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Long term effects of prenatal and postnatal airborne PAH exposures on ventilatory lung function of non-asthmatic preadolescent children. Prospective birth cohort study in Krakow

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HIGHLIGHTS

- This is the first study on PAH exposure on lung function in non-asthmatic children
- Observed deficits of lung function due to PAH exposure persist through childhood
- Prenatal and postnatal PAH exposure compromises the respiratory airways development

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ABSTRACT

The main goal of the study was to test the hypothesis that prenatal and postnatal exposures to polycyclic aromatic hydrocarbons (PAH) are associated with depressed lung function in non-asthmatic children. The study sample comprises 195 non-asthmatic children of non-smoking mothers, among whom the prenatal PAH exposure was assessed by personal air monitoring in pregnancy. At the age of 3, residential air monitoring was carried out to evaluate the residential PAH exposure indoors and outdoors. At the age of 5 to 8, children were given allergic skin tests for indoor allergens; and between 5 and 9 years lung function testing (FVC, FEV_{05}, FEV_1 and FEF_{25-75}) was performed. The effects of prenatal PAH exposure on lung function tests repeated over the follow-up were adjusted in the General Estimated Equation (GEE) model for the relevant covariates. No association between FVC with prenatal PAH exposure was found; however for the FEV_1 deficit associated with higher prenatal PAH exposure (above 37 ng/m^3) amounted to 53 mL ($p = 0.050$) and the deficit of FEF_{25-75} reached 164 mL ($p = 0.013$). The corresponding deficits related to postnatal residential indoor PAH level (above 42 ng/m^3) were 59 mL of FEV_1 ($p = 0.028$) and 140 mL of FEF_{25-75} ($p = 0.031$). At the higher residential outdoor PAH level (above 90 ng/m^3) slightly greater deficit of FEV_1 (71 mL, $p = 0.009$) was observed. The results of the study suggest that transplacental exposure to PAH compromises the normal developmental process of respiratory airways and that this effect is compounded by postnatal PAH exposure.

1. Introduction

Over the last several decades there have been a number of cross-sectional studies, mainly in Europe and North America, on the association between depressed lung function and lung growth in children with postnatal chronic exposure to airborne particulate matter (PM) or other airborne toxins, such as NO_2, SO_2 or ozone (Frye et al., 2003; He et al., 1993; Peters et al., 1999; Raizenne et al., 1996; Speizer et al., 1980). Although the results were not always consistent, they suggested that poor ambient air quality may be causally connected with impaired lung function. Longitudinal studies undertaken on chronic postnatal

Abbreviations: DEP, diesel exhaust particles; ETS, environmental tobacco smoke; FVC, forced volume capacity; FEV_{05}, forced expiratory volume in 0.5 s; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%; GEE, Generalized Estimation Equations; PAH, polycyclic aromatic hydrocarbons.

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exposure provided further evidence on the role of air pollutants in the lung growth, but they did not show the type of lung damage attributable to individual components of ambient air pollution (Detels et al., 1991; Dockery and Brunekreft, 1996; Frischer et al., 1999; Gauderman et al., 2000, 2002, 2004; Jedrychowski et al., 1999). Moreover, the lack of epidemiologic studies on the prenatal effects of air pollutants made it impossible to assess the full impact of ambient air pollution on the natural history of lung function growth. Recently published papers have identified exposure to diesel exhaust particles (DEP) as an important cause of respiratory illness. DEP contain a broad spectrum of PAH and are common outdoor airborne pollutants. Animal and human exposure studies have shown that inhaled DEP induce an inflammatory response in human airways and exert adverse effects on pulmonary function (Brunekreef et al., 1997; Maeda et al., 1991; Sydbom et al., 2001).

Not only diesel exhausts, but also coal combustion is a major source of PAH (Junninen et al., 2009; Lvovsky et al., 2000). Ambient air pollutants penetrate readily into the indoor environment (Junninen et al., 2009; Jung et al., 2010), however, PAH compounds are also generated indoors by residential heating (e.g., coal or wood stoves, fireplaces, kerosene heaters, unvented gas appliances, environmental tobacco smoke (ETS), and fumes from cooking, grilling, and frying (Zedeck, 1980). Up to now, investigations on the specific effects of PAH exposure on children's respiratory health are scarce, though in the last decade the effect of PAH exposure on adverse birth outcomes, including low-birth weight, premature births, slower intrauterine growth and retardation in neurodevelopment was confirmed (Choi et al., 2006; Dejmek et al., 2000; Edwards et al., 2010; Perera et al., 1998, 2005, 2009).

It is realistic to assume that prenatal exposure to ambient air pollutants may have negative consequences for normal fetal development of various organs, such as the lungs, and the immunologic system. These, in turn, may lead to deficient function of the affected organs in postnatal life. For example, studies of ETS in pregnancy suggest that the environmental toxins can interfere with fetal development and their impact can be seen in postnatal life (Cunningham et al., 1994). Eventual changes in lung structure resulting from prenatal exposure can persist and lead to an increased burden of respiratory illness in adult life.

The main goal of this study was to test the hypothesis that lung function in non-asthmatic preadolescent children is associated with prenatal and early postnatal exposures to PAH. Assessment of individual prenatal exposure to airborne PAH compounds in each child under study was performed in the second trimester of pregnancy, a period when branching of the airway system is being completed. However further growth and cellular differentiation continue through postnatal life as well (Bucher and Reid, 1961), so each child's postnatal PAH exposure was also monitored. The study was restricted to non-asthmatic subjects as asthmatic children usually suffer from various chronic respiratory symptoms like wheezing, difficult breathing and attacks of shortness of breath, and for these reasons are under medical treatment with various drugs (bronchodilators and/or anti-allergic specimens) which make them less prone to the effects of air pollutants (Peters et al., 1997).

2. Materials and methods

This study is nested in a birth cohort study of children in Krakow, a collaborative research project of the Jagiellonian University in Krakow and Columbia University in New York. In the city of Krakow, coal combustion for domestic heating represents the major air pollution source, and automobile traffic emissions and coal-combustion for industrial activities are relatively minor contributors (Junninen et al., 2009). During heating seasons (October–April) ambient PAH concentrations within Krakow inner city area reach very high levels.

The present analysis was restricted to 195 non-asthmatic children who took part in the follow-up and performed reliable and acceptable spirometric tests. The design of the study and the detailed selection of the population sample have been described previously (Jedrychowski et al., 2003). In short, pregnant women were recruited from ambulatory prenatal clinics in their first or second trimester of pregnancy. Only women 18–35 years of age, who claimed to be non-smokers, with singleton pregnancies, with no history of illicit drug use and HIV infection, free from chronic diseases such as diabetes or hypertension, and who had resided in Krakow for at least one year prior to pregnancy were eligible for the study. Prior to participation, women read and signed an informed consent. The Ethical Committee of the Jagiellonian University in Krakow and Columbia Presbyterian Medical Center approved the research.

Upon enrollment, a detailed questionnaire was administered to each woman to solicit information on demographic data, home characteristics, medical and reproductive history, occupational hazards, and smoking practices of others present at home. After delivery, every

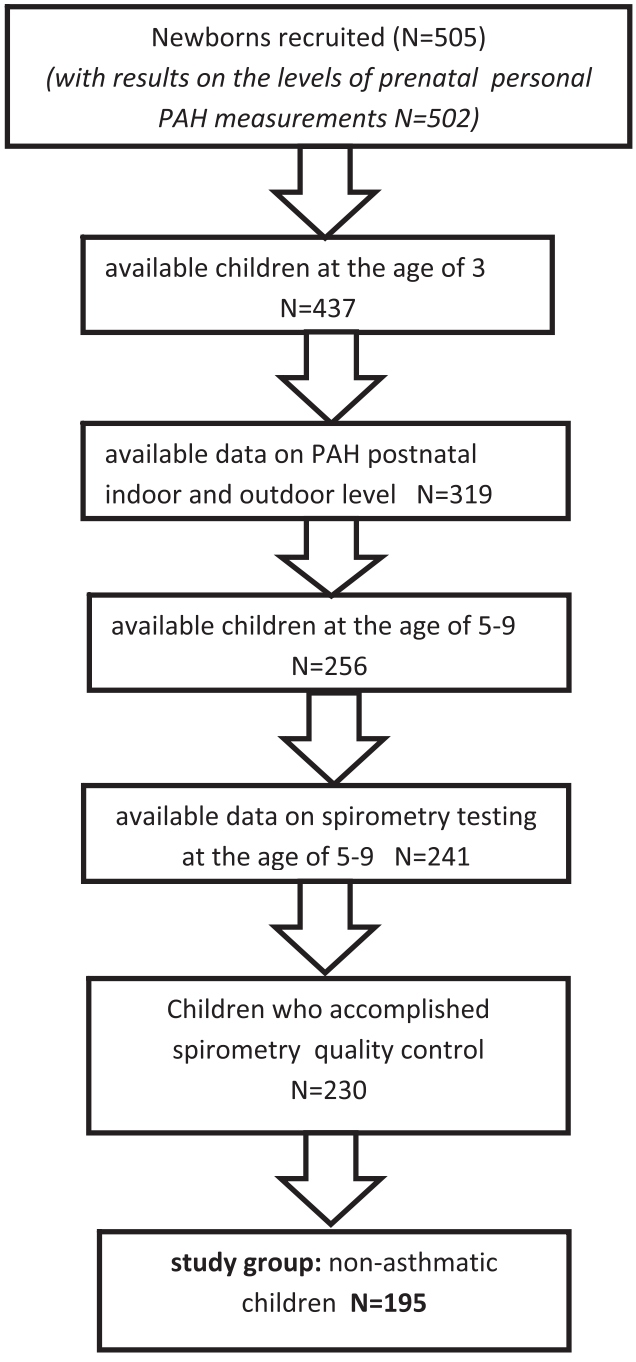

Fig. 1. Flow chart of participant recruitment and derivation of the study population used in the final analysis.

three months in the first two years of the newborn's life and every 6 months thereafter mothers participated in a detailed face-to-face interview on their children's health and environmental conditions, including postnatal ETS exposure in the home. After delivery, the newborns were followed to the age of 9. At the age of 3, residential pollution monitoring was carried out to assess the indoor and outdoor levels of PAH compounds. At ages 5–8 allergic skin tests were performed for indoor allergens; and between ages 5 and 9, children were annually invited for standard lung function testing (FVC, FEV_{05}, FEV_1 and $FEF_{25–75}$). The diagram depicting the availability and data collection at the various ages is presented in Fig. 1.

3. Measurement of prenatal and postnatal PAH airborne concentrations

Monitoring of individual prenatal exposure to airborne PAH over a consecutive 48 hour period was performed with Personal Environmental Monitoring Samplers (PEMS), designed by the Department of Environmental Health, School of Public Health at Harvard University (Dr J. Spengler). The battery operated sampling units collected fine particles on quartz filters and gaseous PAH compounds on polyurethane foam (PUF). Participants were asked to wear the lightweight sampler installed in a backpack wherever they were during the daytime hours and to place it at the bedside at night. The flow rate of the sampling pump was calibrated using a bubble meter prior to the monitoring, and was checked again with a change of the battery pack on the second day.

For each child residential airborne PAH indoor and outdoor monitoring was also conducted with the same samplers and procedures at the age of 3. The indoor samplers were placed for consecutive 48 h in a room where the child spent most of the time. The sampler was placed atop furniture 0.5–2 m above the floor away from the heating sources, about 1 m away from the wall of the home. Outdoor monitoring was performed during the same period and with the same samplers, which were attached to the external wall of the home at the level of the apartment.

The collected filters were sent to the Department of Analytical and Environmental Chemistry, Southwest Research Institute, San Antonio, TX, USA Texas who performed PAH extraction and measured the PAH concentrations of pyrene and the eight carcinogenic PAHs — benz[*a*]anthracene, chrysene/isochrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[a]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenz[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene using gas chromatography/mass spectrometry as described elsewhere (US EPA, 1999). The detection limit for each target PAH was 1.0 ± 0.2 ng/sample; 100% of the air samples were above the detection limit for all PAH. In the study we used the total sum of individual concentrations of all measured PAH compounds (in ng/m^3).

3.1. Assessment of atopic status of children

Children at ages 5–8 were invited to undergo skin prick testing (SPT) for four common domestic aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog and cat hair). The results were read after 15 min by measuring the largest diameter of the wheal. Atopic status was ascertained as a wheal reached a diameter of 3 mm and greater than the histamine control. The participants were defined as atopic if they had at least one positive skin prick test.

4. Spirometric testing

Children were free of respiratory symptoms on the day of testing and prior to spirometric testing the standing height and weight of each child was measured. Subsequently, children were coached to engage in maximal forced expiratory efforts in a standing position without nose clip. All spirometric measurements were carried out by the same staff member (E. Mroz), who was experienced in spirometric testing of children, using a computerized PC QRS Card Spirometer (QRS Diagnostic, Plymouth, MN, USA) with incentive display software. Each day, prior to the lung function examination, the spirometer was calibrated with a 1-L syringe. Each child made at least two good forced exhalation efforts and the primary indicators of lung function were recorded including: FVC, FEV_{05} and FEV_1. In addition, maximal mid expiratory flow over the middle 50% of the FVC ($FEF_{25–75}$) was recorded. Spirometric data were excluded if a submaximal expiratory effort was present in which a peak expiratory flow was not clearly determined, a slow rise of peak expiratory flow was apparent, an expiration time was less than 0.5 s or a cough or an abrupt end of expiration effort appeared in the course of the exhalation effort. In accordance with the American Thoracic Society and European Respiratory Society guidelines on pulmonary function testing in preschool children (Beydon et al., 2007), expiratory flows were reported from the attempt with the best flow (the greatest sum of FEV_1 and FVC) executed by each subject and the spirometric index corrected to recorded body temperature, pressure saturated (BTPS). Spirometric findings were accepted as reliable if the difference between FVCs and the difference between FEV_1 of the two best curves were within the range of 10%. Children at ages 5–6 failed spirometry quality control in 23%, older participants in 11%.

5. Statistical data analysis

The descriptive analysis considered the distribution of various parameters related to women and newborns under study. Initially, the relationship between prenatal PAH exposure and lung function tests was evaluated with linear multivariate regression; however, in order to assess the average effect of the exposure on lung function tests measured at 5, 6, 7, 8 and 9 years of age, the generalized estimating equation (GEE) model was applied to account for the correlation between observations obtained from the same child (Hardin and Hilbe, 2000). Models computed the regression coefficients of the outcome variable on the main predictor variables (prenatal PAH exposure) and potential confounders or modifiers (gestational age, child's gender and their height, weight, residential airborne PAH indoors and outdoors measured at the age of 3, atopic status and ETS). The PAH variables were introduced in the GEE models as ordinal variables (defined by tertiles of distribution) or numeric after transformation to logarithmic values, which normalized their distributions. The preliminary analysis of the data suggested a non-linear dose-dependent effect of prenatal PAH exposure on health outcomes, therefore, in the final multivariable GEE models the PAH levels were inserted in tertiles as it would help in setting up a threshold level of the exposure. In all GEE models a dummy variable defining the season of the residential air pollution monitoring was inserted as well. Interaction between prenatal and postnatal PAH

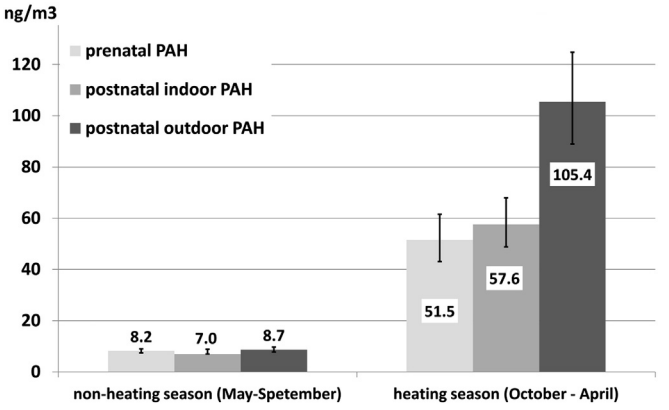

Fig. 2. Prenatal (2000–2003) and postnatal (2003–2007) residential PAH levels (gmean with 95% CI) measured in non-heating and heating seasons.

exposures was verified, however due to lack of significance it was not included in final models. All statistical analyses were carried out with STATA 13.1 version software for Windows.

6. Results

In the study sample the geometric mean (gmean) of the prenatal individual PAH concentrations was 20.1 ng/m^3 (95% CI: 17.1–23.7 ng/m^3) and that of the residential PAH indoor concentration was 21.3 ng/m^3 (95% CI: 17.8–28.5 ng/m^3). The residential outdoor PAH concentrations had a much higher gmean (32.5 g/m^3, 95% CI: 25.5–39.9) and showed a wider range of values. Both the prenatal and residential PAH distributions were skewed. After natural log (ln) transformation, the distribution of the PAH levels measured in all environmental compartments was bimodal, with a lower exposure range in the non-heating season (May–September) and a higher exposure range in the heating season (October–April) (Fig. 2).

Basic characteristics of the children included in the study were presented in Table 1. The average height of children attending spirometric testing was 112.8 ± 4.6 cm at the age of 5 and 137.2 ± 6.3 cm at the age of 9. On average, the annual increase in height was 6.4, 7.5, 5.4, and 5.3 cm between consecutive surveys, suggesting that children under study had not entered their prepubertal growth spurt (Fig. 3). In the follow-up period mean FVC values standardized to height increased from the initial values 1098.4 mL (95% CI: 1052.3–1144.5) to 1827.1 mL (95% CI: 1782.5–1871.7) at the end of the follow-up. The average increase of FVC was 259.5, 310.3, 152.3, and 170.7 mL between consecutive annual surveys.

Table 2 presents the results of the longitudinal GEE regression model for lung function tests recorded annually over the follow-up (FVC, FEV_{05}, FEV_1 and FEF_{25-75}) as they were related to prenatal and residential indoor PAH concentrations (categorized by tertiles) and adjusted for a set of covariates (gestational age, gender, height, atopic status, parity, postnatal ETS and season of residential air pollution monitoring). The results showed insignificant correlation of the prenatal PAH exposure with FVC. In contrast, FEV_{05}, FEV_1 and FEF_{25-75} were inversely correlated with both prenatal and residential indoor PAH levels. The average FEV_{05} deficit amounted to 58 mL at the highest tertile of prenatal PAH exposure ($p = 0.013$); postnatal residential indoor PAH was associated with an FEV_{05} deficit of 59 mL ($p = 0.009$) at the highest exposure tertile. The same regression model applied for FEV_1 also showed significant detrimental effects of both prenatal (coeff = −53 mL, $p = 0.050$) and residential indoor PAH exposures (coeff. = −59 mL, $p = 0.028$).

Table 1
Characteristics of the study sample.

	Size of the study sample (n = 195)
Gender	
Boys: n (%)	89 (45.6%)
Girls: n (%)	106 (54.4%)
Gestational age (weeks): mean (SD)	39.3 (1.53)
Birth weight (g): mean (SD)	3366.1 (480.0)
Length at birth (g): mean (SD)	54.6 (2.73)
Older siblings n (%)	67 (34.4%)
Maternal education (yrs of schooling): mean, SD	17.7 (2.71)
Prenatal ETS: n (%)	49 (25.3%)
Postnatal ETS: n (%)	43 (22.0%)
Prenatal PAH (ng/m^3): gmean (95% CI)	20.1 (17.1–23.7)
Postnatal indoor PAH (ng/m^3): gmean (95% CI)	21.3 (17.8–28.5)
Postnatal outdoor PAH (ng/m^3): gmean (95% CI)	32.5 (25.5–39.9)
Atopy in children: n (%)	30 (15.5%)
Attendance at the lung function testing: n (%)	
5 y	176 (90.3%)
6 y	170 (87.2%)
7 y	158 (81.0%)
8 y	157 (80.5%)
9 y	160 (82.1%)

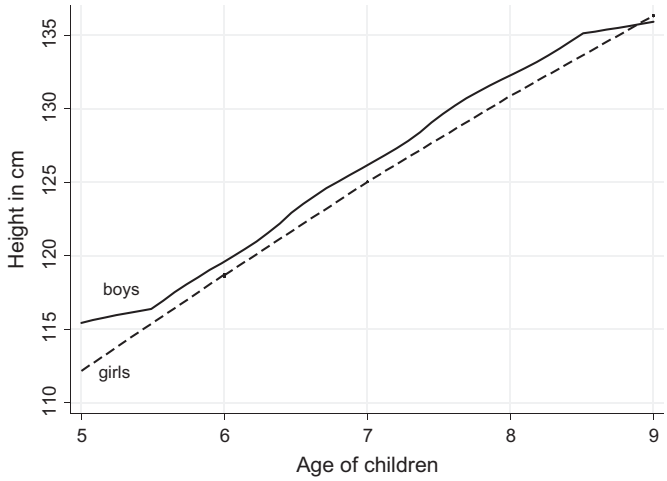

Fig. 3. Height of children measured at various age during the follow-up.

The average FEF_{25-75} level was depressed by 164 mL ($p = 0.013$) at the highest tertile of prenatal PAH exposure; the corresponding FEF_{25-75} deficit was 141 mL ($p = 0.031$) at the third tertile of exposure.

Table 3 displays the effects of prenatal PAH and residential outdoor PAH exposures on lung function tests based on the longitudinal GEE model after adjustment for the same set of covariates as above. As it was found earlier, there was no significant deficit of FVC associated with prenatal PAH, however, postnatal residential outdoor PAH levels affected FVC at the highest tertile of exposure (coeff. = −62 mL, $p = 0.038$). The average FEV_{05} deficit reached 66 mL ($p = 0.005$) at the highest tertile of prenatal PAH exposure; residential outdoor PAH was also inversely associated with FEV_{05} (coeff = −78 mL, $p = 0.001$). Similarly, FEV_1 was significantly related to both prenatal (coeff = −62 mL, $p = 0.021$) and postnatal outdoor exposures (coeff. = −71 mL, $p = 0.009$). The significant FEF_{25-75} deficits amounted to 167 mL at the highest tertiles of prenatal and postnatal outdoor PAH exposures.

Fig. 4 visualizes the relationship between the prenatal PAH concentrations (ln-transformed) and FEV_1 over the follow-up at various levels of the residential outdoor level. There is a clear dose–response relationship between the prenatal PAH exposure and FEV_1 levels both at lower and higher residential PAH concentrations.

7. Discussion

To our knowledge this is the first prospective cohort study to shed light on the natural history of lung growth in the context of prenatal and postnatal PAH exposures observed in preadolescent non-asthmatic children. The study has shown that prenatal PAH exposure is associated with a reduction in FEV05, FEV1 and FEF25–75% but not FVC, suggesting that prenatal PAH exposure inhibits the full development of respiratory airway caliber. The lack of the PAH effects on FVC may result from the fact that in the growth phase not only significant development in size and alveolar number takes place, but also the shape of the thorax and muscular strength undergo a great change. This affects the total lung capacity (TLC) and the FVC, however, in the development of flows, respiratory airway caliber and the elastic properties of airways play a leading part (Grivas et al., 1991; Quanjer et al., 2010; Schrader et al., 1988; Simon et al., 1972). Further, the study suggests that the prenatal effect of PAH exposure on lung growth persists through preadolescence, but the harmful effect of postnatal residential PAH exposure on lung function was noticed. As there were insignificant statistical interaction terms between the estimates of prenatal and postnatal residential PAH exposures, this could indicate that the effects of transplacental and inhaled PAH compounds in the postnatal period were additive, and result from different pathologic pathways. It is

Table 2
Comparison of the effects of prenatal and residential indoor PAH exposure level (defined by tertiles of the distribution) on lung function tests adjusted for gestational age, gender, height, parity, atopic status, postnatal ETS and season of residential air pollution survey (number of observations = 665, number of groups = 194[a], average number of observations per group = 3.4).

	Coef.	z	P > z	[95% conf. interval]	
FVC					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−23.694	−0.86	0.388	−77.475	30.087
3rd tertile >37.0 ng/m^3	−28.216	−0.95	0.344	−86.629	30.197
Residential indoor PAH					
1st tertile <8.0 ng/m^3	Base				
2nd tertile 8.1–42.0 ng/m^3	−21.168	−0.75	0.450	−76.142	33.806
3rd tertile >42 ng/m^3	−45.397	−1.57	0.117	−102.179	11.385
FEV_{05}					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−3.393	−0.16	0.875	−45.628	38.841
3rd tertile >37.0 ng/m^3	−58.137	−2.48	0.013	−103.992	−12.282
Residential indoor PAH					
1st tertile <8.0 ng/m^3	Base				
2nd tertile 8.1–42.0 ng/m^3	−23.438	−1.06	0.287	−66.586	19.710
3rd tertile >42 ng/m^3	−59.222	−2.60	0.009	−103.800	−14.643
FEV_1					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−27.751	−1.11	0.267	−76.783	21.281
3rd tertile >37.0 ng/m^3	−53.343	−1.96	0.050	−106.648	−0.039
Residential indoor PAH					
1st tertile <8.0 ng/m^3	Base				
2nd tertile 8.1–42.0 ng/m^3	−35.275	−1.39	0.165	−85.084	14.533
3rd tertile >42 ng/m^3	−59.348	−2.20	0.028	−112.128	−6.568
$FEF_{25–75}$					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	4.024	0.07	0.947	−114.899	122.947
3rd tertile >37.0 ng/m^3	−163.756	−2.48	0.013	−293.047	−34.465
Residential indoor PAH					
1st tertile <8.0 ng/m^3	Base				
2nd tertile 8.1–42.0 ng/m^3	−62.197	−1.01	0.313	−183.012	58.618
3rd tertile >42 ng/m^3	−141.059	−2.16	0.031	−269.073	−13.044

[a] One child excluded due to the lack of atopic diagnosis.

Table 3
Comparison of the effects of prenatal and residential outdoor PAH exposure level (defined by tertiles of the distribution) on the lung function tests adjusted for gestation age, gender, height, parity, atopic status, ETS and season of residential air pollution survey (number of observations = 665, number of groups = 194[a], average number of observations per group = 3.4).

	Coef.	z	P > z	[95% conf. interval]	
FVC					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−36.376	−1.35	0.178	−89.266	16.513
3rd tertile >37.0 ng/m^3	−39.680	−1.36	0.173	−96.749	17.390
Residential outdoor PAH					
1st tertile <10.0 ng/m^3	Base				
2nd tertile 10.1–92.0 ng/m^3	−19.305	−0.71	0.479	−72.761	34.151
3rd tertile >92 ng/m^3	−61.973	−2.08	0.038	−120.372	−3.574
FEV_{05}					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−7.819	−0.36	0.720	−50.546	34.908
3rd tertile >37.0 ng/m^3	−65.847	−2.80	0.005	−111.951	−19.742
Residential outdoor PAH					
1st tertile <10.0 ng/m^3	Base				
2nd tertile 10.1–92.0 ng/m^3	−36.541	−1.66	0.097	−79.727	6.645
3rd tertile >92 ng/m^3	−77.774	−3.23	0.001	−124.951	−30.598
FEV_1					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	−34.762	−1.41	0.159	−83.138	13.613
3rd tertile >37.0 ng/m^3	−61.626	−2.31	0.021	−113.836	−9.415
Residential outdoor PAH					
1st tertile <10.0 ng/m^3	Base				
2nd tertile 10.1–92.0 ng/m^3	−39.016	−1.56	0.118	−87.934	9.902
3rd tertile >92 ng/m^3	−71.283	−2.62	0.009	−124.664	−17.903
$FEF_{25–75}$					
Prenatal PAH					
1st tertile <9.0 ng/m^3	Base				
2nd tertile 9.1–37.0 ng/m^3	6.788	0.11	0.912	−113.205	126.781
3rd tertile >37.0 ng/m^3	−167.326	−2.53	0.011	−296.854	−37.799
Residential outdoor PAH					
1st tertile <10.0 ng/m^3	Base				
2nd tertile 10.1–92.0 ng/m^3	−119.782	−1.93	0.053	−241.160	1.597
3rd tertile >92 ng/m^3	−167.303	−2.48	0.013	−299.658	−34.948

[a] One child excluded due to the lack of atopic diagnosis.

noteworthy to add that the effects of prenatal exposure on lung function growth measured annually over the follow-up were adjusted for a set of relevant modifiers or confounders.

Although the biological mechanisms whereby transplacental PAH might cause adverse effects on the development of fetal lung have not been yet clearly established, it is known that PAH compounds from maternal blood can easily reach the fetus leading to DNA damage and forming PAH-DNA adducts (Kihlstrom, 1986; Srivastava et al., 1986). The fetal development of the respiratory system involves a complex process that begins one month after fertilization and proceeds from the cellular level to the formation of tissues and morphologic respiratory structures (Bucher and Reid, 1961). The fetal lung develops from the foregut, and undergoes growth and branching morphogenesis in which different factors promote linear growth and branching. Branching of the airway system down to the terminal bronchioles is completed by the second trimester in utero, but further growth continues after birth until early adulthood (Bucher and Reid, 1961). Bronchial linear growth is driven by epidermal- and fibroblast-growth factors (FGF) and branching is promoted by factors of the TGF-β family (Bucher and Reid, 1961). It is possible that transplacental absorption of PAH compounds from maternal blood may alter the release and function of fibroblast-growth factors and consequently compromise the normal lung developmental process leading to remodeling of the fetal lung structure. To which extent epigenetic regulation may be involved in mediating the complex gene–environment interaction in the branching morphogenesis is the new challenge for molecular and environmental epidemiology (Miller and Ho, 2008).

After birth, the remodeling of the airway system may be further continued, presumably through inflammatory mechanisms. Inhaled PAH compounds acting directly on bronchial epithelial cells play a key role in the local synthesis of proinflammatory chemokines. In vitro and in vivo studies have demonstrated that DEP particles have a capacity to up-regulate allergic or proinflammatory cytokines, such as interleukin (IL)-4, IL-2, and IL-8 (Bommel et al., 2000; Heo et al., 2001; Salvi et al., 2000; Takano et al., 1997), enhance local mucosal IgE production, and deviate cytokine production toward a Th2 profile (Diaz-Sanchez, 1997; Romagnani, 1998). In addition, it is well established that PAH compounds can generate reactive oxygen species and initiate an oxidative stress cascade leading to airway inflammation (Whitekus et al., 2002).

To date, only a small number of prospective cohort studies have examined the effect of postnatal exposure to urban air pollutants, such as PM, SO_2, NO_2, NO_x, or ozone on lung function growth in children. For example, a study in Poland (Jedrychowski et al., 1999) assessed lung function growth (FVC and FEV_1) over 3-year follow up (1995–1997) in about 1000 preadolescent schoolchildren from two areas of Krakow differing in levels of PM_{10} and SO_2. The main endpoint variable was slow lung function growth, which was defined as a gain in spirometric values equal to or lower than the first (lowest) quintile of the distribution of a given spirometric test. The adjusted odds ratio for

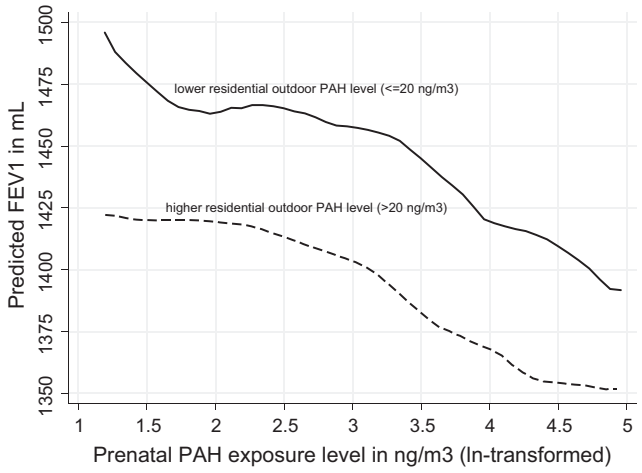

Fig. 4. Predicted average values of FEV_1 (based on GEE model) by the prenatal PAH concentrations (ln-transformed) at various levels of residential outdoor PAH concentrations (local polynomial smoothing).

slow lung growth was significantly higher in the group of children from the more polluted city center than in those who lived in the less polluted peripheral part of the city (OR = 1.80, 95% CI: 1.26–2.58). The estimated OR was adjusted for height velocity, lung function at entry to the study and gender.

In Austria (Frischer et al., 1999) a cohort of 1150 children from nine study areas was investigated to assess the long-term effects of ambient air pollution on lung function measured twice a year (before and after summertime). Those authors found significant deficits of FVC, FEV_1 and $FEF_{25–75}$ that were associated with ozone levels and also some evidence that sulfur dioxide and nitrogen dioxide were associated with deficits in FEV growth. Spirometric data were adjusted for sex, atopy, passive smoking, baseline lung function and increase in height.

In the course of a four-year prospective study of 3000 children from 12 communities in Los Angeles, California (Gauderman et al., 2000), lung function (FVC, FEV_1 and $FEF_{25–75}$) was evaluated and hourly concentrations of ozone, nitrogen dioxide and PM_{10}, $PM_{2.5}$ and acid vapor were measured by outdoor sampling stations in each community. Compared to children living in the least polluted community, those living in the most polluted area had a reduction of 3.4% in FEV_1 and 5.0% in $FEF_{25–75}$ over the four-year study period. Average growth of lung function was adjusted for personal and household characteristics. As the concentrations of air pollutants were very highly correlated, the authors could not identify the independent effects of each of the pollutants measured. A second cohort of more than 1600 fourth-grade children from the same communities followed from 1996 to 2000 confirmed an earlier established association between ambient levels of air pollutants in southern California and impaired lung function growth (Gauderman et al., 2002). Reduced FEV_1 and $FEF_{25–75}$ growth was most strongly correlated with levels of vapor acids, nitrogen dioxide, $PM_{2.5}$ and elemental carbon (a marker for diesel exhaust). The latest report from eight years has shown that deficits in the growth of FEV_1 and $FEF_{25–75}$ were associated with exposure to nitrogen dioxide, acid vapor, $PM_{2.5}$ and elemental carbon among 1759 children followed from 10 to 18 years of age (Gauderman et al., 2004). In a very recently published results of the ESCAPE project based on about 6000 children 6–8 years of age recruited from four European countries (Germany, Netherlands, United Kingdom and Sweden) have shown that estimated levels of NO_2, NO_x, and $PM_{2.5}$ at the current residency, but not at the birth address, were associated with small decreases in lung function (Gehring et al., 2013). For example, changes in forced expiratory volume in 1 s (FEV1) ranged from −0.86% (95% CI: −1.48, −0.24%) for a 20-μg/m^3 increase in NO_x to −1.77% (95% CI: −3.34, −0.18%) for a 5-μg/m^3 increase in $PM_{2.5}$.

Comparisons of our findings with other studies mentioned are very restricted due to the great differences in study designs, various air pollutants considered and methods of their exposure assessment, lung function measurements, and statistical methods used. None of the prior prospective epidemiologic studies considered the prenatal effects of air pollutants on lung function growth using personal monitoring data. The use of personal monitoring of prenatal PAH exposure is a relevant measure of individual exposure incorporating both outdoor and indoor exposures (Choi et al., 2008). As the additional prenatal PAH measurements performed in the subsample of pregnant women both in the second and third trimesters were significantly correlated (Choi et al., 2008), therefore, we considered the single personal monitoring during the second trimester, a reasonable indicator of prenatal airborne PAH exposure over the last two pregnancy trimesters. Despite known seasonal variation in air pollution levels related to heating with coal in Krakow, the potential effect of measurement season on our results was mitigated by the fact that monitoring was evenly distributed across seasons (Choi et al., 2008).

In a parallel cohort study in New York City, we have measured prenatal exposure to 8 nonvolatile PAH using personal air monitoring over a 48-hour period during the third trimester of pregnancy (Choi et al., 2006), which was validated by concurrent 2-week residential air monitoring (Rundle et al., 2012). Levels of PAH measured in sequential 2-week integrated indoor air samples collected from a representative subset of homes ($n = 101$) over six weeks during the final trimester of pregnancy correlated significantly with 48-hour measures ($r = 0.57–0.76$, $p < 0.001$). The indoor air levels of the pollutants over the 6 weeks of monitoring also correlated significantly with PAH levels in the single 48-hour prenatal personal air sample ($r = 0.44$, $p < 0.001$). Concurrent indoor, outdoor, and personal monitoring of PAH in a separate study of pregnant women demonstrated a high correlation between these 3 measures (pairwise Spearman's coefficients ≥ 0.84, $p < 0.01$) (Choi et al., 2006), supporting the use of our single measure of PAH in prenatal personal air as an indicator of chronic prenatal exposure via inhalation. We have also found considerable interindividual variation in PAH concentrations monitored in prenatal air for cohort participants (Perera et al., 2003).

Previous studies relied on area-wide monitoring of residential air pollutants in postnatal period, assigning these residential exposure levels to children under study, or estimating individual exposure from modeling exposure data taken from ambient air-monitoring stations. The postnatal air pollution monitoring in our study was restricted to one 48-h measurement session carried out individually in children's residential environments at the age of 3. Although the level of ambient pollutants varies over time, this mainly depends on the change of residence. In our study sample of 33 children (17%) changed their residence after the age 3, but 16 of them still remained within the same pollution area. One child changed the residence for the area with higher air pollution level, and 16 subjects (8%) moved to the less polluted areas. As the latter group moved at the end of the follow-up period, we do not think that mobility of our children could have significantly biased the estimated exposure effects.

Considerable merit of our study is the restriction of the analysis to the group of non-asthmatic children. The reason for excluding asthmatic children from our study was the fact that they frequently suffer from respiratory symptoms such as wheezing, or attacks of breathlessness and therefore are under medication, making them less prone to ambient air pollution. The important feature of the data is also the comparison of the adjusted effects of indoor and outdoor residential PAH exposures as it puts more weight on the consistency of results and provides an additional value of the paper. As mentioned earlier, several studies have shown that effect estimates of pollutants on health symptoms may be underestimated when medication is used by asthmatic subjects. Recent epidemiologic cohort study by the group at the Columbia Center for Children's Environmental Health (CCCEH) has also found that the associations between

repeated prenatal and residential exposures to PAH compounds such as pyrene were greatest among non-atopic children (Jung et al., 2012). Other strengths of our study are the statistical longitudinal GEE modeling which considered a set of relevant confounders and removal of the confounding effect of maternal active tobacco smoking during pregnancy through entry criteria.

In conclusion, this study in preadolescent children provides evidence that prenatal PAH exposure produces a depressed lung function growth in terms of deficits in FEV_{05}, FEV_1 and FEF_{25-75} and that this effect is compounded by postnatal PAH exposure. The observed deficits appear to persist through childhood, but it is not known whether "catch-up" may occur during adolescence or early adulthood to result in normal attained lung function. The findings are of concern because irreversible impaired development of small bronchial airways could lead to a number of respiratory inflammatory disorders, influence the susceptibility of children to other air pollutants, and contribute to the development of chronic air flow obstruction in adulthood.

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All women participating in the study had read and signed an informed consent.

The Ethical Committee of the Jagiellonian University in Krakow and Columbia Presbyterian Medical Center approved the research.

The authors declare that they have no actual or potential conflict of interest.

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