



Greater susceptibility of girls to airborne Benzo[a]pyrene for obesity-associated childhood asthma

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ARTICLE INFO

Editor: Olga-Ioanna Kalantzi

Keywords:

Polycyclic aromatic hydrocarbon
Benzo[a]pyrene
Obesity
Asthma
Air pollution
Sex-dimorphic

ABSTRACT

Background: Sexually dimorphic risk of obesity-associated asthma is posited to accelerate around puberty. Yet, the role of air pollution on the lean and obese asthmatic children has never been examined.

Objective: To compare whether a unit exposure to airborne benzo[a]pyrene (B[a]P) is associated with altered risks of asthma across the overweight/obese (OV/OB) control, lean asthmatic, and OV/OB asthmatic children, respectively, compared to the lean controls, before and after adjusting for oxidant stress markers (i.e. 15-F2t-IsoP, 8-oxo-dG, and Carbonyl).

Methods: Asthmatic and healthy control children, recruited from polluted urban and rural areas, were matched to ambient concentration of B[a]P. A unit increase in B[a]P and multinomial logistic regression on OV/OB control, lean asthmatic, and OV/OB asthma were compared across the sex- and age-groups.

Results: The median B[a]P was associated with a linear increase among the female children, according to OV/OB and asthma, respectively, and together, compared to the lean control girls ($p = 0.001$). While B[a]P was associated with positive relationship with 15-F2t-IsoP level among the OV/OB boys, the same exposure-outcome association was inverse among the OV/OB girls. One natural log-unit increase in ambient B[a]P was associated with 10.5-times greater odds (95% CI, 2.6–39.6; $p = 0.001$) the adolescent OV/OB boys, compared to the unit odds among the lean controls. In contrast, the adolescent OV/OB girls were associated with highest adjusted odds of the asthma (aOR = 15.4; 95% CI, 2.9–29.1; $p < 0.001$) compared to the lean control girls. An adjustment for 15-F2t-IsoP, and Carbonyls was associated with greater odds of asthma per unit exposure for the adolescent OV/OB girls (aOR = 16.2; 95% CI, 1.4–181.8; $p = 0.024$).

Conclusions: B[a]P exposure was associated with a leap in the odds of asthma among the OV/OB adolescents, particularly the girls, after adjusting for 15-F2t-IsoP and Carbonyls.

1. Introduction

Asthma and obesity represent two diseases with grave public health burden worldwide (Asher and Pearce, 2014). The present global prevalence of 300 million life-time asthma sufferers is expected to grow to 400 million by 2025 (Masoli et al., 2004). As of 2015, an estimated 700 million adults have obesity worldwide (Arroyo-Johnson and Mincey, 2016). While these two diseases have been investigated as two separate diseases with distinct pathophysiological features, growing number of

lifetime asthma sufferers are also obese (Boulet and Boulay, 2011). A segment of the obese asthma patients is associated with most severe and poorly-controlled asthma type (Lang et al., 2013). In fact, the co-morbid condition of severe asthma among obese individuals incurs 50%–80% of the total health care costs for asthma (Accordini et al., 2013, 2008). To date, environmental origin of asthma, which is either induced by or further exacerbated by obesity, has not been extensively investigated. The prevalence of either or both conditions has risen too rapidly within the last few decades, to be adequately explained by genetic

Abbreviations: 8-oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 15-F2t-IsoP, 15-F2t-isoprostane; AP, attributable proportion; B[a]P, benzo[a]pyrene; BMI, body mass index; PAH, polycyclic aromatic hydrocarbon; DEP, diesel exhaust particles; CHMI, Czech Hydrometeorological Institute; RERI, relative excess risk due to interaction; VAPS, versatile air pollution sampler

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<https://doi.org/10.1016/j.envint.2018.08.061>

Received 11 May 2018; Received in revised form 27 August 2018; Accepted 28 August 2018

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susceptibility factors. Accordingly, there is a growing need for a deeper understanding of the environmental etiologies of obesity and asthma co-presentation.

Early-life exposure to air pollution poses risk for not only the childhood asthma onset (Tantisira et al., 2008) and exacerbation (Velická et al., 2015), but also obesity (Chu et al., 2009; Jung et al., 2014; Lang et al., 2013). Furthermore, host susceptibility to air pollution is sexually dimorphic. Excess adiposity in obese rats exacerbate the harmful effects of ozone in female rats more than the males (Gordon et al., 2016). In another study, prenatal exposure to diesel exhaust particles (DEP) predisposed the male rat offspring to heightened calorie intake, weight gain, and neuroinflammation more severely than the female progenies (Bolton et al., 2012). In addition, in utero DEP exposure subsequently induced heightened macrophage infiltration, elevated production of peripheral IL-1 β response to an allergen challenge, insulin resistance, and anxiety-like behavior in male offspring, supporting an early-life “programming” towards metabolic, behavioral, and neuroinflammatory alterations (Bolton et al., 2014). In Boston, US, in utero exposure to elevated PM_{2.5} during second trimester predict asthma by age 6 years in boys, but not in girls (Hsu et al., 2015).

Such growing body of evidence raises a possibility that air pollution exposure among the overweight individuals contributes to asthma onset and/or exacerbation in gender-dimorphic fashion. In support of such hypothesis, adiponectin is observed at a higher absolute concentration in women, than men (Peake et al., 2005). Furthermore, low plasma adiponectin was associated with higher risk for asthma among adolescent girls than boys (Nagel et al., 2009). Accordingly, Sood and Shore (2013) postulated that the loss of the anti-inflammatory effects of adiponectin among obese girls might differ from obese boys, thereby contributing to the difference in asthma susceptibility.

Within Czech Republic, we have been examining early-life exposure to PAHs and their risk on multiple health endpoints by taking advantage of one-of-a-kind routine PAH monitoring program in historically industrial city of Ostrava and rural towns in Southern Bohemia (Dejmek et al., 2000; Sram et al., 2005). Ambient PAH concentrations within Ostrava remain one of the highest observed concentrations within Europe (Sram et al., 2013b). Furthermore, spatial as well as the temporal gradient of ambient B[a]P could vary by > 10-fold (Sram et al., 2013b), thereby allowing us to investigate asthma with relatively modest sample sizes. Within the present case-control study, we investigate air-pollution associated risks of childhood asthma among lean, overweight, and obese children within a heavily polluted city (i.e. Ostrava) vs. background air quality region (i.e. Southern Bohemia) in Czech Republic (Sram et al., 2013a). We postulate that: 1) airborne PAHs are correlated with biomarkers of systemic oxidant stress (i.e. 15-F₂t-IsoP, 8-oxo-dG, and Carbonyl) in a sexually dimorphic fashion; 2) PAH exposures pose different risk of asthma among the OV/OB girls than the boys; and 3) the oxidant stress biomarkers are associated with sexually dimorphic susceptibility to asthma per unit PAH exposure.

2. Material and methods

Target sites, enrollment, and methods have been described. Briefly, 100 asthmatic and 100 healthy control children were enrolled from the city with heavy air pollution (i.e. Ostrava) as well as the background region (i.e. semi-rural towns in Southern Bohemia) in Czech Republic (Sram et al., 2013a). The Ostrava children were enrolled from the primary care clinics within the most polluted districts within Ostrava. According to one estimate, the childhood asthma prevalence in such district was ~30% during 2007 (Sram et al., 2013a). In contrast, the rural background region is marked by overall low annual mean B[a]P concentration in ambient air, comparable to the urban centers in western and northern Europe (Sram et al., 2013a).

2.1. Case definition

We conducted a comprehensive medical record review for each child, which contains records from the primary care physician and allergy specialist. A child was defined as positive case for asthma, if he/she met all of the following conditions: 1) positive diagnosis of ‘current’ asthma using *International Classification of Diseases (ICD), Tenth Revision* (National Center for Health Statistics, 2016) codes within their medical record; 2) currently take asthma medication; 3) positive record of impaired lung function, based on spirometry within the past 12 months; 4) positive record of bronchial hyperresponsiveness in the last 12 months; and 5) positive on allergy skin tests (PhadiatopH, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) for airborne allergens – timothy, mugwort, birch, cat, horse, dog, feathers, pollen, trees, grass, house dust mites (*D. pteronyssinus* and *D. farinae*), and two molds (Penicillium and Cladosporium). Diagnostic reliability across the physicians has been validated (Hertz-Picciotto et al., 2007). The control was free from all of the above conditions. Each case was matched with one healthy control by geographic location, age group, and gender. In addition, all parents answered questionnaire on the children's medical history and life-style choices. The ethics committee of the Institute of Experimental Medicine, Czech Academy of Sciences, approved the study. The parents of the children signed an informed consent.

2.2. Air pollution monitoring of polycyclic aromatic hydrocarbons

We obtained the ambient benzo[a]pyrene data from the Czech Hydrometeorological Institute (CHMI) website. CHMI manages and monitors eight airborne PAHs and PM_{2.5} using Versatile Air Pollution Sampler (VAPS) since 1990s (Pinto et al., 1998). Based on the CHMI protocol, the mean daily level is measured once every three days for total of 10 days/month in southern Bohemia; once every six days for total of 5 days/month in Ostrava. Additional details on quality assurance and control have been described (Choi et al., 2017; Ghosh et al., 2016; Hertz-Picciotto et al., 2007).

2.3. Urinary 8-oxo-dG

The analysis of urinary 8-oxo-dG, using competitive ELISA, has been described (Rossner Jr et al., 2012). Briefly, urine samples were incubated with 50 μ L of 8-oxo-dG standards (concentration range, 1.25–40 ng/mL), 50 μ L of primary antibody (JaICA, Japan, clone N45.1, concentration 0.2 μ g/mL) as well as 100 μ L of secondary antibody conjugated with alkaline phosphatase. The urine samples were repeatedly incubated for 1.5 h at 37 °C and were washed with PBS/Tween and with 0.01% diethanolamine in water. The color absorbance was measured with a microplate reader at 405 nm. Any samples, which demonstrated inhibition < 20% or > 80% were again analyzed with or without further dilution. Urinary 8-oxo-dG concentration was measured as nmol 8-oxo-dG/mmol creatinine. The interassay coefficient of variability was calculated among the triplicate samples as 5.7%.

2.4. 15-F₂t-IsoP immunoassay

Plasma concentrations of 15-F₂t-IsoP were analyzed using immunoassay kits as described (Rossner Jr et al., 2012).

2.5. Protein carbonyl assay

Plasma concentration of carbonyl was analyzed using a non-competitive ELISA, as described (Rossner Jr et al., 2012). The concentration was expressed as nmol carbonyl/mL plasma. The interassay coefficient of variability was 3.1%.

2.6. Cotinine and vitamin assays

A spectrophotometric method for the estimation of urinary creatinine was based on Jaffe's reaction where a colored complex of creatinine with alkaline picrate was formed and then measured at 505 nm (Butler, 1975). Urine sample was used to validate self-reported tobacco exposure. Urinary cotinine levels were analyzed by radioimmunoassay (Rossnerova et al., 2011). Based on an earlier large cross-sectional investigation of tobacco smoke exposure in mother-child pairs, urinary cotinine > 450 ng/mg of creatinine was considered a cut-off value for active smoking status for the child; 20–449 ng/mg was deemed cut-off for passive smoking status (Dostál et al., 2008). Blood sample (~40 mL) was drawn by venipuncture into vacuettes containing lithium heparin (for the vitamin C assay) or EDTA (for the vitamin A, E assays). All blood samples were stored at 4 °C and transported within 18 h of collection to the Department of Genetic Toxicology and Nanotoxicology (Driskell et al., 1982).

2.7. Statistical analysis

We defined teenager those who are ≥ 12 years in age. Body mass index (BMI) was calculated as weight/height². We used an international standard for age and sex specific BMI cut-off points as the definition to categorize children to lean, overweight, or obese category (Cole et al., 2000). Due to sparsity of children who were deemed obese, we combined overweight and obese as single category (and henceforth termed OV/OB). B[a]P association with obesity and asthma were examined by following variables, including age, gender, cotinine, body mass index, season of delivery (indicator variables, fall, winter, and spring), birth-weight and gestational age at delivery. That is, univariate association was examined as Pearson χ^2 test. A linear association between B[a]P and combined outcome of asthma and overweight/obesity outcome were examined through a non-parametric comparison of the distribution, using Jonkheere-Terpestra test with $\alpha = 0.05$. Spearman's ranked agreement coefficient (δ) was examined for continuous scale variable for their association with B[a]P. In the logistic regression model, we retained the variable as a possible confounder in the model if the variable induced > 10% change in the regression coefficients of B[a]P. Modification of B[a]P-overweight/obesity association by a third factor was examined through a test of linear trend with $\alpha = 0.05$. Regression diagnostics were performed by removing the influential values to determine the robustness of the estimated odds ratios. All analyses were performed with SPSS version 20.0.1 for Windows (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Descriptive analysis

The distribution of ambient B[a]P concentration demonstrates large spatial and temporal variability between 2006 and 2008 across the two enrollment sites (JT-test p -value < 0.001; Fig. 1). Table 1 shows the demographic and the exposure traits of the children ($n = 361$), stratified according to their asthma-BMI combined categories (i.e. Lean Control, OV/OB Control, Lean Asthmatic, and OV/OB Asthmatic). Overall, OV/OB asthmatics had the highest prevalence of early-onset diagnoses (of atopic dermatitis, wheezing, or upper respiratory infection, respectively) at ≤ 3 years in age (all p -values < 0.05). For example, OV/OB asthmatic girls had a higher prevalence of the history of atopic dermatitis (65%) and wheezing (70%), respectively, at ≤ 3 yrs., compared to the lean control girls (4% and 3%, respectively, Table S1). Furthermore, OV/OB asthmatic girls also had higher percentages of current doctor-diagnosed allergic rhinitis (40%) or atopic dermatitis (15%), compared to the lean control girls (5% and 0%, respectively, Table S1). The ambient B[a]P (i.e. main exposure variables of interest) was highest near the homes of OV/OB asthmatics (Mean $\pm$ SD,

9.5 ± 8.5 ng/m³), and higher near the homes of lean asthmatics (Mean $\pm$ SD, 7.7 ± 7.3 ng/m³), compared to that for the lean controls (Mean $\pm$ SD, 4.3 ± 2.4 ng/m³; p -value = 0.002). Putative oxidative stress markers (i.e. 15-F2t-IsoP, 8-oxo-dG, and Carbonyl) were not associated with the health outcome (all p -values > 0.05).

3.2. B[a]P and 15-F2t-IsoP associations differ according to children's sex and BMI category

Ambient B[a]P's association with plasma 15-F2t-IsoP level was non-linear and divergent according to the OV/OB-, sex-, and age- of the children (Fig. 2). Among the lean children, the association between ambient B[a]P and the children's 15-F2t-IsoP were null (Fig. 2). However, the pre-adolescent OV/OB girls were associated with -15.1% change (95% CI, -0.9% to -29.3% ; $p = 0.038$) in 15-F2t-IsoP per unit increase in B[a]P, in contrast to -1.3% change (95% CI, -8.8% to 6.2% ; $p = 0.722$) among the lean girls of the same age group. The OV/OB teenage girls were associated with -4.2% change (95% CI, -21.8% to 13.4% ; $p = 0.583$) in 15-F2t-IsoP per B[a]P unit increase. In contrast, the lean teenage girls were associated with 9.6% increase (95% CI, 0.3% to 18.8% ; $p = 0.043$) per unit exposure.

On the other hand, the pre-adolescent OV/OB and adolescent OV/OB boys were respectively associated with -2.1% (95% CI, -36.6% to 32.3% ; $p = 0.888$) and 2.6% (95% CI, -19.7% to 24.9% ; $p = 0.809$) change in plasma 15-F2t-IsoP (Fig. 1). The same unit increase in B[a]P was associated with a negligible change in 15-F2t-IsoP level in the lean pre-adolescent (95% CI, -10.3% to 2.8% ; $p = 0.257$) and the lean adolescent boys (95% CI, -6.6% to 6.2% ; $p = 0.953$). Overall, non-parametric ranked agreement between B[a]P and the oxidant stress markers (i.e. 15-F2t-IsoP, 8-oxo-dG, and Carbonyl) were low (all $\delta < 0.2$; Table S2).

3.3. B[a]P concentration distribution across the children in BMI and atopic march onset and diversity index combined categories

The median and interquartile range of the B[a]P concentration is compared across the age-, gender-, and OV/OB—asthma combined outcome (Fig. 3). Compared to the pre-adolescent lean control girls (median, 4.4 ng/m³ in lower left panel, Fig. 3), both lean (10.3 ng/m³) and OV/OB asthmatic girls (9.5 ng/m³) of the same age range were associated with an elevated an elevated median B[a]P. Compared to the lean adolescent control girls (median, 3.3 ng/m³), the median B[a]P for the lean asthmatic girls (median, 4.4 ng/m³), and OV/OB asthmatic girls (median, 9.5 ng/m³) were significantly higher (JT p -value < 0.001).

The adolescent OV/OB asthmatic boys (2.9 ng/m³ on upper right panel, Fig. 3) were associated with similar median B[a]P as the lean asthmatic (median, 2.7 ng/m³) or the lean controls (median, 3.2 ng/m³) of the same age group (JT p -value > 0.05). The pre-adolescent lean asthmatic boys (upper left panel, Fig. 3) were associated with a higher median B[a]P (median, 7.6 ng/m³), compared to either OV/OB asthmatic (median, 4.3 ng/m³), or the lean control boys (median, 4.2 ng/m³), respectively (JT p -value > 0.05).

3.4. Markers of systemic oxidant stress according to asthma—BMI categories

The median and interquartile ranges of the children's 15-F2t-IsoP, 8-oxo-dG, and Carbonyls are summarized according to the gender-, age-, and asthma-BMI combined categories (Fig. S1). Overall, the OV/OB asthma outcome was associated with lower median 15-F2t-IsoP in the asthmatic children, compared to the lean controls (Fig. S1 (A)). For example, both pre-adolescent OV/OB and lean asthmatic girls were associated with lower 15-F2t-IsoP (median, 148.8 and 140.7 pg/mL, respectively) compared to the pre-adolescent lean control girls (median, 157.7 pg/mL; JT p -value > 0.05). On the other hand, the median

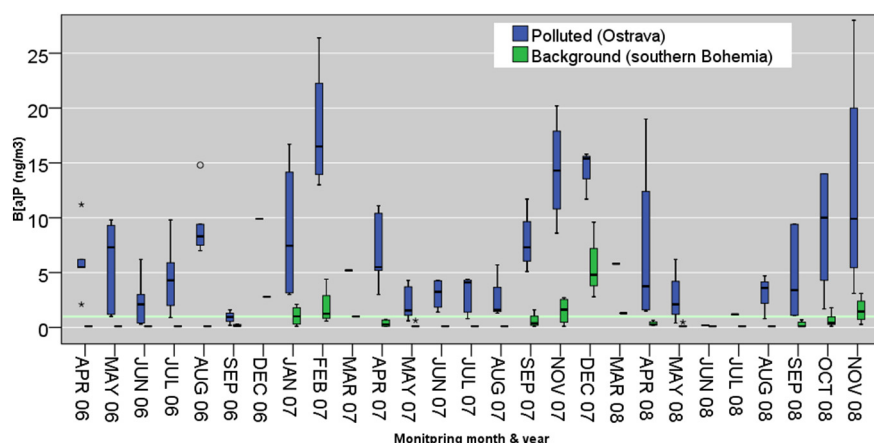


Fig. 1. Distribution of ambient B[a]P concentrations in polluted urban (i.e. blue bar) and rural (i.e. green) monitoring sites between 2006 and 2008 period. Each bar denotes 25th, 50th, and 75th percentile B[a]P value during given month and year at each study site. The edge of the whisker denotes 5th and 95th percentile values. The symbols, O and * represent concentrations > 1.5- and > 3-fold of the 75th percentile value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

15-F2t-IsoP was higher in the adolescent OV/OB asthmatic boys (median, 167.5 pg/mL), compared to the lean controls (median, 150.1 pg/mL).

The median 8-oxo-dG was not associated with a clear trend according to the gender and asthma-BMI combined categories (Fig. S1 (B)). However, consistent with the trend for 15-F2t-IsoP, the median Carbonyls were significantly reduced in the OV/OB asthmatic children (Fig. S1 (C)).

3.5. Modification of B[a]P—asthma association according to demographic traits

The adjusted odds ratio of multinomial asthma—BMI combined outcomes per (ln)-unit increase in B[a]P concentrations is shown in Table 2. Compared to the lean controls (i.e. reference group), the unit B[a]P exposure was associated with 4.3-times higher odds of asthma diagnosis (95% CI, 2.6–7.4; $p < 0.001$) among the lean children (Model A, Table 2). However, similar diagnosis was 5.7-times greater (95% CI, 2.8–11.7; $p < 0.001$) among the OV/OB children per (ln) unit

Table 1
Demographic and exposure traits of the four demographic groups.

		Lean control		OV/OB control		Lean asthma		OV/OB asthma		p-Value
		N	%	N	%	N	%	N	%	
Enrollment site	Rural	82	50%	11	58%	72	54%	19	43%	0.593
	Urban	82	50%	8	42%	62	46%	25	57%	
Age group	Children	84	51%	10	53%	71	53%	21	48%	0.944
	Adolescent	80	49%	9	47%	63	47%	23	52%	
Gender (female)		78	48%	10	53%	47	35%	20	46%	0.132
Used antibiotic in past < 2 weeks (yes)		3	2%	0	0%	2	1%	2	5%	0.552
MD-diagnosed atopic dermatitis, ≤ 3 yrs (yes)		5	3%	0	0%	48	36%	20	46%	< 0.001
MD-diagnosed wheezing, ≤ 3 yrs (yes)		2	1%	1	5%	91	68%	28	64%	< 0.001
Upper respiratory infection, < 3 yrs (yes)		3	2%	0	0%	12	9%	2	5%	0.025
IUGR		147	88%	17	90%	115	89%	39	91%	0.899
	Not IUGR	17	12%	2	10%	13	11%	4	9%	
	Mild	7	4%	1	5%	7	5%	2	5%	
	Moderate	6	4%	1	5%	4	3%	2	5%	
	Severe	7	4%	0	0%	4	3%	0	0%	0.121
Smoking	Non-smoker	123	75%	12	63%	94	70%	32	73%	
	Passive smoker	41	25%	7	37%	40	30%	12	27%	
Born at < 37 weeks (Yes)		8	5%	2	11%	12	9%	4	9%	0.477

	Mean ± SD (Min, Max)	Mean ± SD (Min, Max)	Mean ± SD (Min, Max)	Mean ± SD (Min, Max)	
Age	11 ± 3 (6, 16)	11 ± 2 (7, 14)	11 ± 3 (6, 16)	11 ± 3 (6, 16)	0.492
Birthweight (g)	3353 ± 585 (1470, 5150)	3301 ± 556 (2500, 4800)	3305 ± 566 (1080, 4800)	3308 ± 499 (2100, 4700)	0.288
Body mass index (kg/m ²)	18 ± 2 (13, 23)	24 ± 3 (18, 30)	17 ± 2 (7, 22)	24 ± 4 (18, 38)	< 0.001
Gestational age	40 ± 2 (32, 49)	39 ± 2 (35, 41)	39 ± 3 (26, 50)	40 ± 2 (33, 50)	0.358
B[a]P (ng/m ³)	4.3 ± 2.4 (0.5, 11.2)	3.4 ± 1.7 (0.5, 6.0)	7.7 ± 7.3 (0.5, 28.0)	9.5 ± 8.5 (1.0, 28.0)	0.002
15-F2t-IsoP (pg/mL plasma)	154.3 ± 36.9 (79.9, 317.4)	171.1 ± 40.1 (121.2, 255.4)	156.7 ± 61.6 (71.5, 604.0)	164.4 ± 66.9 (41.6, 400.4)	0.872
8-oxo-dG (nmol/mmol creatinine)	6.0 ± 2.3 (0.3, 14.7)	6.3 ± 1.8 (3.2, 9.5)	5.9 ± 2.4 (0.1, 14.4)	5.5 ± 2.1 (0.4, 10.1)	0.195
Carbonyls (nmol/mL plasma)	21.2 ± 4.8 (12.7, 38.0)	24.4 ± 5.0 (14.7, 35.3)	20.8 ± 4.1 (14.4, 34.5)	21.4 ± 3.5 (15.4, 29.6)	0.768

Categorical variables were tested using Chi-square test of linear-by-linear association. Distribution of the continuous variables was tested using Jonkheere-Terpstra test.

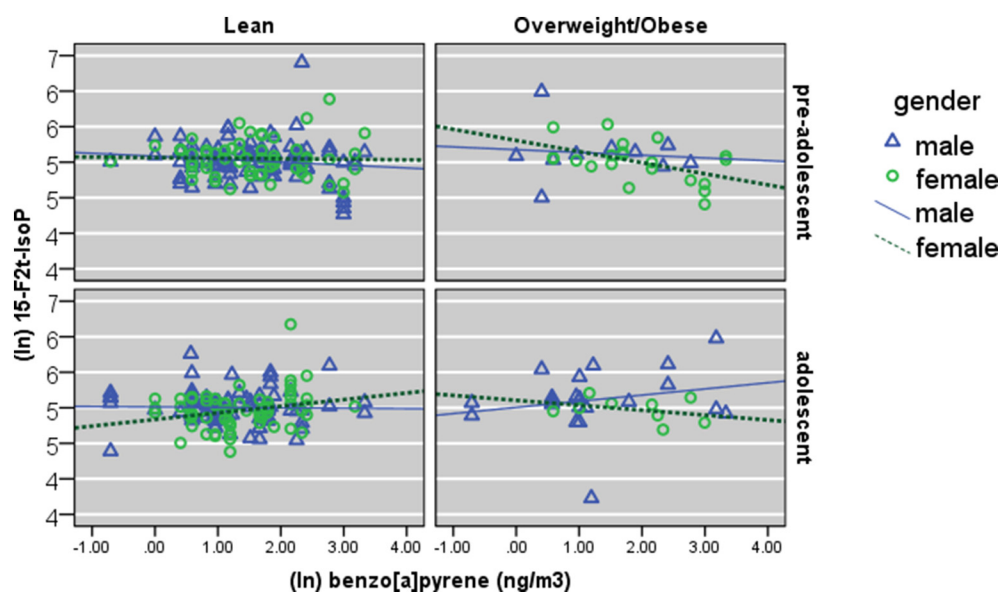


Fig. 2. Scatter plot of ambient B[a]P (ng/m³) and plasma 15-F2t-IsoP in all children. The children are stratified according to OV/OV status, gender-, and age group. The lines represent children's plasma (ln) 15-F2t-IsoP as a linear function of (ln) B[a]P.

exposure (Model A, Table 2).

When we further stratified the children according to their gender (Model B, Table 2), the odds ratio of asthma was comparable between the lean boys (aOR = 5.0; 95% CI, 2.5–9.9; $p < 0.001$) and the OV/OB boys (aOR = 4.2; 95% CI, 1.6–11.0; $p = 0.004$) per unit exposure. However, the same unit increase among the girls was associated with considerably higher odds of asthma among the OV/OB girls (aOR = 5.9; 95% CI, 1.9–17.7; $p = 0.002$) than the lean girls (aOR = 3.8; 95% CI, 1.9–17.7; $p = 0.002$), compared to the lean controls (Model B, Table 2).

Following an additional stratification of the children according to their age and gender (Model C, Table 2), the adolescent OV/OB boys were associated with 10.5-times greater odds (95% CI, 2.6–39.6; $p = 0.001$), compared to the unit odds among the lean controls. In contrast, the adolescent OV/OB girls were associated with highest adjusted odds of the asthma (aOR = 15.4; 95% CI, 2.9–29.1; $p < 0.001$)

compared to the lean control girls.

3.6. Direct effect of B[a]P on asthma outcome, following oxidative stress marker adjustment

We compared the total vs. the direct effect of (ln)-unit increase in ambient B[a]P on the multinomial outcomes, by postulating the total B[a]P effect (Model C, Table 2) as the one which adjusts only for the demographic traits (but not the oxidative stress markers). In contrast, the direct effect models were postulated as those which additionally adjust for the children's 15-F2t-IsoP (Model A, Table 3), and 15-F2t-IsoP and Carbonyls (Model B, Table 3), respectively.

An additional adjustment for 15-F2t-IsoP was associated with a highest adjusted odds of OV/OB asthma outcome among the adolescent girls (aOR = 15.3; 95% CI, 1.4–164.4; $p = 0.024$; Model A, Table 3), which was comparable to total effect model estimate (aOR = 15.4; 95%

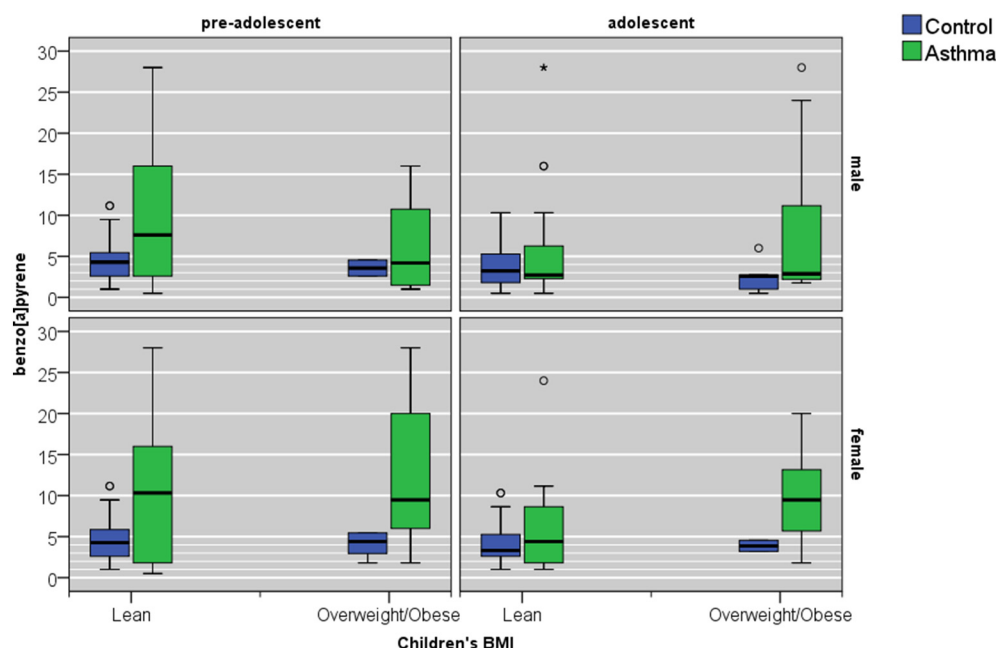


Fig. 3. Ambient benzo[a]pyrene distribution (ng/m³) near children's residence. The children are stratified according to OV/OV status and atopic march onset and diversity index. They are further grouped by age group and gender. Box shows 25th, 50th, and 75th percentile; the whiskers show the 5th and the 95th percentiles, respectively. The symbols, O and * represent concentrations > 1.5- and > 3-fold of the 75th percentile value. Jonckheere–Terpestra (JT) test was used to assess a linear trend of B[a]P, without assuming a normal distribution of the underlying the sample distributions, using $\alpha = 0.05$.

Table 2

Ambient B[a]P (coded as either continuous or tertile categories) and adjusted odds ratio of multinomial outcomes in overall (Model A), gender-stratified (Model B), and age- and gender-stratified participants (Model C), using multinomial logistic regression models.

			Continuous B[a]P			Categorical coding of B[a]P					
			n	One (ln) unit		1st tertile	2nd tertile		3rd tertile		
				aOR	(95% CI) <i>p</i> -Value		aOR	(95% CI) <i>p</i> -Value	aOR	(95% CI) <i>p</i> -Value	
Model A ^a		Lean controls (reference)	164	1.0			1.0		1.0		
		OV/OB controls	19	0.6	(0.2 to 1.6) <i>p</i> = 0.348	Reference	1.2	(0.3 to 4.7) <i>p</i> = 0.764			
		Lean asthmatics	134	4.3	(2.6 to 7.4) <i>p</i> < 0.001	Reference	0.5	(0.2 to 1.0) <i>p</i> = 0.037	6.9	(2.5 to 19.5) <i>p</i> < 0.001	
		OV/OB Asthmatics	44	5.7	(2.8 to 11.7) <i>p</i> < 0.001	Reference	0.8	(0.3 to 2.4) <i>p</i> = 0.720	8.7	(2.2 to 33.8) <i>p</i> = 0.002	
Model B ^b	Male	Lean controls (reference)	86	1.0			1.0		1.0		
		OV/OB controls	9	0.6	(0.2 to 2.0) <i>p</i> = 0.408	Reference	0.9	(0.2 to 5.4) <i>p</i> = 0.911	0.0	(0.0 to 0.0)	
		Lean asthmatics	87	5.0	(2.5 to 9.9) <i>p</i> < 0.001	Reference	0.6	(0.2 to 1.6) <i>p</i> = 0.318	10.6	(2.7 to 41.8) <i>p</i> = 0.001	
		OV/OB Asthmatics	24	4.2	(1.6 to 11.0) <i>p</i> = 0.004	Reference	1.1	(0.3 to 4.1) <i>p</i> = 0.850	9.8	(1.6 to 58.9) <i>p</i> = 0.013	
	Female	Lean controls (reference)	78	1.0		Reference	1.0		1.0		
		OV/OB controls	10	0.9	(0.2 to 4.8) <i>p</i> = 0.870	Reference	2.7	(0.3 to 27.0) <i>p</i> = 0.394	0.0	(0.0 to 0.0)	
		Lean asthmatics	47	3.8	(1.6 to 9.0) <i>p</i> = 0.002	Reference	0.3	(0.1 to 1.0) <i>p</i> = 0.058	3.6	(0.6 to 20.2) <i>p</i> = 0.149	
		OV/OB asthmatics	20	5.9	(1.9 to 17.7) <i>p</i> = 0.002	Reference	0.3	(0.0 to 3.8) <i>p</i> = 0.365	3.3	(0.2 to 48.3) <i>p</i> = 0.387	
	Model C ^c	Pre-adolescent Male	Lean controls (reference)	45	1.0		Reference	1.0		1.0	
			OV/OB controls	2	0.7	(0.0 to 12.9) <i>p</i> = 0.809	Reference	0.0	(0.0 to 0.0) <i>p</i> = 0.997	0.0	(0.0 to 0.0) <i>p</i> = 0.997
			Lean asthmatics	48	5.2	(2.0 to 13.6) <i>p</i> = 0.001	Reference	0.2	(0.1 to 1.0) <i>p</i> = 0.043	3.2	(0.5 to 17.8) <i>p</i> = 0.140
			OV/OB asthmatics	8	1.1	(0.2 to 5.4) <i>p</i> = 0.903	Reference	0.0	(0.0 to 0.0) <i>p</i> = 0.997	0.0	(0.0 to 0.0) <i>p</i> = 0.997
		Pre-adolescent Female	Lean controls (reference)	39	1.0		Reference	1.0		1.0	
			OV/OB controls	8	0.8	(0.1 to 5.1) <i>p</i> = 0.809	Reference	1.1	(0.1 to 18.5) <i>p</i> = 0.931	0.0	(0.0 to 0.0) <i>p</i> = 0.997
			Lean asthmatics	23	4.8	(1.6 to 14.0) <i>p</i> = 0.001	Reference	0.3	(0.1 to 2.5) <i>p</i> = 0.250	2.3	(0.2 to 22.5) <i>p</i> = 0.140
			OV/OB asthmatics	13	5.8	(1.7 to 20.2) <i>p</i> = 0.005	Reference	0.0	(0.0 to 0.0) <i>p</i> = 0.997	0.0	(0.0 to 0.0) <i>p</i> = 0.997
Adolescent Male		Lean controls (reference)	41	1.0		Reference	1.0		1.0		
		OV/OB controls	7	0.6	(0.2 to 2.3) <i>p</i> = 0.363	Reference	2.4	(0.3 to 17.9) <i>p</i> = 0.469	0.0	(0.0 to 0.0)	
		Lean asthmatics	39	5.0	(1.8 to 13.8) <i>p</i> = 0.001	Reference	1.7	(0.4 to 6.7) <i>p</i> = 0.439	32.0	(3.6 to 281.5) <i>p</i> = 0.002	
		OV/OB asthmatics	16	10.1	(2.6 to 39.6) <i>p</i> = 0.001	Reference	4.9	(1.0 to 25.6) <i>p</i> = 0.057	56.2	(4.8 to 655.9) <i>p</i> = 0.001	
Adolescent Female		Lean controls (reference)	39	1.0		Reference	1.0		1.0		
		OV/OB controls	2	0.9	(0.2 to 1.9) <i>p</i> = 0.363	Reference	to	(0.3 to 17.9) <i>p</i> = 0.998	1.8	(0.0 to 0.0)	
		Lean asthmatics	24	2.4	(1.7 to 8.4) <i>p</i> = 0.001	Reference	0.2	(0.0 to 1.2) <i>p</i> = 0.086	7.4	(0.4 to 139.2) <i>p</i> = 0.180	
		OV/OB asthmatics	7	15.4	(2.9 to 29.1) <i>p</i> < 0.001	Reference	1.1	(0.1 to 20.2) <i>p</i> = 0.950	–	–	

^a All ORs are adjusted for the enrollment region, age group, creatinine adjusted cotinine. Actively smoking pre-adolescent was excluded from the analysis.

^b Low range include < 6.3 ng/m³ and high includes 6.3–8.5 ng/m³.

^c Pre-adolescent and adolescents are those < 12 yrs and ≥ 12 yrs in age at the time of the sampling.

CI, 2.9–29.1; *p* = 0.001; Model C, Table 2). Additional adjustment of the model for Carbonyls, in addition to 15-F2t-IsoP, was not associated with a considerable change in aORs for all sub-groups (Model B, Table 3).

4. Discussion

The question regarding early-life air pollution exposure and the developmental risk on the lean asthma and/or the obesity-associated

Table 3

Direct effect of (ln)-unit increase in ambient B[a]P and the multinomial outcomes, following the adjustment for 15-F2t-IsoP (Model A), 15-F2t-IsoP and Carbonyls (Model B), and 15-F2t-IsoP, Carbonyls, and 8-oxo-dG (Model C), respectively.

	Pre-adolescent male			Pre-adolescent female			Adolescent male			Adolescent female		
	N	aOR	(95% CI) p-Value	N	aOR	(95% CI) p-Value	N	aOR	(95% CI) p-Value	N	aOR	(95% CI) p-Value
Model A (adjusts for 15-F2t-IsoP)												
Lean controls	45	1.0		38	1.0		41	1.0		39	1.0	
(reference)	2	0.7	(0.0 to 15.4)	8	0.8	(0.1 to 5.1)	7	0.6	(0.2 to 2.3)	2	0.6	(0.0 to 91.8)
OV/OB control			<i>p</i> = 0.838			<i>p</i> = 0.830			<i>p</i> = 0.480			<i>p</i> = 0.822
Lean asthmatic	48	5.0	(1.9 to 13.0)	23	4.4	(1.5 to 13.3)	39	5.1	(1.9 to 14.2)	24	2.9	(0.7 to 12.3)
			<i>p</i> = 0.001			<i>p</i> = 0.008			<i>p</i> = 0.002			<i>p</i> = 0.154
OV/OB asthmatic	8	1.1	(0.2 to 5.7)	13	4.6	(1.3 to 16.8)	16	8.5	(2.2 to 33.3)	7	15.3	(1.4 to 164.4)
			<i>p</i> = 0.879			<i>p</i> = 0.021			<i>p</i> = 0.002			<i>p</i> = 0.024
Model B (adjusts for 15-F2t-IsoP and Carbonyls)												
Lean controls	45	1.0		38	1.0		41	1.0		39	1.0	
(reference)	2	1.7	(0.1 to 35.3)	8	1.6	(0.2 to 12.0)	7	0.8	(0.2 to 3.1)	2	0.7	(0.0 to 129.4)
OV/OB control			<i>p</i> = 0.743			<i>p</i> = 0.648			<i>p</i> = 0.736			<i>p</i> = 0.887
Lean asthmatic	48	5.2	(2.0 to 13.7)	23	4.5	(1.5 to 13.8)	39	5.4	(1.9 to 15.5)	24	2.8	(0.7 to 12.3)
			<i>p</i> = 0.001			<i>p</i> = 0.008			<i>p</i> = 0.002			<i>p</i> = 0.165
OV/OB asthmatic	8	1.3	(0.3 to 6.9)	13	4.8	(1.2 to 18.4)	16	8.5	(2.2 to 32.7)	7	16.2	(1.4 to 181.8)
			<i>p</i> = 0.723			<i>p</i> = 0.024			<i>p</i> = 0.002			<i>p</i> = 0.024

In addition to the oxidative stress markers, the three models adjust for the enrollment region and creatinine adjusted cotinine. All active smokers were removed from the analysis.

asthma, remains unanswered. Such question is important because higher exposure to air pollution and high-fat food items are disproportionately more prevalent in the socio-economically disadvantaged groups (Choi and Perera, 2012). Furthermore, obesity-associated asthma phenotype is typically more severe (Accordini et al., 2008) and poorly controlled with pharmaceutical therapies (Nagel et al., 2009). To date, various markers of air pollution exposure have been shown to pose greater asthma risk on the overweight/obese children (Agrawal and Prakash, 2014; Dong et al., 2013; Lang et al., 2013). However, the sources of greater vulnerability of the overweight/obese children to air pollution have never been systematically investigated. Within the present investigation, we demonstrate for the first time that the OV/OB girls might represent the most vulnerable subgroup to airborne B[a]P. This is particularly notable, considering that adolescent OV/OB girls were associated with overall lower B[a]P distribution, compared to the pre-adolescent OV/OB girls (Fig. 3). Specifically, highest ambient B[a]P distribution among the pre-adolescent OV/OB girls were associated with a largest reduction in their 15-F2t-IsoP level (Fig. 3 and Fig. S1 (A)). In turn, a unit B[a]P exposure was associated with highest adjusted odds of asthma (aOR = 16.2; 95% CI, 1.4 to 181.8; *p* = 0.024) among the adolescent girls, who were also OV/OB, compared to the lean controls. In contrast, same unit of B[a]P exposure was associated with a non-significant increase in the odds of asthma among the lean adolescent girls (aOR = 2.8; 95% CI, 0.7 to 12.3; *p* = 0.165), compared to the lean controls.

Obesity-mediated inflammation and its role in asthma onset and/or exacerbation is characterized by a complex interplay of pro- and anti-inflammatory adipokines and cytokines (Dixon et al., 2010). Within our data, adolescence (≥ 12 yrs. in age) was associated with a leap in the odds of asthma among the OV/OB females, compared to that among the preadolescent OV/OB girls (Table 3). Such trend is counter-intuitive, because pre-adolescent OV/OB girls experienced a highest median B[a]P in all demographic groups. Accordingly, we expected the pre-adolescent girls to show a greatest adjusted-OR per unit exposure, rather than the adolescent OV/OB girls (Fig. 2). Such paradoxical association might reflect greater suppression of 15-F2t-IsoP, Carbonyls, and/or 8-oxo-dG among the pre-adolescent children, compared to the adolescent children (Figs. 3–5). That is, in spite of highest interquartile range of B[a]P exposure, an inverse association between 15-F2t-IsoP and asthma severity among pre-adolescent OV/OB children, suggest

possible suppression of 15-F2t-IsoP as well as an activation of other anti-inflammatory effect of B[a]P among younger and/or mildly affected (e.g. OV/OB controls) children. Such anti-inflammatory mechanisms among the pre-adolescent OV/OB female children might confer at least partial protection against B[a]P. In contrast, approximately U-shaped association between 15-F2t-IsoP and asthma-OV/OB combined outcome among the adolescent OV/OB females (most of whom experienced somewhat lower median B[a]P than the pre-adolescent girls) suggest exhaustion of such anti-inflammatory protection, and greater susceptibility to asthma (Tables 2 and 3).

In addition, preponderance of seminal allergic events (e.g. atopic dermatitis, wheezing, allergic rhinitis, and upper respiratory infection) among the adolescent OV/OB girls and the lean pre-adolescent boys suggest that the asthma mechanisms are preceded by allergic march during the first three years of life (Table 1). It is possible that the lean male children, and adolescent OV/OB female children are associated with different stages of incremental cellular oxidative stress induced by PAHs, roughly distinguished in terms of three tiers (Li et al., 2008). The preliminary, tier 1 response is characterized by mounting of anti-oxidant defense by the host. Our observation of stronger correlation between 15-F2t-IsoP and B[a]P among the pre-adolescent OV/OB children support possibly negative mediation by 15-F2t-IsoP, 8-oxo-dG, Carbonyls, and/or their proximal correlates, consistent with tier 1 oxidative stress response. In contrast, tier 2 and 3 are characterized by inflammatory responses, depletion of intracellular glutathione reserve, and a cascade of reactions, resulting in mitochondrial injury (Li et al., 2003). Some of the traits of the adolescent OV/OB asthmatic girls are consistent with tier 2 and/or 3 responses by initiating heme catabolism, upregulate heme oxygenase-1 (HO-1) expression, and produce anti-inflammatory substances (manuscript under review). Our observation of puberty as a second critical developmental time-point also suggest that sexually dimorphic interaction between air pollution and obesity might manifest at certain critical life stages. Deeper clarification of the age- and sex-dependent mechanisms of PAH is warranted using a prospective cohort design.

Our observation concurs with a growing body of evidence that early-life exposures to environmental toxics ‘programs’ the host to a dysregulated state of metabolism, thereby predisposing the host to obesogenic effects. For example, in utero exposure to ambient air polycyclic aromatic hydrocarbons (PAHs), significantly predicts

childhood obesity (Jung et al., 2014). Furthermore, maternal exposure to cigarette smoke during pregnancy not only increase the risk of asthma (Weitzman et al., 1990), but also the obesity in the child (Power and Jefferis, 2002; von Kries et al., 2002). Post-natal exposure to air pollution has been associated with exacerbation of inflammation and insulin unresponsiveness in a mouse model (Liu et al., 2014; Sun et al., 2009). Conversely, pre-existing obesity could worsen inflammatory effects of the air pollution (Guarnieri and Balmes, 2014). Adult male offspring of the rats, which were prenatally exposed to diesel exhaust followed by postnatal high-fat diet tended to be heavier, less active, and more anxious than their air-pollution unexposed counterparts. The adult female rats, which were prenatally exposed to diesel exhaust, in combination with postnatal high fat diet showed 340% higher rate of weight gain than the unexposed females (Bolton et al., 2014). In a large, cross-sectional examination of 30,000 children, a unit exposure to particulate matter with an aerodynamic diameter < 10 µm (PM₁₀), was associated with greater risk of respiratory symptoms and asthma among the OV/OB children than the lean children (Dong et al., 2013).

Our observation of non-linear and divergent association between the oxidative stress markers (i.e. 15-F2t-IsoP, 8-oxo-dG, and Carbonyls) with B[a]P and asthma outcome, respectively, according to the sex-, age-, and adiposity- warrant caution in their application as biomarkers. Other, earlier investigations, for example, on white male workers within plastic laminate factories (who also smoked) noted significantly elevated 15-F2t-IsoP among the smoke and formaldehyde-exposed workers (Romanazzi et al., 2013). The difference in the results between our and Romanazzi et al. (2013) investigation might be due to greater heterogeneity of air pollution exposure range and history within our case-control study.

Strengths of the present investigation include the availability of one-of-its-kind stationary air monitoring of airborne PAHs. To our knowledge, Czech Republic remains the only nation, which conducts a nationwide routine monitoring program of airborne PAHs. Outside of Czech Republic, high cost of conducting personal and/or ambient monitoring of PAHs represent one of the most critical barriers to deeper understanding of the PAH risk on young children. In addition, large spatial variability in ambient B[a]P concentration between high vs. low polluted region in Czech Republic helped us to overcome the need for a large sample size for children, typically expected for gene × environment interaction research.

At the same time, our sample size is modest, and thus, did not allow direct testing of the mediation of the B[a]P effect by the oxidative stress markers among the overweight/obese children. In addition, we used B[a]P concentration from the stationary monitor closest to the child's residence as a proxy for his/her personal exposure level. Therefore, our approximation of personal exposure is overall crude. As the sources of PAHs differ between Ostrava (i.e. urban polluted region) and Southern Bohemia (i.e. rural background), other proximal correlate of B[a]P (e.g., metals) could have confounded the association in our analysis. Within Ostrava region, major sources of PAHs include industrial power plants, coal combustion for domestic heating, automobile and truck emissions (Binkova et al., 2003; Sram et al., 2005). Within Southern Bohemia, coal combustion for domestic heating is considered the main source of B[a]P. Therefore, the difference in the relative abundance of the toxic substances between the urban and the rural region might have contributed to the variability of the risk. In addition, the data on home indoor environment, such as presence of molds or indicators of exposure to house dust mite, and pet ownership were not collected. Other important sources of PAHs, such as dietary sources, might have contributed to the outcome. We are currently conducting a prospective birth cohort study of human body burden of polycyclic aromatic hydrocarbons of the Czech population (Srám et al., 2016). That is, we compare inhalational vs. dietary exposure to PAHs in a small subset of mother-newborn cohort (n = 40). Our preliminary findings indicate that both the dietary intake and the exposure via inhalation contribute to human body burden (Pulkrabova et al., 2016). However, during the

heating season, majority of exposure occurs through inhalation, particularly in the polluted urban environment (Pulkrabova et al., 2016). During the summer, the major part of exposure (60–90%) occurs through diet intake in both the polluted urban and the rural population (Pulkrabova et al., 2016). In newborns, maternal breast milk contribute by about 20–50% of the total PAH exposure depending on the season (Pulkrabova et al., 2016).

We carefully considered, but decided against, conducting a mediation analysis by systemic oxidant stress markers (i.e. 15-F2t-IsoP, Carbonyls, and 8-oxo-dG). This was due to extreme assumptions the mediation analysis poses on our modest sample size. First assumption requires that there be no other confounding factors, other than the above three oxidant stress markers. Second assumption requires that there be no interaction between the exposure and the intermediates. Our Fig. 1 demonstrates that our data violate the second assumption. Third assumption requires that there be little to no measurement error associated with the mediators. We could not rule out that systemic oxidant stress (measured in terms of plasma 15-F2t-IsoP and Carbonyls, as well as the urinary 8-oxo-dG) might be associated with some degree of measurement error on the impairments of the respiratory system. Finally, our study is a cross-sectional in design. Therefore, reverse causality of oxidant stress as consequence, rather than the cause, could not be ruled out. Finally, we did not use precise indicators of children's adiposity (e.g. truncal adiposity). Rather, we used BMI as a proxy for overweight and obesity status.

5. Conclusion

B[a]P exposure was associated with a large leap in the odds of asthma among the OV/OB adolescents, particularly the girls, after adjusting for 15-F2t-IsoP, Carbonyls, and 8-oxo-dG.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2018.08.061>.

Statement of author contributions

H. Choi conducted all analysis and wrote all sections of the manuscript. R. J. Sram coordinated the study. M. Dostal and A. Pastorkova collected health status and biological material from the children. Pavel Rossner, Jr., analyzed oxidative damage in urine and plasma. S. Ho contributed to interpretation of the data.

Acknowledgements

We thank Dr. Eva Schallerova, Ostrava-Radvanice, and other pediatricians from the district of Prachatice for facilitating the collection of blood and sputum samples from the children. This work was supported by the Czech Ministry of the Environment, Czech Republic [grant number SP1b35007]; Ministry of Education, Czech Republic [grant number 2B08005]; and Academy of Sciences, Czech Republic [grant number AVOZ50390703].

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Online Supporting Information

Table S1: Exposure and lifestyle characteristics of the four demographic sub groups, stratified by gender.

		Lean Control			OV/OB Control			Lean Asthmatic			OV/OB Asthmatic			P
Enrollment site (urban)	male	41	48%		2	22%		37	43%		9	38%		0.368
	female	41	53%		6	60%		25	53%		16	80%		0.125
atopic dermatitis ≤3 yrs of age (yes)	male	2	2%		0	0%		31	36%		7	29%		< 0.001
	female	3	4%		0	0%		17	36%		13	65%		< 0.001
wheezing ≤3 yrs of age (yes)	male	0	0%		0	0%		62	71%		14	58%		< 0.001
	female	2	3%		1	10%		29	62%		14	70%		< 0.001
upper resp. infection at <3 yrs (yes)	male	1	1%		0	0%		9	10%		1	4%		0.041
	female	2	3%		0	0%		3	6%		1	5%		0.338
allergic rhinitis in past 12 mo. (yes)	male	4	5%		1	11%		24	28%		8	33%		< 0.001
	female	4	5%		0	0%		15	32%		8	40%		< 0.001
atopic dermatitis in past 12 mo. (yes)	male	1	1%		0	0%		10	12%		1	4%		0.031
	female	0	0%		0	0%		5	11%		3	15%		0.001
Fetal growth restriction (severe)	male	4	5%		0	0%		4	5%		0	0%		0.789
	female	3	4%		0	0%		0	0%		0	0%		0.101
Smoking (passively exposed)	male	23	27%		1	11%		27	31%		9	38%		0.290
	female	18	23%		6	60%		13	28%		3	15%		0.833
Child born preterm (Yes)	male	5	6%		0	0%		10	12%		3	13%		0.139
	female	3	4%		2	20%		2	4%		1	5%		0.866
age group (adolescent)	male	41	48%		7	78%		39	45%		16	67%		0.494
	female	39	50%		2	20%		24	51%		7	35%		0.469
Percentiles														
B[a]P (ng/m ³)		5 th	50 th	95 th	5 th	50 th	95 th	5 th	50 th	95 th	5 th	50 th	95 th	
	male	1.0	3.8	9.5	0.5	2.6	.	1.2	3.3	24.0	1.1	2.9	27.0	0.121

	female	1.5	3.8	10.3	1.8	4.4	.	1.0	5.5	24.0	1.8	9.5	28.0	0.001
15-F2t-IsoP (pg/ml plasma)	male	105.9	153.9	240.7	121.2	159.2	.	91.7	148.2	223.7	53.8	167.1	399.1	0.556
	female	101.5	147.7	217.0	139.8	168.1	.	99.2	150.6	326.8	83.0	144.7	208.0	0.496
8-oxo-dG (nmol/mmol creatinine)	male	2.9	5.9	10.4	3.2	5.7	.	2.4	5.8	10.3	1.0	5.1	8.9	0.205
	female	1.8	5.5	10.7	5.0	6.5	.	1.0	5.4	9.4	1.6	5.5	10.0	0.995
Carbonyls (nmol/ml plasma)	male	14.5	19.9	31.6	14.7	26.4	.	14.8	20.1	30.1	16.2	22.8	29.3	0.126
	female	15.1	21.6	32.2	17.0	24.0	.	15.8	20.5	28.5	15.4	19.9	25.8	0.239
Cotinine (µg/g creatinine)	male	3.5	10.5	97.3	1.8	10.0	.	3.4	9.4	129.2	3.0	10.2	121.6	0.598
	female	3.2	8.8	80.9	7.8	28.2	.	2.5	6.2	103.2	4.7	9.2	58.4	0.777
Vitamin A (mg/l plasma)	male	0.3	0.7	1.3	0.5	0.7	.	0.3	0.6	1.3	0.3	0.8	1.7	0.532
	female	0.2	0.6	1.8	0.4	0.6	.	0.3	0.7	1.2	0.4	0.7	1.2	0.476
Vitamin C (mg/l plasma)	male	3.8	7.7	14.8	4.0	6.7	.	2.7	6.7	11.8	2.9	6.9	.	0.124
	female	2.1	7.2	12.6	6.4	8.3	.	3.3	5.7	.	3.5	6.3	.	0.634
Vitamin E (mg/l plasma)	male	5.3	9.9	15.7	6.9	9.9	.	4.1	9.4	19.4	3.9	10.9	20.5	0.746
	female	4.1	10.5	16.3	7.7	9.6	.	3.4	10.6	18.9	5.7	9.0	14.0	0.952

Table S2: Spearman's ranked agreement coefficient (δ) and sample size (N) among children's exposure and potential susceptibility factors.

	<i>B[a]P</i>	<i>15-F2t-IsoP</i>	<i>8-oxo-dG</i>	<i>Carbonyl</i>	<i>Cotinine</i>
	δ (N)	δ (N)	δ (N)	δ (N)	δ (N)
<i>B[a]P</i>	1.000 (385)	-0.059 (384)	0.087 (385)	-0.146 ** (384)	-0.019 (385)
<i>15-F2t-IsoP</i>		1.000 (384)	0.127 * (384)	-0.094 (384)	0.011 (384)
<i>8-oxo-dG</i>			1.000 (385)	0.022 (384)	0.070 (385)
<i>Carbonyl</i>				1.000 (384)	0.109 * (384)
<i>Cotinine</i>					1.000 (385)