptional age. ‡ Outliers included moderate to late preterm infants, which had severe asphyxia and therefore needed mechanical ventilation >20days and/or supplementary oxygen >80days.

Figure E2 Duration of mechanical ventilation (defined as cPAP and intubation) (**A**) and of supplementary oxygen (**B**) according to gestational age at birth shown for preterm infants. Vertical lines represent the three groups of preterm infants (extremely, very early and moderate to late).

Figure E3 Adjusted effect of PM_{10} during the second trimester of pregnancy on lung function parameters in term, preterm, moderate to late, very early and extremely preterm infants. Effect estimates of (**A**) minute ventilation (mL/min), (**B**) tidal volume (mL) and (**C**) FeNO (ppb) are presented as coefficient (β) and 95% confidence intervals (CIs) for each 10 $\mu g/m^3$ increase in PM_{10} during second trimester of pregnancy for term, preterm, moderate to late, very early and extremely preterm infants. The model was adjusted for sex, weight at lung function measurement, season and postconceptional age at lung function measurement, gestational age at birth, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP and intubation). Analyses for FeNO were additionally adjusted for minute ventilation (mL/min).

Figure E4 Pathway of mediation analysis. The mediation effect of gestational age (GA) at birth on the association of air pollution exposure with lung function was examined using the

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Structural Equation Modeling (SEM) approach. Left side: Pathway of mediation effect of PM_{10} on V_E. Right side: Pathway of mediation effect of NO_2 on V_E in preterm infants; c'= total effect of air pollution on V_E; c' = c+a*b; c = the direct effect of air pollution on V_E after controlling for gestational age (GA); a*b = indirect effect of air pollution on V_E; (i) Mixed regression of the mediator GA on the independent variable air pollution exposure in the second trimester adjusted for season; $GA_{ij} = \alpha+b_1PM_{10ij}+b_{2seasonij}+u_j+e_{ij}$; Where *u* are *e* residuals for individual *i* and study center *j*; (ii) Mixed regression of the lung function (here we showed the effect on V_E) on the independent variable air pollution exposure in the second trimester and potential mediator GA adjusted for season; $V_{Eij}=\alpha+b_1PM_{10ij}+b_{2seasonij}+b_3GA_{ij}+ u_j+e_{ij}$

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Table E1 Anthropometric data and considered risk factors

	Very early preterm	Extremely preterm
Study participants, n	99	65
Gestational age at birth, w	29.9 (1.1)	26.4 (1.1)
Postconceptional age at lung function measurement, w	44.9 (1.3)	44.5 (1.1)
Weight at lung function measurement, g	4172.8 (779.3)	3843.7 (655.0)
Length at lung function measurement, cm	53.0 (3.2)	51.3 (2.8)
Male sex	53 (59)	35 (54)
Maternal smoking during pregnancy	10 (11)	12 (18)
Maternal asthma *	7 (8)	4 (6)
Duration of oxygen supplementation, d	28.7 (34.9)	77.1 (60.2)
Duration of mechanical ventilation †, d	16.4 (13.3)	45.3 (20.6)

Values are mean (standard deviation) or number (percentage). * Asthma was defined as self-reported or doctor-diagnosed asthma. † mechanical ventilation was defined as cPAP or intubation

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Table E2 Comparison between participants lost to follow-up / excluded vs. included participants

	Included	**Lost to follow-up / excluded**	**p-value**
Study participants, n	771	329	
Gestational age at birth, weeks	36.7 (4.8)	34.4 (5.6)	<0.001
Weight at study date, g	4327.8 (631.4)	4179.6 (835.1)	0.003
Length at study date, cm	54.2 (2.7)	53.7 (4.0)	0.027
Male sex	413 (54)	190 (58)	0.126
Maternal smoking during pregnancy *	59 (8)	29 (11)	0.102
Maternal asthma †	85 (11)	12 (5)	0.028
Duration of oxygen supplementation, d ‡	10.2 (30.7)	52.3 (80.4)	<0.001

Values are mean (standard deviation) or number (percentage). * information is missing for 74 children lost to follow-up. † Asthma was defined as self-reported or doctor-diagnosed asthma, missing information for 100 children lost to follow-up. ‡ information is missing for 144 children lost to follow-up.

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Table E3 Inter-center differences in characteristics and lung function at 44 weeks of postconceptional age in term and preterm infants

	Term			Preterm		
	Bern n = 391	**Basel n = 126**	**p-value**	**Bern n = 146**	**Basel n = 108**	**p-value**
Characteristics						
Male sex	204 (52)	65 (52)	0.909	85 (58)	59 (55)	0.570
Gestational age at birth, w	39.8 (1.1)	39.7 (1.1)	0.426	29.1 (2.9)	32.5 (2.6)	<0.001
Postconceptional age at lung function measurement, w	44.8 (1.1)	44.9 (1.2)	0.680	44.7 (1.2)	44.7 (1.1)	0.982
Postnatal age at lung function measurement, d	35.7 (4.5)	36.3 (7.4)	0.315	108.7 (21.3)	85.2 (20.7)	<0.001
Weight at lung function measurement, g	4377.4 (531.7)	4447.9 (595.2)	0.209	3949.3 (750.7)	4520.2 (634.1)	<0.001

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Maternal smoking during pregnancy	29 (7)	4 (3)	0.091	17 (12)	9 (8)	0.392
Tidal breathing						
Minute ventilation, mL/min	1418.1 (268.0)	1554.7 (392.6)	<0.001	1423.0 (332.8)	1617.5 (339.8)	<0.001
Respiratory rate, /min	44.7 (10.2)	46.2 (11.7)	0.175	51.7 (13.2)	49.2 (11.9)	0.130
Tidal volume, mL	32.6 (5.3)	34.7 (7.4)	<0.001	28.8 (8.0)	34.1 (8.4)	<0.001
T_{PTEF}/T_E, %	36.1 (10.9)	37.8 (10.8)	0.126	28.2 (9.1)	29.7 (8.1)	0.192
FeNO, ppb *	13.4 (6.0)	14.6 (7.4)	0.078	12.7 (7.3)	17.8 (7.4)	<0.001

Values are mean (standard deviation) or number (percentage). * information is missing for 71 children (26 term, 45 preterm infants)

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Table E4 Distribution of estimated residential outdoor air pollutants over all time windows

	Overall	Term	Preterm	Moderate to late preterm (32 – 36 weeks)
PM_{10} µg/m^3				
PM_{10} 1st trimester	20.2 (6.3–44.6)	20.1 (9.0–44.6)	20.2 (6.3–41.8)	18.5 (6.3–36.1)
PM_{10} 2nd trimester	19.9 (6.1–44.7)	19.9 (7.5–37.7)	20.0 (6.1–44.7)	18.5 (6.3–38.3)
PM_{10} 3rd trimester	19.5 (6.6–62.7)	19.8 (8.2–40.4)	18.7 (6.6–62.7)	17.1 (6.6–36.6)
Postnatal PM_{10} *	19.1 (6.8–54.7)	18.9 (6.8–54.7)	19.5 (10.0–43.9)	17.1 (10.0–43.9)
NO_2 µg/m^3				
NO_2 1st trimester	17.6 (5.1–46.4)	17.7 (6.5–46.4)	17.5 (5.1 - 45.4)	16.6 (5.1–45.4)
NO_2 2nd trimester	17.6 (5.3–52.8)	17.9 (5.5–40.7)	16.9 (5.3–52.8)	16.1 (5.3–52.8)
NO_2 3rd trimester	16.9 (4.9–49.4)	17.3 (5.4–41.5)	15.9 (4.9–49.4)	14.6 (4.9–49.4)
Postnatal NO_2 *	17.4 (4.8–42.3)	16.7 (5.3–41.8)	18.8 (4.8–42.3)	15.2 (4.8–42.3)

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Values are mean (range). * postnatal was defined as time between birth and lung function measurement.

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Table E5 Associations of pre- and postnatal air pollution exposure (for all time windows) with lung function at 44 weeks of postconceptional age

	Term n = 517				**Preterm n = 254**				**Moderate to late preterm (32 – 36 weeks) n = 99**			
Minute ventilation, mL/min	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value
PM_{10} 1st trimester	22.43	(-21.39,66.24)	0.316	0.483	6.51	(-53.84,66.86)	0.833	0.737	160.66	(51.74,269.59)	0.004	0.056
PM_{10} 2nd trimester	75.27	(19.74,130.79)	0.008	0.070	88.62	(18.57,158.68)	0.013	0.086	162.62	(59.37,265.87)	0.002	0.035
PM_{10} 3rd trimester	13.11	(-32.32,58.53)	0.572	0.680	-35.94	(-86.18,14.31)	0.161	0.373	-86.4	(-185.16,12.36)	0.086	0.278
PM_{10} postnatal *	33.27	(-7.24,73.77)	0.107	0.316	0.83	(-75.43,77.08)	0.983	0.790	20.73	(-106.33,147.80)	0.749	0.738
NO_2 1st trimester	3.97	(-31.57,39.52)	0.827	0.737	11.74	(-40.52,63.99)	0.66	0.700	75.25	(0.29,150.22)	0.049	0.213
NO_2 2nd trimester	-2.45	(-43.79,38.88)	0.907	0.764	-12.74	(-71.52,46.04)	0.671	0.701	45.42	(-32.35,123.20)	0.252	0.417

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NO_2 3rd trimester	-3.53	(-40.37,33.32)	0.851	0.744	-36.93	(-91.16,17.30)	0.182	0.383	-4.44	(-78.71,69.82)	0.907	0.759
NO_2 postnatal *	7.27	(-35.18,49.73)	0.737	0.742	-44.16	(-107.92,19.60)	0.175	0.374	-15.47	(-103.51,72.57)	0.731	0.742
Tidal volume, mL												
PM_{10} 1st trimester	0.52	(-0.30,1.34)	0.215	0.404	-0.1	(-1.44,1.24)	0.888	0.762	1.37	(-0.87,3.61)	0.23	0.410
PM_{10} 2nd trimester	1.22	(0.18,2.26)	0.022	0.122	1.33	(-0.18,2.85)	0.084	0.285	3.82	(1.57,6.08)	0.001	0.046
PM_{10} 3rd trimester	-0.96	(-1.80,-0.12)	0.025	0.133	-0.1	(-1.22,1.02)	0.856	0.744	-2.55	(-4.62,-0.48)	0.016	0.097
PM_{10} postnatal *	-0.92	(-1.68,-0.17)	0.016	0.101	0.08	(-1.61,1.78)	0.924	0.765	-0.22	(-2.92,2.48)	0.873	0.754
NO_2 1st trimester	0.09	(-0.57,0.76)	0.787	0.729	-0.17	(-1.33,0.99)	0.772	0.740	0.45	(-1.18,2.07)	0.589	0.682
NO_2 2nd trimester	-0.58	(-1.36,0.19)	0.139	0.372	-0.42	(-1.73,0.88)	0.527	0.660	-0.02	(-1.69,1.64)	0.98	0.792
NO_2 3rd trimester	-1.1	(-1.78,-0.41)	0.002	0.040	-0.59	(-1.79,0.62)	0.339	0.496	-0.87	(-2.44,0.70)	0.277	0.443
NO_2 postnatal *	-1.04	(-1.83,-0.25)	0.01	0.077	-0.58	(-2.00,0.84)	0.422	0.581	-1.25	(-3.13,0.64)	0.194	0.397

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FeNO, ppb												
PM_{10} 1st trimester	0.06	(-0.89,1.00)	0.909	0.757	-0.45	(-2.22,1.32)	0.622	0.686	0.46	(-2.42,3.33)	0.756	0.740
PM_{10} 2nd trimester	-0.32	(-1.54,0.89)	0.601	0.674	2.84	(0.84,4.84)	0.005	0.053	3.38	(-0.08,6.83)	0.056	0.222
PM_{10} 3rd trimester	-0.71	(-1.72,0.29)	0.162	0.369	1.19	(-0.26,2.64)	0.109	0.316	2.39	(-0.16,4.94)	0.066	0.255
PM_{10} postnatal *	-0.13	(-1.04,0.77)	0.771	0.744	0.84	(-1.52,3.21)	0.485	0.642	1.67	(-2.10,5.43)	0.385	0.546
NO_2 1st trimester	-0.22	(-0.98,0.55)	0.576	0.678	0.63	(-0.87,2.12)	0.412	0.573	0.48	(-1.51,2.47)	0.637	0.692
NO_2 2nd trimester	-0.55	(-1.44,0.34)	0.226	0.408	1.46	(-0.15,3.07)	0.075	0.274	0.53	(-1.39,2.45)	0.591	0.671
NO_2 3rd trimester	-0.43	(-1.23,0.37)	0.288	0.450	0.87	(-0.64,2.38)	0.26	0.425	0.21	(-1.59,2.00)	0.822	0.737
NO_2 postnatal *	-0.47	(-1.39,0.44)	0.312	0.482	0.43	(-1.43,2.29)	0.651	0.696	0.00	(-2.23,2.23)	0.999	0.789
Respiratory rate, /min												
PM_{10} 1st trimester	-0.46	(-2.03,1.11)	0.567	0.679	0.43	(-2.12,2.97)	0.741	0.741	2.00	(-1.33,5.32)	0.239	0.415

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PM_{10} 2nd trimester	-0.27	(-2.19,1.66)	0.787	0.724	0.29	(-2.60,3.19)	0.843	0.742	0.66	(-2.87,4.18)	0.715	0.731
PM_{10} 3rd trimester	1.75	(0.11,3.38)	0.036	0.173	-1.42	(-3.53,0.69)	0.186	0.386	1.17	(-1.98,4.33)	0.467	0.624
PM_{10} postnatal *	2.12	(0.65,3.58)	0.005	0.058	-0.37	(-3.59,2.84)	0.820	0.740	1.93	(-2.06,5.92)	0.343	0.497
NO_2 1st trimester	-0.34	(-1.63,0.95)	0.609	0.677	0.77	(-1.43,2.97)	0.494	0.648	1.79	(-0.59,4.18)	0.141	0.363
NO_2 2nd trimester	0.47	(-1.03,1.96)	0.539	0.657	0.17	(-2.31,2.65)	0.893	0.762	1.77	(-0.67,4.22)	0.156	0.368
NO_2 3rd trimester	1.36	(0.03,2.70)	0.045	0.209	-0.75	(-3.04,1.53)	0.517	0.665	1.5	(-0.83,3.82)	0.206	0.403
NO_2 postnatal *	1.51	(-0.03,3.04)	0.054	0.227	-0.85	(-3.54,1.85)	0.539	0.651	2.07	(-0.72,4.86)	0.145	0.366
t_{PTEF}/t_E												
PM_{10} 1st trimester	-0.91	(-2.51,0.70)	0.267	0.432	0.7	(-1.00,2.39)	0.422	0.575	1.88	(-1.21,4.98)	0.233	0.410
PM_{10} 2nd trimester	0.27	(-1.74,2.28)	0.794	0.726	-0.66	(-2.59,1.27)	0.506	0.657	-0.49	(-3.71,2.74)	0.767	0.746
PM_{10} 3rd trimester	0.55	(-1.12,2.21)	0.519	0.662	-0.73	(-2.17,0.71)	0.323	0.488	-0.43	(-3.37,2.51)	0.774	0.737
PM_{10} postnatal *	0.04	(-1.46,1.54)	0.959	0.784	-1.88	(-4.02,0.25)	0.084	0.278	-2.68	(-6.45,1.09)	0.164	0.362

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NO_2 1st trimester	0.50	(-0.82,1.83)	0.454	0.613	1.24	(-0.23,2.70)	0.098	0.303	1.78	(-0.43,3.99)	0.114	0.317
NO_2 2nd trimester	1.07	(-0.46,2.61)	0.17	0.369	0.82	(-0.83,2.47)	0.331	0.489	1.25	(-1.02,3.51)	0.281	0.444
NO_2 3rd trimester	1.14	(-0.23,2.51)	0.102	0.308	0.19	(-1.37,1.75)	0.813	0.739	0.36	(-1.80,2.53)	0.743	0.738
NO_2 postnatal *	1.19	(-0.39,2.76)	0.139	0.365	0.06	(-1.74,1.87)	0.944	0.776	0.39	(-2.29,3.08)	0.774	0.732

Estimates derived from mixed modelling for the association per 10 μg/m^3 increase of PM_{10} and NO_2 with lung function measurements at 44 weeks of postconceptional age. Multivariable model adjusted for sex, weight at lung function measurement, gestational age at birth, season at lung function measurement, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP and intubation). Analyses for FeNO were additionally adjusted for minute ventilation (mL/min). * postnatal was defined as time between birth and lung function measurement. Results are presented as coefficient (Coef) and 95% confidence intervals (95% CIs) and adjusted p-values after Benjamini-Hochberg correction (for 176 tests).

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Table E6 Multipollutant models of pre- and postnatal air pollution exposure with lung function at 44 weeks of postconceptional age

Minute ventilation, mL/min	**Term n = 517**				**Preterm n = 254**				**Moderate to late preterm (32 – 36 weeks) n = 99**			
	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value	Coef	95% CIs	p-value	Adj. p-value
PM_{10} 2nd trimester	95.3	(33.3, 157.2)	0.003	0.046	96.0	(22.4,169.7)	0.011	0.080	184.5	(71.8, 397.2)	0.001	0.028
NO_2 2nd trimester	-33.2	(-79.0, 12.6)	0.155	0.371	-45.0	(-108.1,18.1)	0.162	0.363	0.8	(-78.1, 79.7)	0.984	0.786
PM_{10} postnatal *	40.0	(-7.1, 87.1)	0.096	0.303	66.9	(-35.8,169.6)	0.202	0.407	37.4	(-112.0, 186.8)	0.623	0.682
NO_2 postnatal *	-13.9	(-63.1, 35.4)	0.581	0.679	-82.1	(-35.8,169.6)	0.202	0.401	-22.2	(-127.3, 82.8)	0.678	0.703
Tidal volume, mL												
PM_{10} 2nd trimester	1.9	(0.8, 3.1)	0.001	0.035	1.8	(0.2,3.5)	0.031	0.160	4.4	(2.0, 6.7)	<0.001	0.023

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NO_2 2nd trimester	-1.2	(-2.1, -0.3)	0.006	0.060	-1.0	(-2.4,0.4)	0.152	0.377	-1.1	(-2.7, 0.6)	0.207	0.394
PM_{10} postnatal *	-0.6	(-1.4, 0.3)	0.207	0.400	1.0	(-1.3, 3.3)	0.386	0.542	1.0	(-2.2, 4.1)	0.537	0.661
NO_2 postnatal *	-0.7	(-1.7, 0.2)	0.111	0.315	-1.2	(-3.1, 0.8)	0.239	0.410	-1.6	(-3.8, 0.6)	0.152	0.371
FeNO, ppb												
PM_{10} 2nd trimester	-0.8	(-2.2, 0.6)	0.240	0.407	2.3	(0.2, 4.4)	0.035	0.174	3.1	(-0.4, 6.6)	0.083	0.296
NO_2 2nd trimester	-0.3	(-1.4, 0.7)	0.529	0.657	1.1	(-0.6, 2.8)	0.215	0.398	0.0	(-2.0, 2.0)	0.993	0.789
PM_{10} postnatal *	-0.2	(-1.3, 0.9)	0.699	0.720	0.4	(-2.5, 3.4)	0.774	0.727	2.1	(-2.1, 6.4)	0.329	0.492
NO_2 postnatal *	-0.5	(-1.6, 0.6)	0.364	0.522	0.4	(-1.9, 2.7)	0.774	0.722	-0.6	(-3.1, 1.9)	0.641	0.691

Estimates derived from mixed modelling for the association per 10 $\mu g/m^3$ increase of either trimester or postnatal PM_{10} and NO_2 with lung function measurements at 44 weeks of postconceptional age. Multivariable model adjusted for sex, weight at lung function measurement, season and postconceptional age at lung function measurement, gestational age at birth, maternal smoking during pregnancy, maternal asthma (defined as self-reported or doctor-diagnosed), days of supplementary oxygen, days of mechanical ventilation (defined as cPAP or intubation). Analyses for FeNO were additionally adjusted

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for minute ventilation (mL/min). Results are presented as coefficient (Coef) and 95% confidence intervals (95% CIs) and adjusted p-values after Benjamini-Hochberg correction (for 176 tests). * postnatal was defined as time between birth and lung function measurement.

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	PM_{10} 1st trimester	PM_{10} 2nd trimester	PM_{10} 3rd trimester	PM_{10} Postnatal
PM_{10} 1st trimester	1.0000			
PM_{10} 2nd trimester	0 .2051	1.0000		
PM_{10} 3rd trimester	0.0025	0.2114	1.0000	
PM_{10} Postnatal *	0.1518	-0.0019	0.2672	1.0000
NO_2 1st trimester	0.7328	0.2110	-0.3809	-0.2155
NO_2 2nd trimester	-0.1463	0.7135	0.2425	-0.3089
NO_2 3rd trimester	0.0246	0.2335	0.5860	0.4063
NO_2 Postnatal *	0.2907	0.0194	0.3067	0.6598

	NO_2 1st trimester	NO_2 2nd trimester	NO_2 3rd trimester	NO_2 Postnatal *

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NO_2 1st trimester	1.0000			
NO_2 2nd trimester	0.7154	1.0000		
NO_2 3rd trimester	0.4502	0.7662	1.0000	
NO_2 Postnatal *	-0.1508	-0.3361	0.1935	1.0000

Table E7 Pearson's correlation of air pollutants (PM_{10} and NO_2) among different time windows. * postnatal was defined as time between birth and lung function measurement.

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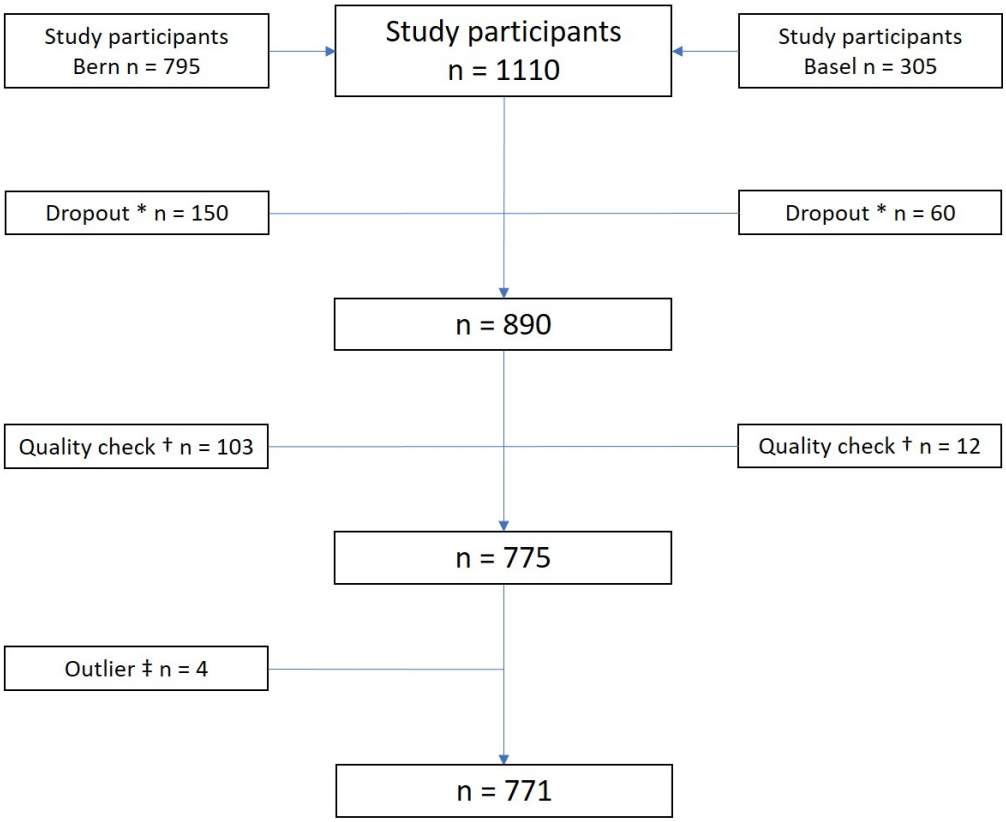

Figure E1 Flow chart of the study population. *Dropout due to missing lung function measurement at 44 weeks of postconceptional age, missing air pollution exposure values, missing information on important risk factors and dropout of the child from the study. † Quality check included lung function quality or gestational age at lung function measurement <42 or >48 weeks of postconceptional age. ‡ Outliers included moderate to late preterm infants, which had severe asphyxia and therefore needed mechanical ventilation >20days and/or supplementary oxygen >80days.

197x163mm (150 x 150 DPI)

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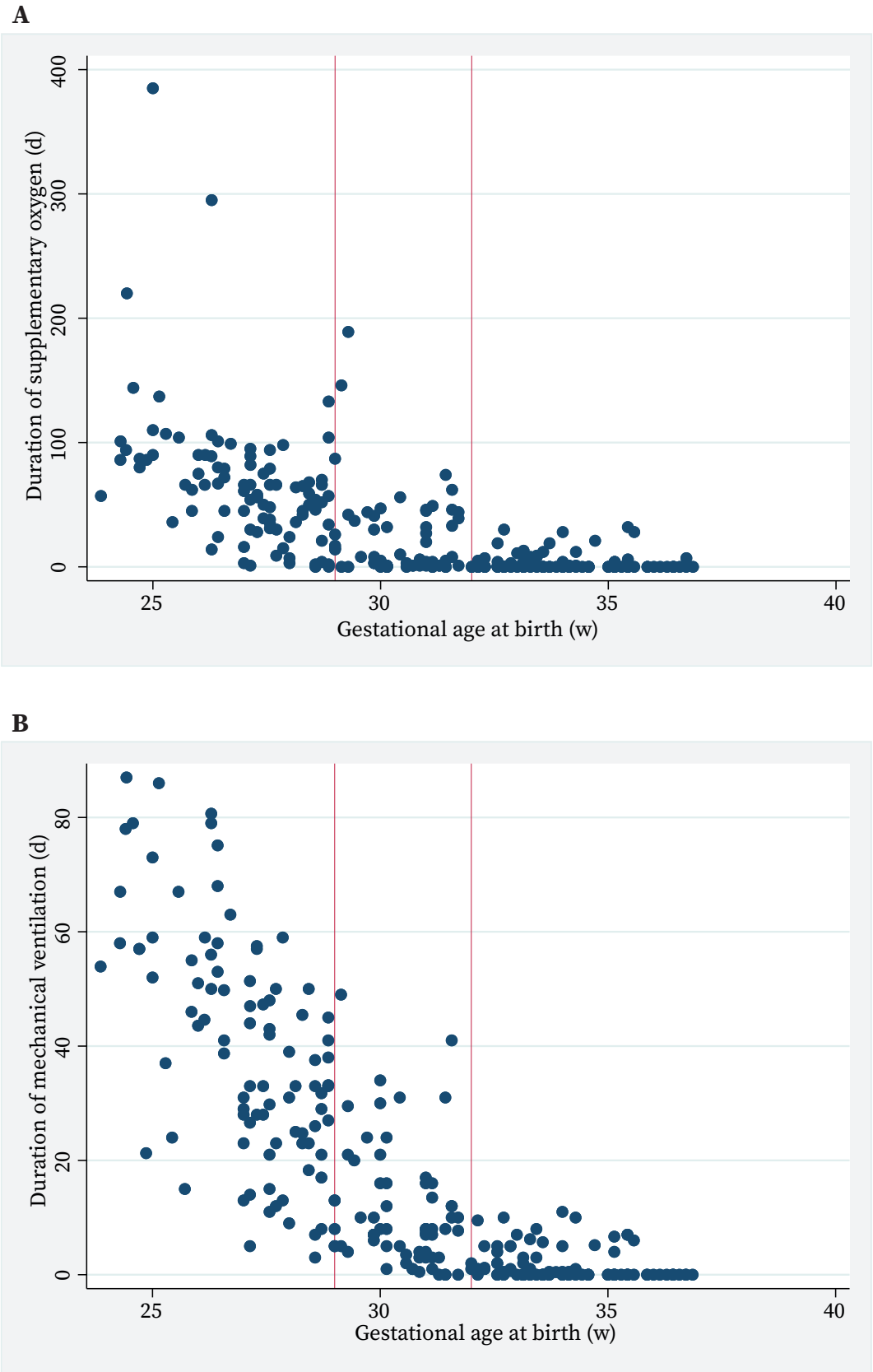

Figure E2

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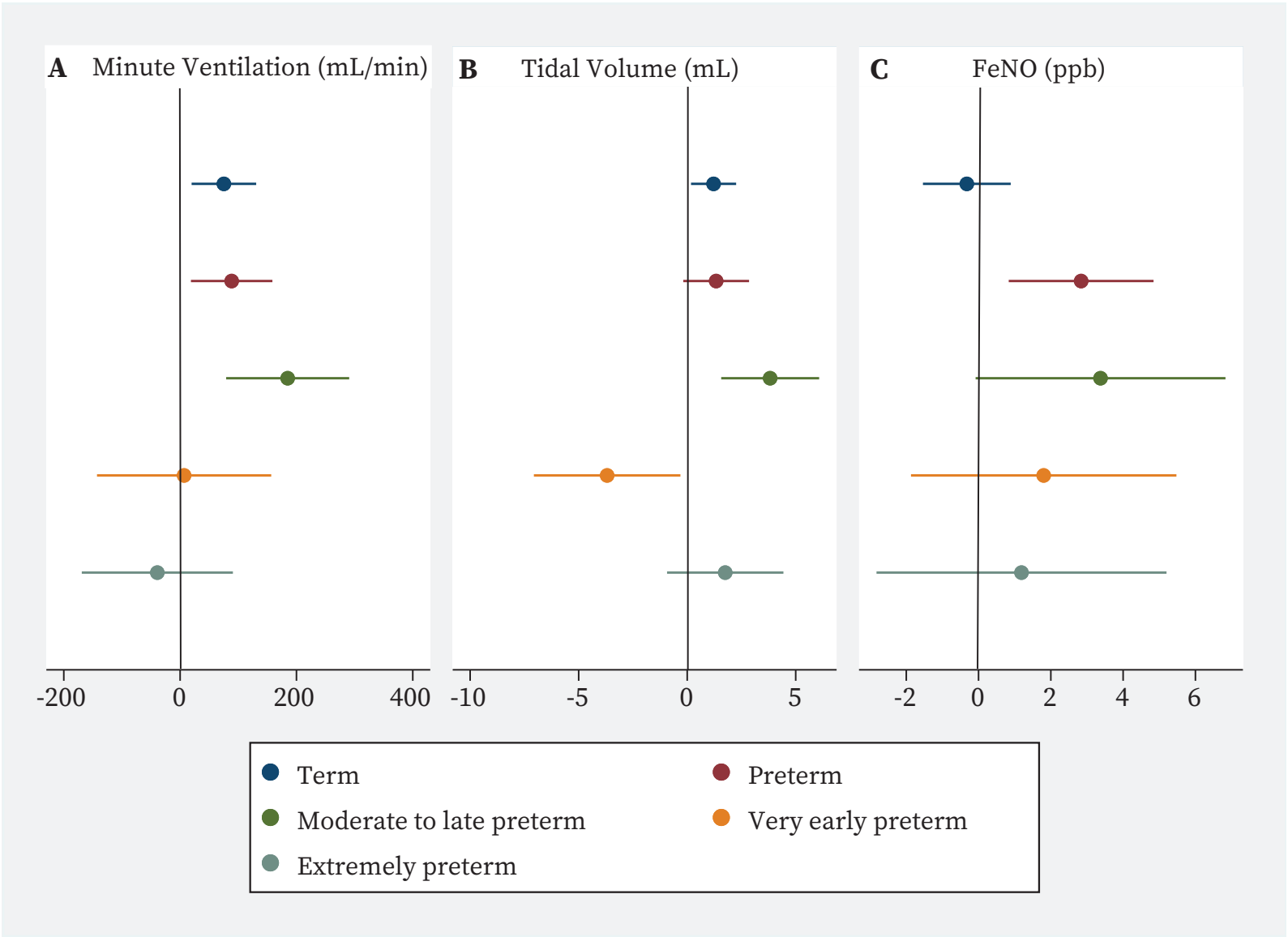

Figure E3

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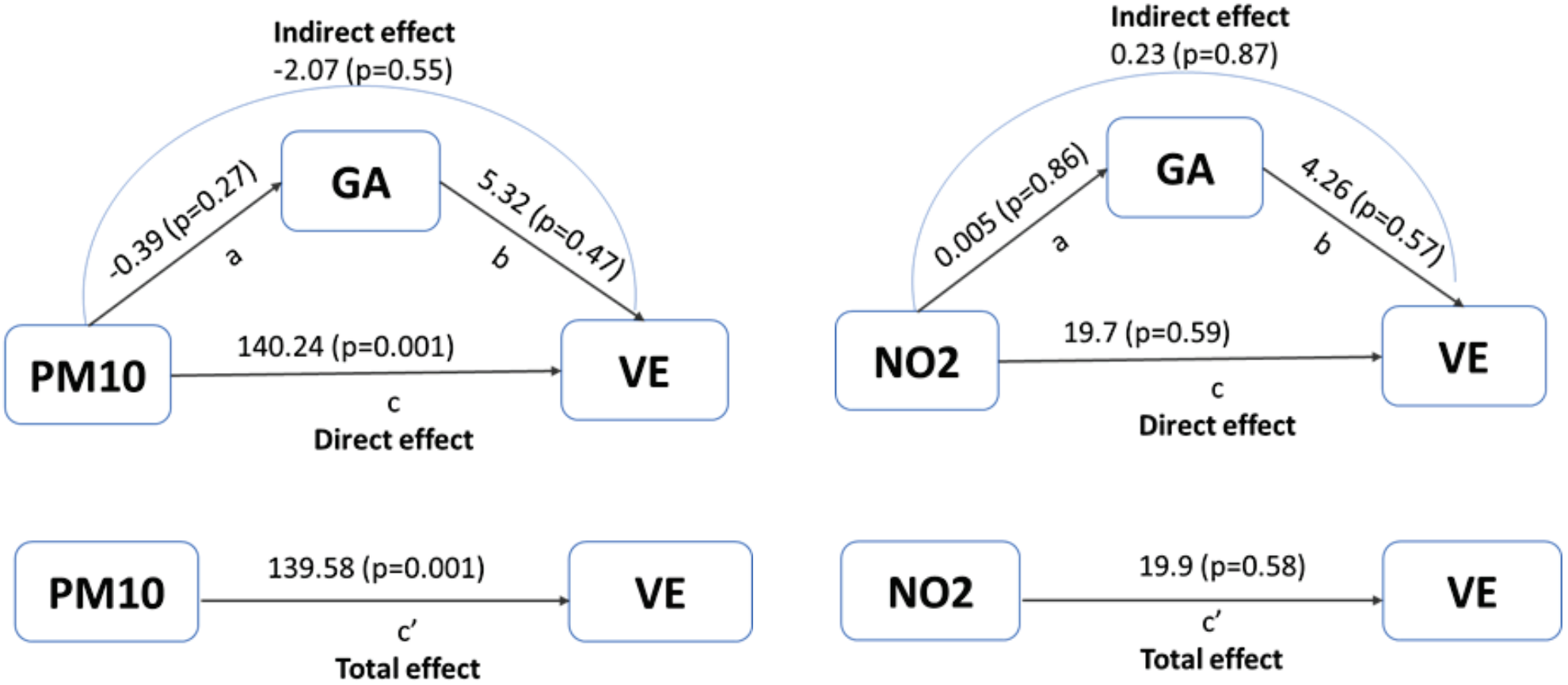

Figure E4

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