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Prenatal Polycyclic Aromatic Hydrocarbon (PAH) Exposure, Antioxidant Levels and Behavioral Development of Children Ages 6–9

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Abstract

Purpose—Prenatal polycyclic aromatic hydrocarbon (PAH) exposure has been shown to increase DNA adduct levels and to affect neurodevelopment. Micronutrients may modify the adverse effect of PAH on neurodevelopment. Thus, we examined if micronutrient concentrations modified the association between PAH exposure and neurodevelopmental outcomes.

Methods—151 children from a birth cohort who had micronutrient concentrations measured in cord blood and completed the Child Behavioral Checklist (CBCL), between the ages of 6 and 9

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Conflicts of Interest

The authors declare that they have no conflict of interest.

years, were evaluated. Prenatal airborne PAH exposure was measured by personal air monitoring. The betas and 95% CI for the associations of antioxidant concentrations and PAH exposure with each of the outcomes of CBCL raw score and dichotomized standardized T-score (based on clinical cutpoints) were estimated, respectively, by multivariable poisson and logistic models.

Results—Children below the median for alpha-tocopherol and gamma-tocopherol concentrations, compared to those above, were more likely to have thought problems, aggressive behavior and externalizing problems ($p<0.05$). Lower carotenoid concentration was associated with more thought problems ($MV\beta=0.60$, $p<0.001$) and externalizing problems ($MV\beta=0.13$, $p<0.05$) for the same contrast. No statistically significant associations were observed between retinol concentrations and neurodevelopmental symptoms. Overall, no consistent patterns were observed when we examined the interaction between antioxidants (e.g., alpha-tocopherol) and PAH in relation to CBCL symptoms (e.g., internalizing and externalizing problems, $p<0.05$).

Conclusions—Lower alpha-tocopherol, gamma-tocopherol and carotenoid levels may adversely affect healthy neurodevelopment, even after accounting for PAH exposure. Future research to confirm these findings are warranted given the importance of identifying modifiable factors for reducing harmful PAH effects.

Keywords

Antioxidants; Neurodevelopment; PAH; Cohort; Children

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are a group of pollutants due to incomplete combustion of carbon sources stemming from industrial activities, coal heating, vehicle exhaust, as well as tobacco smoke, and grilled foods (Guo et al., 2011). Exposure to PAHs can result in genotoxic effects, DNA damage through DNA adduct formation, and dysregulation of the epigenome (Farmer et al., 2003; Jedrychowski et al., 2013; Perera and Herbstman, 2011; Perera et al., 2005). Most prior research has been conducted in adults; however, evidence over the last decade has emerged showing that prenatal and early life exposure to PAH has effects on fetuses and infants, and may result in long term detrimental effects (Perera et al., 1999). For example, higher prenatal PAH exposure was inversely associated with measures of fetal growth, such as birth weight and head circumference, and neurodevelopment outcomes in children (Jedrychowski et al., 2003). Within two birth cohorts in the United States and Poland, prenatal PAH exposure was associated with poor cognitive development and intelligence, as well as behavioral problems, in children measured at three to seven years of age (Edwards et al., 2010; Perera et al., 2009; Perera et al., 2006; Perera et al., 2012; Perera et al., 2013). Given the potential long term consequences of prenatal and early life exposure to PAH, reduction of exposure to PAH and identification of modifiable risk factors that modulate downstream effects of PAH exposure are crucial.

Micronutrients, particularly antioxidants, found in fruits and vegetables, oils and nuts and supplemental forms, may modulate PAH exposure by quenching free radicals, reducing DNA adduct formation (Kelvin et al., 2009), and ultimately preventing harmful downstream

effects, such as poor health (Masters et al., 2007; Pedersen et al., 2013) and neurodevelopmental outcomes. In particular, alpha-tocopherol (Vitamin E) has been shown to be critical for fetal growth, normal neurological function and development of the nervous system (Kiely et al., 1999a; Kiely et al., 1999b; Masters et al., 2007). Similarly, retinol (Vitamin A) is essential for postnatal brain development activities such as learning and spatial memory (Haga et al., 1982). In addition, carotenoids seem to exhibit protective effect against Benzo(a)Pyrene (B(a)P)-induced oxidative stress and DNA damage (Azqueta and Collins, 2012), possibly by combating oxidative stress via free radical scavenging activity (Baydas et al., 2002a; Baydas et al., 2002b). However, to date, no study has analyzed the modulating effect of micronutrient levels on the association between prenatal and early life exposure to PAH and the behavioral development of children. Thus, within a well characterized birth cohort based in Krakow, Poland, we examined the interaction effects of antioxidant micronutrient concentrations and prenatal PAH exposure, measured by air concentrations of eight PAH carcinogens, on neurodevelopment of children.

2. MATERIALS AND METHODS

2.1 Population

A prospective birth cohort study conducted by the Columbia Center for Children's Environmental Health (CCCEH) and Jagiellonian University was designed to study the health effects of prenatal exposure to air pollution in children in Krakow, Poland (Jedrychowski et al., 2004; Jedrychowski et al., 2003). Non-smoking women ages 18 to 35 year with singleton pregnancies, during the 1st or 2nd trimester of pregnancy, who lived within a 0.5 km radius of the ambient air monitoring stations (ródmie cie and Krowodrza district) were recruited for this study (n=535 maternal-newborn pairs). Informed consent was obtained from all participants after a full description of the study was given. The study was approved by the ethics committees of the Jagiellonian University in Poland and Columbia University in the United States. Details of the study are described elsewhere (Jedrychowski et al., 2003).

2.2 Exclusion Criteria for the Cohort

Women were excluded from the cohort study if they had a history of illicit drug use, an incomplete or unreliable record for determining gestational age, a history of potential occupational exposure to PAH or any chronic health condition. As only non-smoking women were eligible for the cohort study, we excluded 33 mother-newborn pairs for whom the maternal blood sample had cotinine levels ≥ 25 ng/mL, indicating active smoking during pregnancy despite their self-reported non-smoking status. In addition, as the overarching aim of the parent study was to examine the association of PAH with health outcomes, we excluded 112 mother-infant pairs whose PAH measurement did not meet quality control criteria, which included problems with the PEMS filter, PUF filter, pump or battery, flows during measurements or average flow; quality control exclusions were not based on low prenatal PAH measurements. Thus, this resulted in an analytic sample of 423 maternal-newborn pairs.

2.3 Study Procedures

At entry into the study and during the 3rd trimester of pregnancy, a comprehensive questionnaire was administered to study participants. The questionnaire included questions on, but was not limited to, demographics (e.g., age, sex), lifestyle factors (e.g., exposure to environmental tobacco smoke), socioeconomic status (e.g., maternal education, occupation, household income), medical history, as well as a 27-item questionnaire, Psychiatric Epidemiology Research Instrument – Demoralization (Peri-D) to assess maternal demoralization (Clarke and Kissane, 2002; Dohrenwend et al., 1980), a standard measure of maternal psychologic distress (Perera et al., 2013). The questionnaire also ascertained dietary habits for foods (poultry, beef, pork, sausage) likely to contain PAH (e.g., type of cooking process (smoked, fried, broiled, and barbecued) for specific types of meat, and the frequency at which they consumed those food items ranging from “never” to “daily”); similar tools have shown to be valid instruments for measuring dietary PAH exposure (John et al., 2011; Sinha et al., 2005). Dietary PAH was calculated as the sum of the frequency of consumption of each type of food multiplied by the PAH value for that food item under that specific cooking process (Jedrychowski et al., 2012; Kelvin et al., 2009). Cord blood, at the time the umbilical cord was cut, and maternal venous blood, within 1 day of delivery, was collected. Plasma was separated at the Jagiellonian University Clinical Lab, then stored at -70°C , and shipped to Columbia University in New York City for analysis. Personal inhalation exposure and indoor exposure were measured during the 2nd trimester.

Following the birth, health and lifestyle questionnaires were administered to the mothers every 3 months until the age of 4 and every year until the age of 9. Medical chart reviews were also completed for the mother and newborn to collect information on factors such as fetal growth (e.g., gestational age) and medical complications.

2.4 Exposure Assessment

2.4.1. Micronutrient Concentrations—Plasma micronutrients concentrations from maternal and cord blood samples were analyzed at the Centers for Disease Control and Prevention Nutritional Biomarkers Laboratory. Retinol, alpha (α)-tocopherol, gamma (γ)-tocopherol, and a composite variable for carotenoids, which was calculated as the sum of the plasma α -carotene, beta(β)-carotene, β -cryptoxanthin, lutein/zeaxanthin and lycopene concentrations (Sowell et al., 1994), were measured using a minor modification of isocratic high-performance liquid chromatography and multi-wave length detection. Briefly, a 100- μL aliquot of plasma, precipitated with a mixture of two internal standards (nonapreno- β -carotene and retinyl butyrate) dissolved in ethanol, was extracted with hexane, dried, redissolved in equal parts ethanol and acetonitrile, and it was then filtered to remove any insoluble material. Fat soluble micronutrients were released from their binding proteins (RBP, albumin, lipoproteins, etc) when the internal standard was added to the serum. The internal standard serves two functions, namely, 1) protein precipitation because it is diluted in ethanol, which precipitates proteins, and 2) addition of unique compounds that behave similarly to the micronutrients and are tracked throughout the assay to assess the recovery of the micronutrients and allow for adjustment of the final concentrations based on recovery. An aliquot of the filtrate was injected into a C18 reversed phase column (Phenomenex Ultracarb; 4.6×150 mm; 3- μm particle size) maintained at 25°C and eluted with 50%: 50%

ethanol in acetonitrile for 15 minutes. Micronutrient quantitation was accomplished by comparing the peak height or area of the analyte in the plasma extract with the peak height or area of a known amount of standard in a calibrator solution; corrections were made based on the peak height or peak area of an internal standard. Retinol, and α -tocopherol and γ -tocopherol were compared with retinyl butyrate at 325 and 300 nm, respectively. Carotenoids were compared with nonapreno- β -carotene at 450 nm. Three in-house serum pools were used as quality control measures for the fat-soluble micronutrient assay and the target values for these pools were verified against National Institute of Standards and Technology (NIST), Standard Reference Material (SRM) 968c and through overlap comparison with three previous quality control pools. Additional external quality assurance was provided through participation in semiannual NIST (Gaithersburg, MD) round-robin exercises for all fat-soluble micronutrients. The inter-day coefficients of variation for three levels of quality control materials ranged from 3.5% to 3.8% for retinol A, 2.6% to 3.0% for α -tocopherol, 3.0% to 4.0% for γ -tocopherol, and 6.0% to 13.1% for the carotenoids based on quality control rules established by Caudill et al (Caudill et al., 2008). There were no results less than the limit of detection for vitamins A and E. Individual carotenoid results less than the limit of detection were assigned half the limit of detection for calculating total carotenoid estimates. The limit of detection values were as follows: retinol (1.03 $\mu\text{g/dL}$), α -tocopherol (40.67 $\mu\text{g/dL}$), γ -tocopherol (10.72 $\mu\text{g/dL}$), α -carotene (0.71 $\mu\text{g/dL}$), trans- β -carotene (0.79 $\mu\text{g/dL}$), β -cryptoxanthin (0.85 $\mu\text{g/dL}$), lutein/zeaxanthin (2.43 $\mu\text{g/dL}$), and trans-lycopene (0.77 $\mu\text{g/dL}$). The limits of detection were established by measuring a pool of specimens from severely micronutrient deficient populations in 7 runs in duplicate. The LOD was estimated as three times the standard deviation of the average of 14 estimates of these low analytes levels.

2.4.2. Lead Concentrations—Lead was measured using inductively coupled plasma mass spectrometry, DLS method code (CDC, 2003).

2.4.3. Personal Ambient Air Monitoring—During the 2nd trimester of pregnancy, personal air monitoring to capture mother's inhalation PAH exposure was conducted for a forty-eight-hour consecutive period. Personal ambient air monitors in small backpacks were worn by mothers during the day, and positioned next to their bed at night. The polyurethane foam cartridges were analyzed at Southwest Research Institute for levels of pyrene and eight carcinogenic PAH [benzo(a)anthracene, chrysene/iso-chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, B(a)P, indeno(1,2,3-cd)pyrene, dibenz (a,h)anthracene, and benzo(g,h,i)perylene] as previously described (Choi et al., 2006; Tonne et al., 2004). The air concentrations of eight carcinogenic PAH were summed to calculate the total airborne PAH exposure (Choi et al., 2006). The prenatal air monitor measure of PAH exposure was not correlated with dietary PAH exposure (Perera et al., 2013).

2.5. Outcome

Behavioral development was measured by maternal report on the 118-item Child Behavior Checklist (CBCL) for children at 6–9 years of age (Achenbach and Edelbrock, 1981; Achenbach and Rescorla, 2001; Achenbach et al., 1987; Hudziak et al., 1999). The CBCL assesses 8 different behavioral domains, which include Withdrawn/Depressed, Somatic

Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior and Aggressive Behavior. From the CBCL assessment, a raw and a standardized T-score are calculated. The raw scores range from 0 to a maximum raw scores of 26 for anxious/depressed domain, 16 for withdrawn/depressed domain, 22 for somatic complaints domain, 22 for social problems domain, 30 for thought problems domain, 20 for attention problems domain, 34 for rule-breaking behavior domain, and 36 for aggressive behavior domain. Internalizing problems is the sum of the scores on the anxious/depressed, withdrawn/depressed and somatic complaints scales; a higher score denotes more symptoms or problems. Externalizing problems is the sum of scores on the rule-breaking and aggressive behavior scales. The standardized T scores range from 50 to 100; 65 represent the presence of borderline/clinical range or greater than the 93rd percentile for that domain, symptom and/or composite score (Achenbach et al., 1991; Doyle and McCarthy, 2001).

2.6. Exclusion Criteria for the Analytic Sample

Of the 423 mother-child pairs, women and children were excluded from the analyses if the child had missing CBCL results between ages six and nine years (n=122), or either the mother or child was missing any of the micronutrient measurements (n=105) or any of the covariate information (n=45), leaving 151 mother-child pairs for the final analytic sample.

2.7. Statistical Analyses

Frequencies and mean levels of demographic, health and environmental characteristics were compared between those subjects who were in the analysis compared to those who were excluded using a t-test for continuous variables and chi-squared test for categorical variables. Dietary PAH exposure was examined as a continuous variable, while total airborne PAH exposure was examined as a continuous variable, that was natural log transformed to stabilize the variation. The carotenoids, retinol and α -tocopherol and γ -tocopherol were examined as continuous and dichotomized variables; micronutrient variables were dichotomized into low versus high categories based on the median in cord blood of 10.9 mg/dL for γ -tocopherol, 271.3 mg/dL for α -tocopherol, 19 mg/dL for cord retinol, and 1.37 mg/dL for the carotenoids compound. The raw score for each component of the CBCL was examined as a count variable, while the standardized T score was treated as a dichotomized variable representing those classified as borderline/clinical range for that domain based on the threshold of >65, versus not. Previous research using latent class analysis has shown that the domains or symptoms measured by the CBCL can also be examined as continuous or counts, instead of just based on the clinical range, to look at the full range of values, as determined in a large representative sample of US children (Hudziak et al., 1999).

Using Poisson regression, we examined the association between blood cord concentration of each micronutrient and the raw score of each of the eight domains from the CBCL. We used the Poisson model for the raw scores outcome, as they are counts data with a right skew. We applied the same approach for examining the dietary and airborne PAH exposure with each of the eight domains from the CBCL. For the dichotomized outcome of T-score, we used logistic regression to calculate odds ratios and 95% confidence intervals. In the regression models, we adjusted for the sex of the newborn (male, female), maternal gestational age

(continuous), maternal years of education (continuous), maternal psychological distress as measured by maternal demoralization score (continuous), the age of the child at the time of the assessment (continuous), heating season (yes, October to April; no, May to September), lead exposure ($\mu\text{g}/\text{dl}$, continuous), prenatal and postnatal (at three years of age of child) environmental tobacco smoke in the household (yes, no), as well as dietary PAH (continuous). We also assessed if the associations between continuous log PAH exposure and CBCL domain raw and standardized T scores were modified by levels of micronutrient concentrations (low versus high micronutrient concentrations dichotomized at the median). We assessed the statistical significance of the cross-product term between the PAH exposure (modeled as a log transformed continuous variable) and the micronutrient concentrations (low versus high (reference category) micronutrient concentrations dichotomized at the median) using a Wald test. Statistical significance was set at the 0.05 level. SAS software, version 9.2, was used.

3. Results

151 mother-child pairs were included in our analysis (Table 1). We did not observe any statistically significant differences between those included versus those not included in the study by maternal (e.g., maternal years of education, maternal psychological distress as measured by the demoralization score) and offspring characteristics (e.g., sex of the newborn). Similarly, there were no differences in the mean log prenatal air PAH concentrations or in the percentage exposed to prenatal and postnatal environmental tobacco smoke. However, offspring included in the analysis had slightly higher mean log cord lead concentrations ($0.36 \pm 0.45 \mu\text{g}/\text{dl}$) compared to those not included in the analysis ($0.27 \pm 0.42 \mu\text{g}/\text{dl}$, $p\text{-value} = 0.05$) and were less likely to have their prenatal air monitoring during the heating season (50% vs 60%, $p\text{-value} = 0.04$). The Pearson correlation coefficient for α -tocopherol, γ -tocopherol, carotenoids and retinol ranged from 0.29 to 0.77 ($p\text{-value} < 0.001$; data not shown).

In Table 2, the frequency and distribution of the symptoms and problems of the CBCL are presented for those excluded from the analysis and those included in the analysis. The mean age of CBCL assessment within our analysis was 7.6 year and ranged in age from 6.1–9.3 years. The majority of the children were ages 6 and 7 (73%). Overall, the mean raw scores were similar for each of the eight domains and the two summary scales, internalizing problems and externalizing problems, measured by the CBCL for those included compared to those not included. However, the percent of children classified as borderline or clinical for the CBCL was slightly higher for four out of the eight domains and the two summary scales for those included compared to those not included.

For this analytic sample, we observed positive associations between each unit increase in continuous natural log PAH exposure from air measurement and more adverse score on CBCL for the domains of withdrawn/depressed ($\beta = 0.34$, $p = 0.0002$), social problems ($\beta = 0.18$, $p = 0.009$), attention ($\beta = 0.17$, $p = 0.003$), aggressive behavior ($\beta = 0.17$, $p = 0.0002$), internalizing problems ($\beta = 0.12$, $p = 0.004$), and externalizing problems ($\beta = 0.17$, $p < 0.0001$); these estimates were adjusted for alpha-tocopherol concentrations, in addition to all the main covariates described in the methods. We observed a statistically significant association

between continuous dietary PAH and more adverse score on the CBCL for the domain of attention problems ($\beta = -0.02$, $p=0.03$); however, there was no statistically significant associations observed for any other domain. The risk estimates were similar (data not shown) when we included a different cord micronutrient (e.g., gamma-tocopherol, total carotenoids) as a covariate in separate regression models in place of alpha-tocopherol concentrations or when we examined airborne PAH exposure as a dichotomized variable based on the median.

When examining the association between dichotomized cord micronutrients levels (cut at median of the subjects included in the study) and CBCL syndrome raw scores (Table 3), low cord concentrations of alpha-tocopherol levels, compared to high micronutrient concentrations, were statistically significantly associated with higher domain scores for thought problems ($\beta = 0.68$, $p<0.001$), aggressive behavior ($\beta = 0.16$, $p=0.04$) and externalizing problems ($\beta = 0.14$, $p=0.04$). Similar inverse associations were observed for low cord concentrations, compared to higher cord concentrations, of gamma-tocopherol for domain of thought problems ($\beta = 0.57$, $p=0.002$), aggressive behavior ($\beta = 0.40$, $p<0.001$), and externalizing problems ($\beta = 0.32$, $p<0.001$). The results also showed significant inverse associations between low levels of cord carotenoids, compared to high concentrations, and higher (more adverse) scores on the domains of thought problems ($\beta = 0.60$, $p<0.001$) and externalizing problems ($\beta = 0.13$, $p=0.05$). No statistically significant results were found for any of the symptoms or problems within the CBCL when examining cord concentrations of retinol levels. When examining the domain score as a standardized T score, which classifies children as within the borderline/clinical range, these aforementioned statistically significant findings were not observed (data not shown), except for the association between gamma-tocopherol and aggressive behavior ($\beta = 0.13$, $p=0.05$). However, we had small number of children classified as borderline/clinical range and limited power to assess these associations.

We observed statistically significant effect modification by dichotomized alpha-tocopherol, gamma-tocopherol and carotenoid micronutrient levels on the association between continuous log airborne PAH exposure and select behavioral domains of the CBCL (Table 4). Low, compared to high, alpha-tocopherol concentrations modified the association between continuous log airborne PAH exposure and the behavioral domains of Withdrawn / Depressed (β for interaction $=0.25$, p -value for interaction $=0.02$), Aggressive Behavior (β for interaction $=0.15$, $p=0.02$), Internalizing Problems (β for interaction $=0.11$, p -value for interaction $=0.03$) and Externalizing problems (β for interaction $=0.11$, p -value for interaction $=0.04$). Compared to high concentrations, low cord concentrations of gamma-tocopherol modified the association between log airborne PAH exposure and internalizing problems (β for interaction $=0.14$, p -value for interaction $=0.007$). In addition, low cord concentrations of carotenoids levels modified the association between log airborne PAH exposure and the domains of Aggressive Behavior (β for interaction $=0.14$, p -value for interaction $p=0.02$) and Externalizing problems (β for interaction $=0.12$, p -value for interaction $=0.02$).

When we stratified on micronutrient status, we observed a slightly stronger adverse association of continuous log PAH exposure on Withdrawn / Depressed in low alpha-

tocopherol levels ($\beta = 0.35$, p -value = 0.004) than in the high alpha-tocopherol levels ($\beta = 0.17$, p -value = 0.26) (Table 5). When stratifying by gamma-tocopherol, the association between log airborne PAH exposure and internalizing problems for those with low micronutrient concentrations was stronger ($\beta = 0.13$, p -value = 0.03) compared to high micronutrient concentrations ($\beta = 0.09$, p -value = 0.10). This was also observed for social problems, in which the low gamma-tocopherol group had a stronger positive association for log airborne PAH exposure compared to the higher gamma-tocopherol group. For anxious/depressed, aggressive behavior, rule breaking, internalizing problems and externalizing problems, we observed a stronger positive association between log airborne PAH exposure and the domain score in the low carotenoid group compared to the high carotenoid group. When stratifying by retinols, we observed a stronger positive association for log airborne PAH exposure with anxious/depressed, withdrawn/depressed, social problems, and internalizing problems for the low retinol group compared to the high retinol group. However, for the other domains of CBCL, we observed no difference between high versus low micronutrient status or observed a stronger positive PAH-CBCL association in the high micronutrient arm compared to the lower micronutrient arm.

4. DISCUSSION

This study provides evidence that low prenatal micronutrient concentrations of alpha-tocopherol, gamma-tocopherol and carotenoids at birth were associated with adverse children's behavioral neurodevelopment as measured by the CBCL, at the age of 6 to 9 years. In particular, statistically significant findings were observed for less favorable scores for the domains of thought problems, aggressive behavior and externalizing problems for low compared to high alpha-tocopherol, gamma-tocopherol and carotenoids. In addition, PAH measures from prenatal personal air monitoring, but not dietary PAH, was positively associated with adverse children's neurodevelopment. Overall, we did not observe a consistent pattern with regards to the interaction effect between airborne PAH exposure and micronutrient concentrations on each of these neurodevelopment domains.

A number of previous studies have examined the association between prenatal micronutrient supplementation of the mother or newborn cord micronutrient levels and neurodevelopment and behavior of the offspring. Most prior studies, particularly randomized clinical trials, examined retinol, B-vitamins, folic acid, iron or other micronutrients, macronutrients or fatty acids (Leung et al., 2011). In a large systematic review of 18 studies, conducted by Leung et al. (Leung et al., 2011), higher fatty acids and multi-micronutrients intakes and/or concentrations were more consistently associated with better mental development; results from studies examining single supplements were mixed. Few studies have examined cord micronutrient levels of alpha-tocopherol, gamma-tocopherol, or carotenoids. Our results showed that low concentrations of alpha-tocopherol, gamma-tocopherol and carotenoids in cord blood were associated with higher symptom scores for select domains on the CBCL. Similar to our study, two recent analyses within the same study (Kiely et al., 1999a; Zhang et al., 2009), suggested that higher vitamin A and E in cord blood were associated with children's cognitive as well as behavioral development quotients, as measured by Gesell development Schedules at age 2. These micronutrients, alpha-tocopherol, gamma-tocopherol and carotenoids, are important for fetal growth, brain development, and reduction in

oxidative stress and DNA damage (Azqueta and Collins, 2012; Baydas et al., 2002a; Baydas et al., 2002b; Chen et al., 2009; Haga et al., 1982; Jedrychowski et al., 2013; Kiely et al., 1999b; Masters et al., 2007; Zhang et al., 2009). Although we examined the four micronutrients individually, we were unable to tease apart the independent effect of each micronutrient due to the high correlation among some of the micronutrient concentrations (r ranged from 0.29–0.77). Additionally, we could not examine the associations of each micronutrient stratified by other micronutrient levels (e.g., high/low tocopherol by high/low carotenoids) due to limited power and a smaller sample size. Future examination of multi-micronutrient concentrations and supplementation with multi-micronutrients may be more beneficial to reducing harmful effects of PAH, particularly given a number of these micronutrient have overlapping or interacting biological effects.

Besides examination of the range of scores for each domain for the CBCL, our study observed no statistically significant association when the outcome was based on the standardized T score. In our study, the number of children who were classified within the borderline/clinical range varied from one to twenty cases, depending on the domain. Due to the small number of cases classified as borderline/clinical range within each domain, we had limited power to examine the standardized T score. However, examining across the range of scores for each domain may provide opportunities for understanding more subtle changes in neurodevelopment and behavior.

To our knowledge, no previous study has examined whether micronutrients, particularly antioxidants, modifies the association between PAH exposure and child neurodevelopment. PAH exposure has previously been shown to be associated with DNA adduct formation, epigenetic alterations, and has detrimental effects on neurodevelopment and behavior (Alshaarawy et al., 2013; Edwards et al., 2010; Perera et al., 2009; Perera et al., 2006; Tang et al., 2012). In the Columbia Center for Children's Environmental Health longitudinal cohort study in New York City, a statistically significant positive association was observed between higher prenatal PAH exposure and symptoms of Anxious/Depressed and Attention problems (Perera et al., 2012). In this analysis, we observed positive associations between PAH exposure and other domains on the CBCL, namely withdrawn/depressed, social problems, aggressive behavior, internalizing problems and externalizing problems. In addition, we observed inverse associations between low micronutrient concentrations and other domains of the CBCL (e.g., thought problems, aggressive behavior) in our population. Certain micronutrients or combinations of micronutrients may be beneficial to neurodevelopment, even in the presence of PAH exposure, due to their antioxidant capacity, potential ability to reduce DNA adducts (Kelvin et al., 2009), DNA damage (Azqueta and Collins, 2012; Collins et al., 2012), and inflammation (Cooney et al., 2008; Jiang et al., 2009; Rubin et al., 2012; Singh et al., 2005; Singh and Jialal, 2004). This hypothesis has been supported by the following: 1) research in adults in which higher fruit and/or vegetable or related micronutrient intake or concentration is associated with lower DNA damage (e.g., DNA adducts) in most studies (Palli et al., 2003; Palli et al., 2000; Peluso et al., 2008), 2) related research in which the effect of other air pollutants on neurodevelopment has been modified by antioxidant levels (Guxens et al., 2012), and 3) within our cohort, Kelvin et al. (Kelvin et al., 2009) observed a stronger association between PAH exposure and PAH-DNA adducts when concentrations of alpha-tocopherol and carotenoid were below the

median compared to above the median. These results (Kelvin et al., 2009) suggest that one mechanism in which micronutrient levels may benefit neurodevelopment is through preventing the formation of PAH-DNA adducts.

As we observed adverse effects on neurodevelopment due to low micronutrient status, and nutritional intake is modifiable through improved dietary and supplemental intake, a plausible strategy for reducing the harmful effects of PAH could be targeted prevention methods, particularly for pregnant women in areas of higher PAH concentrations. However, we interpret our results with caution. Within this manuscript, we examined a large number of associations, and we observed a limited number of significant associations. Thus, due to the issues of multiple comparisons, some of the statistically significant associations we observed may be explained by chance. Specifically, we conducted 40 statistical tests for Table 3, 40 statistical tests for Table 4, and 80 tests for Table 5. However, even after applying the Bonferroni corrections to Table 3, 4 and 5 ($0.05/40 = 0.00125$; $0.05/40 = 0.00125$; $0.05/80 = 0.000625$), four out of eight associations for Table 3, zero out of seven for Table 4, and three out of 27 would still be considered statistically significant. These statistically significant findings are further supported by biologic rationale (e.g. antioxidant capacity to reduce DNA adducts formation). Due to our small sample size and concern about multiple comparisons, these results require replication in larger studies. Furthermore, we did not have information on dietary intake of micronutrients, related food items and supplemental intakes within this population. However, we believe that serum levels of micronutrients captures information beyond just intake levels, such as biological dose, that may be more relevant for our research question.

This study has several strengths including that our analyses were conducted within a well-characterized cohort with detailed epidemiologic, environmental and neurodevelopmental information. We also adjusted our analyses for a number of known and suspected confounders that had been previously identified in this cohort. However, to do this, we excluded individuals missing any covariate information, thus limiting our sample size to 151 mother-children pairs. When comparing the 151 mother-children pairs to the larger 423 mother-children pairs, we did not observe any statistically significant differences for a number of important demographic, lifestyle and environmental factors at birth and during the first year of follow-up. Nonetheless, we cannot rule out uncontrolled confounding by an unknown or unmeasured factor or residual confounding from measurement error in the included covariates.

Furthermore, we cannot rule out misclassification of or changes in PAH exposure and micronutrient concentrations during childhood as our analyses are based on only baseline information, which might result in greater misclassification of these levels compared with data that is based on multiple assessments throughout follow-up. In particular, we measured plasma vitamin A using HPLC, a standard and valid measure of vitamin A; however, as we are not measuring vitamin A concentrations through hepatic biopsy, an invasive measure that would be unsuitable in children, our measure may not adequately reflect vitamin A deficiency. We also did not measure serum cholesterol in our analytic sample and are unable to normalize the alpha-tocopherol measures as has been suggested in some prior publications to account for the high correlation between vitamin E and cholesterol (Ford et

al., 2006; Schleicher et al., 2013). However, any misclassification of these levels should not have varied by outcome status in this prospective study and, as such, may only have resulted in non-differential misclassification. The effect of non-differential misclassification would have tended to attenuate the associations. Further, single measurements of PAH exposure has been linked to long term health consequences and shown to be important to neurodevelopment in this and other cohorts (Edwards et al., 2010; Perera et al., 2009; Perera et al., 2006; Perera et al., 2012).

As PAH exposure has been shown to increase DNA adduct levels, and to have long-term detrimental effects on health, research to identify modifiable factors to reduce the adverse effects from PAH exposure is critical. Our results suggest that micronutrients, such as alpha-tocopherol, gamma-tocopherol and carotenoids may be important for neurodevelopment and behavior and that these micronutrients may protect against the adverse effects of PAH on neurodevelopment and behavior. However, due to the small sample size and limited power, future research is needed to confirm these findings.

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Highlights

- Lower antioxidants levels may harm neurodevelopment after adjusting for PAH levels.
- No interaction was present between antioxidants and PAH on CBCL symptoms.
- Future research is needed to confirm these findings.

Table 1

Demographic, health and environmental characteristics of the Polish Birth Cohort

	N	Mean + SD or %	Subjects who have a PAH measurement ¹	Subjects excluded from Analysis	Subjects included in the Analysis ²	P-value, test for equality ³
Maternal						
Education (Years)	423	15.53 (2.81)	272	15.62 (2.84)	151	15.38(2.76) 0.40
Demoralization ⁴	423	1.03 (0.43)	272	1.02 (0.45)	151	1.03 (0.41) 0.85
Dietary PAH ⁵	380	42.75 (5.89)	229	42.68 (5.89)	151	42.85 (5.90) 0.77
Offspring						
% Female	423	49.6%	272	48.5%	151	51.7% 0.54
Gestational Age ⁶	423	39.39 (1.53)	272	39.33 (1.51)	151	39.48 (1.56) 0.33
Log Cord Lead (µg/dl)	396	0.30 (0.43)	245	0.27 (0.42)	151	0.36 (0.45) 0.05
Log Prenatal PAH ⁷	423	3.03 (1.29)	272	3.07 (1.30)	151	2.96 (1.27) 0.38
Prenatal ETS (%) ⁸	423	21.28%	272	20.2%	151	23.2% 0.48
Postnatal ETS (%) ⁸	418	26.3%	267	24.0%	151	30.5% 0.15
Heating Season (%) ⁹	422	56.4%	271	60.1%	151	49.7% 0.04

¹ . The whole cohort includes those individuals who were enrolled and had an airborne PAH measurement (n=423).

² . The second column (n=151) represents those mother-child pairs who completed a CBCL assessment at age 6–9 years, had a prenatal PAH measurement and blood cord micronutrient measurement, and complete data on the covariates included in the regression models.

³ . P-value, test for equality was based on a chi-square test for categorical variables and a t-test for continuous variables.

⁴ . Maternal demoralization was measured by 27-item PERI-D administered during pregnancy

⁵ . To calculate dietary PAH, dietary questionnaires during pregnancy ascertained the consumption of grilled, fried and broiled meat.

⁶ . Gestational age defined and calculated by obstetrician and based on the last menstruation date.

⁷ . Prenatal air monitoring of polycyclic aromatic hydrocarbons (PAH)

⁸ . Environmental tobacco smoke (ETS) was based on maternal report of ETS exposure in the household during pregnancy (prenatal) and at three year of age (postnatal)

The percentage of mother-child dyads were the prenatal air monitored PAH was measured during the heat season of October-April

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Table 2

Frequency and Distribution of the Child Behavior Checklist (CBCL) measures in the Polish Birth Cohort.

Domain	Those with a CBCL and PAH measurement ¹ (N=301)		Those Included in the Analysis ² (N=151)	
	Mean age 7.3 yrs (6.1–9.3)		Mean age 7.6 yrs (6.1–9.3)	
	Raw Score Range	Raw Score Mean	Borderline or clinical range % ²	Borderline or clinical range % ¹
Anxious/depressed	0–17	3.97	12.6	0–16 4.35 13.2
Withdrawn/depressed	0–14	1.51	6.6	0–8 1.42 7.9
Somatic Complaints	0–16	1.46	6.6	0–8 1.44 6.6
Social problems	0–13	2.33	5.0	0–11 2.45 4.6
Thought problems	0–11	1.27	1.3	0–11 1.09 0.7
Attention problems	0–13	3.37	3.7	0–13 3.70 6.0
Rule-breaking behavior	0–31	2.97	3.7	0–6 1.87 2.0
Aggressive Behavior	0–21	5.46	6.3	0–20 5.16 9.3
Internalizing Problems	0–40	7.67	12.6	0–31 7.22 13.2
Externalizing Problems	0–58	10.47	3.7	0–24 7.03 5.3

¹ . This first column (n=301) represents those mother-child pairs who completed a CBCL assessment at age 6–9 years and had a prenatal PAH measurement from air monitors. The second column (n=151) represents those mother-child pairs who completed a CBCL assessment at age 6–9 years, had a prenatal PAH measurement and blood cord micronutrient measurement, and complete data on the covariates included in the regression models.

² . The raw scores range from 0 to a maximum raw scores of 26 for anxious/depressed domain, 16 for withdrawn / depressed domain, 22 for somatic complaints domain, 22 for social problems domain, 30 for thought problems domain, 20 for attention problems domain, 34 for rule breaking behavior domain, and 36 for aggressive behavior domain; a higher score denotes more symptoms or problems.

³ . The standardized T scores range from 50 to 100; 65 represent the borderline/clinical range or greater than the 93rd percentile for that symptom.

Table 3

The association between dichotomized low versus high cord micronutrient concentrations and CBCL Syndrome raw score in children ages 6–9 years, adjusting for select covariates (N=151)^{1,2}

Domain	α -tocopherol		γ -tocopherol		Carotenoids		Retinol	
	β	p-value	β	p-value	β	p-value	β	p-value
Anxious/Depressed	-0.05	0.56	-0.01	0.93	-0.06	0.45	-0.08	0.33
Withdrawn /Depressed	0.15	0.34	0.14	0.37	0.06	0.67	-0.01	0.95
Somatic Complaints	-0.02	0.89	-0.09	0.54	0.02	0.92	-0.002	0.99
Social Problems	0.09	0.46	0.10	0.39	-0.09	0.44	0.04	0.70
Thought Problems	0.68	<0.001	0.57	0.002	0.60	<0.001	0.0001	1.00
Attention Problems	0.18	0.06	0.10	0.27	0.08	0.40	-0.12	0.17
Rule-breaking Behavior	0.08	0.56	0.09	0.50	0.10	0.45	-0.15	0.23
Aggressive Behavior	0.16	0.04	0.40	<0.001	0.14	0.07	-0.06	0.44
Internalizing Problems	-0.002	0.98	0.003	0.96	-0.02	0.77	-0.05	0.41
Externalizing Problems	0.14	0.04	0.32	<0.001	0.13	0.05	-0.09	0.19

¹ . Poisson models were used to examine the association between low compared to high micronutrients concentrations and CBCL Syndrome Raw Score, controlling for sex of the newborn, maternal gestational age, maternal years of education, maternal psychological distress, the age of the child at the time of the CBCL assessment, heating season, lead exposure, prenatal and postnatal environmental tobacco smoke, continuous natural log transformed prenatal PAH exposure measured from air monitors and dietary PAH.

² . The cutpoint for the low vs high micronutrient group was determined by the median value of the subjects included in the study with high micronutrient as the reference level. Micronutrient variables were dichotomized into high/low categories based on the median in cord blood of 10.9 mg/dL for cord γ -tocopherol, 271.3 mg/dL for α -tocopherol, 19 mg/dL for cord retinol, and 1.37 mg/dL for the carotenoids compound

Interaction effects of the micronutrient¹ and PAH1 on CBCL syndrome raw scores in children ages 6–9 years, adjusting for select covariates² (N=151)

Table 4

Domain	α -tocopherol and PAH		γ -tocopherol and PAH		Carotenoids and PAH		Retinol and PAH	
	β inter	p-value	β inter	p-value	β inter	p-value	β inter	p-value
Anxious/Depressed	0.11	0.08	0.13	0.06	0.03	0.60	0.07	0.29
Withdrawn /Depressed	0.25	0.02	0.20	0.08	0.04	0.73	0.01	0.94
Somatic Complaints	-0.10	0.39	0.07	0.55	-0.23	0.05	-0.02	0.87
Social Problems	0.03	0.72	0.02	0.85	0.10	0.25	-0.02	0.83
Thought Problems	0.02	0.91	-0.05	0.73	-0.06	0.64	0.03	0.80
Attention Problems	-0.07	0.34	-0.09	0.19	-0.05	0.46	-0.20	0.01
Rule-breaking Behavior	0.002	0.99	0.13	0.22	0.06	0.56	0.001	1.00
Aggressive Behavior	0.15	0.02	-0.03	0.62	0.14	0.02	0.02	0.69
Internalizing Problems	0.11	0.03	0.14	0.007	-0.02	0.77	0.04	0.42
Externalizing Problems	0.11	0.04	0.01	0.82	0.12	0.02	0.02	0.74

¹ . The interaction term included in the model is dichotomized micronutrients*continuous log PAH (prenatal PAH exposure measured from air monitors). The cutpoint for the dichotomized micronutrient group (low versus high) was determined by the median value of the subjects included in the study with high micronutrient as the reference level.

² . Poisson models were used to examine the interaction between dichotomized low/high micronutrients and continuous log PAH exposure on CBCL, controlling for sex of the newborn, maternal gestational age, maternal years of education, maternal psychological distress, the age of the child at the time of the CBCL assessment, heating season, lead exposure, prenatal and postnatal environmental tobacco smoke and dietary PAH.

The association/ between continuous log PAH exposure and CBCL Syndrome Raw Score in Children Ages 6–9 years stratified by Micronutrient Status², adjusting for select covariates

Table 5

Domain	Stratified by Alpha-Tocopherol			Stratified by Gamma-Tocopherol			Stratified by Carotenoids			Stratified by Retinols		
	N	β for PAH	p-value	N	β for PAH	p-value	N	β for PAH	p-value	N	β for PAH	p-value
<i>Anxious/Depressed</i>												
High Micronutrient	73	0.15	0.06	73	0.10	0.19	68	0.08	0.32	62	0.05	0.56
Low Micronutrient	78	0.09	0.20	78	0.11	0.15	83	0.16	0.03	89	0.17	0.02
<i>Withdrawn/Depressed</i>												
High Micronutrient	73	0.17	0.26	73	0.44	0.004	68	0.26	0.08	62	0.30	0.07
Low Micronutrient	78	0.35	0.004	78	0.32	0.01	83	0.35	0.005	89	0.41	0.0008
<i>Somatic Complaints</i>												
High Micronutrient	73	-0.08	0.57	73	-0.17	0.16	68	-0.17	0.26	62	-0.09	0.60
Low Micronutrient	78	-0.19	0.15	78	-0.02	0.90	83	-0.18	0.15	89	-0.19	0.11
<i>Social Problems</i>												
High Micronutrient	73	0.15	0.19	73	0.12	0.22	68	0.23	0.04	62	0.20	0.08
Low Micronutrient	78	0.09	0.35	78	0.19	0.06	83	0.17	0.06	89	0.26	0.003
<i>Thought Problems</i>												
High Micronutrient	73	0.09	0.63	73	0.37	0.04	68	0.21	0.27	62	0.12	0.49
Low Micronutrient	78	0.08	0.55	78	0.09	0.49	83	0.12	0.34	89	0.22	0.09
<i>Attention Problems</i>												
High Micronutrient	73	0.24	0.01	73	0.30	0.001	68	0.17	0.07	62	0.27	0.003
Low Micronutrient	78	0.03	0.67	78	0.01	0.90	83	0.16	0.03	89	0.10	0.17
<i>Rule-breaking</i>												
High Micronutrient	73	0.19	0.14	73	0.22	0.07	68	0.04	0.75	62	0.14	0.28
Low Micronutrient	78	0.07	0.51	78	0.11	0.34	83	0.21	0.05	89	0.10	0.34
<i>Aggressive Behavior</i>												
High Micronutrient	73	0.17	0.04	73	0.32	<0.0001	68	0.09	0.25	62	0.24	0.002
Low Micronutrient	78	0.15	0.01	78	0.11	0.07	83	0.23	0.0001	89	0.08	0.18
<i>Internalizing</i>												

Domain	Stratified by Alpha-Tocopherol			Stratified by Gamma-Tocopherol			Stratified by Carotenoids			Stratified by Retinols		
	N	β for PAH	p-value	N	β for PAH	p-value	N	β for PAH	p-value	N	β for PAH	p-value
High Micronutrient	73	0.10	0.11	73	0.09	0.10	68	0.07	0.29	62	0.08	0.26
Low Micronutrient	78	0.10	0.08	78	0.13	0.03	83	0.13	0.02	89	0.14	0.009
Externalizing												
High Micronutrient	73	0.18	0.01	73	0.29	<0.0001	68	0.08	0.25	62	0.22	0.001
Low Micronutrient	78	0.13	0.01	78	0.11	0.04	83	0.23	<0.0001	89	0.09	0.09

¹ . Multivariable Poisson models were used to examine the association between continuous log PAH (prenatal PAH exposure measured from air monitors) and CBCL, stratified by micronutrient concentration, controlling for sex of the newborn, maternal gestational age, maternal years of education, maternal psychological distress, the age of the child at the time of the CBCL assessment, heating season, lead exposure, prenatal and postnatal environmental tobacco smoke and dietary PAH.

² . The cutpoint for the low vs high micronutrient group was determined by the median value of that micronutrient for the subjects included in the study.