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Modulation of the Effect of Prenatal PAH Exposure on PAH-DNA Adducts in Cord Blood by Plasma Antioxidants

Elizabeth A. Kelvin[1,2], **Susan Edwards**[1], **Wieslaw Jedrychowski**[3], **Rosemary L. Schleicher**[4], **David Camann**[5], **Deliang Tang**[1], and **Frederica P. Perera**[1]

[1]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York [2]Data Coordinating Center, New York State Psychiatric Institute, New York, New York [3]Department of Epidemiology and Preventive Medicine, Jagiellonian University College of Medicine, Krakow, Poland [4]National Center for Environmental Health, Centers for Disease Control & Prevention, Atlanta, Georgia [5]Department of Analytical and Environmental Chemistry, Southwest Research Institute, San Antonio, Texas

Abstract

The fetus is more susceptible than the adult to the effects of certain carcinogens, such as polycyclic aromatic hydrocarbons (PAH). Nutritional factors, including antioxidants, have been shown to have a protective effect on carcinogen-DNA adducts and cancer risk in adults. We investigated whether the effect of prenatal airborne PAH exposure, measured by personal air monitoring during pregnancy, on the level of PAH-DNA adducts in a baby's cord blood is modified by the concentration of micronutrients in maternal and cord blood. The micronutrients examined were: retinol (vitamin A), α-tocopherol and γ-tocopherol (vitamin E), and carotenoids. With the use of multiple linear regression, we found a significant interaction between prenatal PAH exposure and cord blood concentration of α-tocopherol and carotenoids in predicting the concentration of PAH adducts in cord blood. The association between PAH exposure and PAH adducts was much stronger among those with low α-tocopherol ($\beta = 0.15$; $P = 0.001$) and among those with low carotenoids ($\beta = 0.16$; $P < 0.001$) compared with babies with high levels of these micronutrients (among those with high α-tocopherol: $\beta = 0.05$; $P = 0.165$; among those with high carotenoids: $\beta = 0.06$; $P = 0.111$). These results suggest a protective effect of micronutrients on the DNA damage and potential cancer risk associated with prenatal PAH exposure.

Requests for reprints: Frederica Perera, Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, 25F Tower III, 100 Haven Avenue, New York, NY 10032. Phone: 212-304-7280; Fax: 212-544-1943. fpp1@columbia.edu.

Note: Laboratories: PAH-DNA adducts were analyzed at the Columbia Center for Children's Environmental Health Laboratory, New York, NY; environmental PAH exposure at Southwest Research Institute (SwRI), San Antonio, TX; and micronutrient concentrations at the Centers for Disease Control & Prevention (CDC), Atlanta, GA. F. P. Perera designed the parent cohort study, obtained funding for the study, contributed to the development of the research question and statistical analysis plan, and made extensive revisions to the manuscript; E.A. Kelvin contributed to the development of the research question, conducted the statistical analysis, and wrote the manuscript; S. Edwards also contributed to the development of the research question and the writing of the manuscript; R.L. Schleicher supervised the micronutrient analyses at the CDC; D. Camann supervised the analysis of PAHs at SwRI; D. Tang supervised the sample storage and shipments, and managed the vitamin databases; and W. Jedrychowski supervised the conduct of the study, including all data and sample collection. All authors contributed to the revision of the manuscript.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are a group of pollutants commonly found in air, food, and drinking water (1), a number of which are mutagenic and carcinogenic (2, 3). PAHs bind covalently to DNA to form adducts, which are an indicator of DNA damage and are thought to be a critical step in many carcinogenic pathways (4). Associations between PAH-DNA adduct levels and cancer risk have been seen in both experimental (5) and epidemiologic studies (6). Benzo(*a*)pyrene [B(a)P], a PAH commonly found in air from fossil fuel combustion, has also been found to be a transplacental carcinogen in animal studies (7). Molecular epidemiologic studies have provided evidence that the fetus is more susceptible than the adult to the effects of certain carcinogens, such as PAHs (8–10).

Nutrition may influence susceptibility to carcinogens. Studies have shown that higher consumption of fruits and vegetables, which are rich in antioxidants and other micronutrients, has a protective effect against the development of DNA adducts (11) and risk for certain cancers (12). A randomized controlled trial looking at the effect of antioxidant vitamin supplementation on the level of smoking-related B(a)P-DNA adducts found a significant protective effect but only among women (13), and a cross-sectional study found an inverse association between DNA adducts and plasma levels of certain micronutrients, specifically retinol, α-tocopherol, and γ-tocopherol (14). However, previous research on nutrition and the development of PAH-DNA adducts has focused primarily on adult populations. The aim of this study was to investigate whether the effect of prenatal environmental PAH exposure on the level of PAH-DNA adducts in babies' cord blood is modified by concentrations of retinol (vitamin A), α-tocopherol and γ-tocopherol (vitamin E), and/or carotenoids in maternal or cord blood.

Materials and Methods

Enrollment

We analyzed data from an ongoing cohort study of the health effects of prenatal exposure to outdoor/indoor air pollution on infants and children in Krakow, Poland (15). Nonsmoking women ages 18 to 35 y with singleton pregnancies who were attending prenatal healthcare clinics in their 1st or 2nd trimester of pregnancy were eligible for this study. Women were excluded from the 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 PAHs (including coke oven, or chemical or rubber production; environments with dust, fumes, or solvents; toll taking; and bus, tram, or taxi driving), diabetes, hypertension, or some other chronic health condition.

Eligible women were given a full description of the study and were then invited to participate. Informed consent was obtained from all participants. The study was approved by the ethics committees of the Jagiellonian University in Poland and Columbia University in the United States.

A detailed questionnaire was given to study participants upon entry into the study and during the 3rd trimester of pregnancy to collect information on demographics and lifestyle characteristics, including exposure to environmental tobacco smoke and frequency of consumption of smoked, fried, broiled, and barbequed foods likely to contain PAHs.

Biological Sample Collection

After delivery, a blood sample was collected from the umbilical cord immediately after the umbilical cord was cut. A venous blood sample was also obtained from each mother within 1 d of delivery. Plasma was separated in Krakow at the Jagiellonian University Clinical Lab,

supervised by Dr. Danuta Fedak, stored at −70°C, and then shipped to Columbia University in New York City for analysis.

PAH-DNA Adducts

B(a)P-DNA adducts were measured in all maternal and cord blood samples that had sufficient quantities of DNA. B(a)P is widely used as a representative PAH because concentrations of individual PAHs in the urban setting are highly correlated (9). Thus B(a)P-DNA adducts can serve as a proxy for PAH-DNA adducts (16). B(a)P-DNA adducts in extracted WBC DNA were measured with the use of the high-performance liquid chromatography–fluorescence method of Alexandrov et al. (17), which detects B(a)P tetraols. The method has a coefficient of variation of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides. As in prior analyses (9), samples below the limit of detection were assigned a value midway between the limit of detection and zero (0.125 adducts per 10^8 nucleotides).

Micronutrient Concentrations

Plasma concentrations of micronutrients were measured in maternal and cord blood samples at the Centers for Disease Control and Prevention Nutritional Biomarkers Laboratory with the use of isocratic high-performance liquid chromatography and multiwavelength detection. The method involved minor modifications of a published method (18). Briefly, a 100-uL aliquot of plasma precipitated with a mixture of two internal standards (nonapreno-β-carotene and retinyl butyrate) dissolved in ethanol was extracted with the use of hexane, dried, redissolved in equal parts ethanol and acetonitrile, and filtered to remove any insoluble material. 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 min. 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. Calculations were corrected 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.

Quality control for the fat-soluble micronutrient assay was established with the use of replicate analysis of three in-house serum pools in each assay. 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 National Institute of Standards and Technology (NIST) (Gaithersburg, MD) round-robin exercises for all fat-soluble micronutrients. The coefficients of variation for three levels of quality control materials ranged from 3.5% to 3.8% for vitamin 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. 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: vitamin A (1.03 μg/dL), α-tocopherol (40.67 μg/dL), γ-tocopherol (10.72 μg/dL), α-carotene (0.71 μg/dL), trans-β-carotene (0.79 μg/dL), β-cryptoxanthin (0.85 μg/dL), lutein/zeaxanthin (2.43 μg/dL), and trans-lycopene (0.77 μg/dL).

Personal Ambient Air Monitoring

Forty-eight–hour personal air monitoring was conducted during the 2nd trimester of pregnancy. The women wore small backpacks holding personal ambient air monitors during the daytime hours for 2 consecutive days and kept the monitors near their beds at night to

determine their inhalation exposure to PAHs. The backpack was designed so that the sampling inlet was positioned in the woman's breathing zone. Pumps operated continuously at 2 L/min collecting semivolatile vapors and aerosols on a polyurethane foam cartridge. During the morning of the 2nd d, the air monitoring technician and an interviewer visited each woman's home to change the battery pack and administer a full questionnaire. The polyurethane foam cartridges were analyzed at Southwest Research Institute for levels of pyrene and eight carcinogenic PAHs [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 (19, 20).

Statistical Analyses

Data analysis was conducted in SPSS version 16.0 (SPSS Inc.). Statistical significance was set at a *P*-value < 0.05. For these analyses, we excluded 4 newborns with major birth defects and 33 mother-new-born pairs for whom the maternal blood sample had cotinine levels ≥25 ng/mL, indicating active smoking during pregnancy. The micronutrients examined in these analyses were retinol, α-tocopherol, γ-tocopherol, and a composite variable for carotenoids, which was calculated as the sum of the plasma α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene concentrations. As in prior analyses, total airborne PAH exposure was calculated by taking the sum of the air concentrations of the eight carcinogenic PAHs [benz(*a*)anthracene, chrysene, benzo(*b*)fluroanthene, benzo(*k*)fluroanthene, B (a)P, indeno(1,2,3-*cd*)pyrene, disbenz(*a*,*h*)anthracene, and benzo(*g*,*h*,*i*)perylene] measured from personal air samples (20).

We used linear regression to examine the association of adduct concentration in cord blood as a continuous outcome with prenatal PAH exposure, micronutrient concentration (in cord and maternal blood), and the interaction of PAH and micronutrient concentrations. All continuous variables were natural log-transformed to more closely approximate a normal distribution. We also ran the models looking at the association of adduct concentration in cord blood with prenatal PAH exposure stratified on micronutrient concentration. For these stratified models, each micronutrient was dichotomized as high or low at the median (cord blood dichotomization: retinol >20.9 μg/dL, α-tocopherol >273.6 μg/dL, γ-tocopherol >11.20 μg/dL, and carotenoids >0.35 μg/dL; maternal blood dichotomization: retinol >37.2 μg/dL, α-tocopherol >1,631.35 μg/dL, γ-tocopherol >79.00 μg/dL, and carotenoids >3.0 μg/dL). In addition, we examined the presence of detectable cord blood adducts as a dichotomous outcome with the use of logistic regression. For the logistic regression models, we also dichotomized PAH and micronutrient concentration and looked at the interaction of these two exposures. Micronutrient variables were dichotomized into high/low categories based on the median as described above, and environmental PAH exposure was dichotomized at the 3rd quartile (>56 ng/m^3). When significant interactions were not observed in a model, we ran the main effect models (the unstratified model for the independent effect of PAH exposure and micronutrient concentration without the interaction term). Reported prenatal exposure to environmental tobacco smoke (ever versus never) and dietary PAHs, calculated as the sum of the frequency of consumption of each type of food associated with PAH exposure (smoked, fried, broiled, blackened, and barbequed food) and dichotomized at the median (>42 portions of food high in PAH per week), were also included in all the models to control for confounding. In addition, we controlled for the gender of the newborn.

Results

Description of the Sample

A description of the participants who were included and excluded in the analyses is provided in Table 1. There were no statistically significant differences between the women and their newborns enrolled in this study who are included in these analyses (e.g., who meet the inclusion criteria and for whom we have biomarker data) versus those excluded from analysis with respect to maternal education, environmental tobacco smoke exposure, proportion with high dietary PAH, environmental PAH exposure, gestational age, and birth weight or gender of the baby. However, the mothers included in these analyses were significantly older, and a higher proportion reported alcohol consumption during pregnancy than the mothers excluded from these analyses.

Concentrations of Adducts and Micronutrients

One hundred and seventy-nine (62.2%) of the cord blood samples and 209 (65.5%) of the maternal blood samples had detectable PAH-DNA adducts (data not shown). Table 2 shows the concentrations of, and correlations among, environmental PAHs, maternal and cord PAH-DNA adducts, and micronutrients. There was little difference in the mean concentration of PAH-DNA adducts in maternal and cord blood, and the two were significantly correlated with each other. The mean concentrations of all micronutrients were significantly higher in maternal blood than in cord blood, although the correlation between cord and maternal blood micronutrient concentration was statistically significant for α-tocopherol, γ-tocopherol, and carotenoids, and of borderline significance for retinol (P = 0.053; Table 2).

Multivariate Linear Regression Models

In the linear regression models (Table 3) controlling for environmental tobacco smoke, dietary PAHs, and infant gender, there was a statistically significant interaction between prenatal PAH exposure and both α-tocopherol and carotenoid concentration in cord blood in predicting the concentration of PAH adducts in cord blood. In the stratified models, the association between prenatal PAH exposure and adducts in cord blood was much stronger among infants with low levels of these two micronutrients (among those with low α-tocopherol: β = 0.15; P = 0.001; among those with low carotenoids: β = 0.16; P = 0.000) compared with infants with high levels of these micronutrients (among those with high α-tocopherol: β = 0.05; P = 0.165; among those with high carotenoids: β = 0.06; P = 0.111). Although the P-value for the interactions of PAH exposure with retinol and with γ-tocopherol concentration in cord blood in predicting adducts in cord blood were not statistically significant (P = 0.207 and P = 0.103, respectively), the stratified models showed a pattern similar to that for α-tocopherol and carotenoids, with a stronger association between PAH exposure and adducts in cord blood among those with low levels of cord blood retinol (β = 0.13; P = 0.001) compared with those with high levels of cord blood retinol (β = 0.08; P = 0.041), and among those with low concentration of γ-tocopherol (β = 0.10; P = 0.017) compared with those with high concentration (β = 0.07; P = 0.070).

There were no significant interactions between prenatal PAH exposure and maternal blood micronutrient concentration in predicting cord blood adducts, although the stratified models showed a pattern similar to that seen when looking at micronutrient concentrations in cord blood for all micronutrients except for γ-tocopherol. There was a stronger association between prenatal PAH exposure and cord blood adduct concentration among those with low concentrations of carotenoids, α-tocopherol, and retinol in maternal blood compared with those with higher concentrations of the micronutrients.

In the unstratified models looking at the main effects (without the interaction term) of PAH exposure and micronutrient concentration, higher PAH exposure was significantly associated with higher PAH adduct concentration in cord blood in all the models. Retinol concentration in cord blood was positively associated with adducts in cord blood, but none of the micronutrients in maternal blood were significantly associated with adducts in cord blood.

Multivariate Logistic Models

In the logistic regression models (Table 4), there were no statistically significant interactions between PAH exposure and micronutrient concentration in predicting the presence of detectable cord blood PAH adducts. However, when stratified on cord blood micronutrient concentration, the pattern of the associations between PAH exposure and cord blood adducts in the logistic models was similar to that seen in the linear regression models, with much higher odds ratios (OR) for PAH adducts seen among those with low concentrations of cord blood α-tocopherol (OR, 3.96; $P = 0.025$) and low concentration of cord blood carotenoids (OR, 7.89; $P = 0.008$) compared with those with high concentrations of α-tocopherol (OR, 1.86; $P = 0.196$) or high concentration of cord blood carotenoids (OR, 1.81; $P = 0.184$). The same pattern was seen when stratifying on maternal concentration of α-tocopherol or carotenoids. However, the models stratified on retinol and γ-tocopherol (cord blood or maternal blood concentration) did not show this pattern.

In the main effects models (unstratified and without the interaction term), high PAH exposure was significantly associated with a higher odds of having detectable PAH adducts in cord blood in all the models (controlling for each micronutrient).

Discussion

Our analyses indicate that prenatal PAH exposure is a significant risk factor for PAH-DNA adducts in cord blood and that certain micronutrients are protective against adduct formation in the fetus. Elevated concentrations (above the median) of α-tocopherol and carotenoid in a baby's cord blood mitigated the effect of increasing PAH exposure on cord blood adducts. In the linear regression models, the positive association between prenatal PAH exposure and PAH adducts in cord blood was weaker among the babies with higher cord blood concentrations of α-tocopherol, carotenoid, γ-tocopherol, and retinol; the interaction (PAH × micronutrient) was statistically significant for α-tocopherol and carotenoid concentrations. Furthermore, a similar pattern was seen about PAH exposure and adducts in the baby's cord blood in models stratifying on maternal micronutrient concentrations of α-tocopherol and carotenoid.

Micronutrients may prevent the formation of adducts due to PAH exposure by inducing the detoxifying enzyme glutathione *S*-transferase (4, 21, 22). Although it is not yet known if and by how much the presence of PAH adducts at birth increases cancer risk later in life, these results suggest that maternal nutrition during pregnancy may have a long-term impact on the child's health in terms of risk for cancer during childhood and possibly into adulthood.

Although micronutrient levels in cord and maternal blood were correlated with each other, the concentrations of all four micronutrients in maternal blood were significantly higher than in the baby's cord blood (Table 2). This finding has been previously reported and is thought to be due to inefficient placental transfer of lipid-soluble antioxidants (23). Thus, although maternal micronutrient consumption may help prevent adduct formation in the fetus, the dose of micronutrients received by the fetus from the mother is significantly reduced during the placental transfer process.

The median PAH concentration in the air samples was much higher in this Polish sample compared with a sample of pregnant Dominican and African-American women in New York City (17.96 ng/m^3 in Polish sample versus 2.27 ng/m^3 in the New York sample; ref. 24). In addition, the median micronutrient concentrations in maternal blood in that New York sample were lower for retinol and α-tocopherol (29.96 and 838.80 μg/dL, respectively) but higher for carotenoids (9.24 μg/dL)6 than in the Polish sample (retinol, 37.00 μg/dL; α-tocopherol, 1,654.05 μg/dL; and carotenoids, 3.0 μg/dL). However, the median micronutrient concentrations in the pregnant Polish women in our study were lower than median concentrations found among the general white, non-Hispanic female population ages 20 to 39 years in the United States for carotenoids (range of 2.3–22.4 μg/dL for each of five carotenoids in U.S. sample), retinol (51.7 μg/dL in U.S. sample), and γ-tocopherol (79.70 μg/dL in Polish sample versus 217 μg/dL in U.S. sample), but higher for α-tocopherol (982 μg/dL in U.S. sample; ref. 25). These differences in PAH and micronutrient concentrations may limit the generalizability of our results if the interaction between these factors only occurs within a certain exposure range. Furthermore, studies suggest that polymorphisms in the glutathione *S*-transferase genes involved in carcinogenic detoxification may also be important modifiers of the risk of developing PAH-DNA adducts upon exposure to environmental PAH (4, 26, 27). Therefore, our findings in this Polish sample of pregnant women are unlikely to be generalizable to populations with a different prevalence of glutathione *S*-transferase polymorphisms.

This study has a number of limitations that most likely bias our results toward the null. First, given our modest sample size, we had low statistical power for some of the models examined in which we assessed interactions or stratified on micronutrient concentration. The decreased statistical significance of the interaction terms in the logistic regression models compared with the linear regression models is likely an indication of this low statistical power, which is exacerbated when the variables are dichotomized. Second, because biologically meaningful cut-points in infants have not been established for micronutrients, we dichotomized micronutrient concentration at the median, as has been done in prior analyses (28), which may not be the most appropriate categorization. Third, our exposure and outcome variables were measured at a single time point and may not accurately represent the changing distribution of these variables over the entire 9 months of pregnancy. In addition, the time between sample collection and processing for storage was, in some cases, somewhat longer than the optimal 2-to-3-hour range. For the cord blood samples, the blood was stored at 4°C for a mean of 5 hours (median of 4 hours and a range of <1–13 hours) and maternal blood for a mean of 4 hours (median of 2 hours and a range of <1–13 hours). One study found that, compared with processing samples within 2 hours, a delay of 24 hours in suboptimal storage temperatures (32°C) led to a statistically significant increase in the concentration of vitamin E (α-tocopherol and γ-tocopherol) and in some carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, and lycopene. However, these changes, although statistically significant, were deemed to be too small to be of clinical importance (29). Retinol concentration was not significantly changed after a delay of 24 hours. Thus, it is possible that our processing procedures lead to some changes in the biomarker concentrations, but the effect would likely be minimal.

Despite these limitations, most of which would tend to bias our results toward the null, we found a clear pattern of effect modification by α-tocopherol and carotenoid concentration on the association between prenatal PAH exposure and PAH-DNA adducts in the baby's cord blood. The strengths of this study include the measurement of antioxidants in both cord blood and maternal blood, and the ability to control for confounding variables, such as

[6]Unpublished data.

environmental tobacco smoke and dietary PAHs. In conclusion, the results of this study indicate a protective effect of micronutrients on the DNA damage and potential cancer risk associated with prenatal PAH exposure.

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Table 1

Characteristics of participants included in analyses compared with those excluded

Maternal characteristics	Subjects not in analyses (*n* = 182)	Subjects in analyses (*n* = 328)*	*P*
Mean age, y (SD)	27.3 (3.4)	28.2 (3.7)	0.036†
Percent with > high school diploma	47.5	41.2	0.303‡
Percent reporting ETS exposure	31.2	36.6	0.371‡
Percent reporting alcohol consumption during pregnancy	48.8	63.7	0.014‡
Percent with high dietary PAH consumption	45.0	50.6	0.368‡
Mean environmental PAH concentration, ng/m^3 (SD)	26.3 (30.6)	39.6 (48.6)	0.418‡
Newborn characteristics			
Mean gestational age, wk (SD)	39.2 (1.8)	39.4 (1.6)	0.570†
Mean birth weight, g (SD)	3313.6 (512.2)	3437.7 (493.4)	0.057†
Percent female	40.0	51.2	0.072‡

*Participants included in these analyses had maternal cotinine levels <25 ng/mL, no major birth defects in the baby, and valid data on at least one biomarker examined (PAH-DNA adducts or micronutrient concentration in cord or maternal blood).

†Wilcoxin rank sum test *P*-value.

‡χ^2-test *P*-value.

Table 2

Concentrations of, and correlations among, environmental PAHs, maternal and cord PAH-DNA adducts, and micronutrients

	Sample size	Median	Mean concentration (95% CI)	Correlation (Spearman's rho) with maternal blood concentration (*P*-value)
Environmental PAH in air (ng/m^3)	328	17.96	39.60 (34.32–44.88)	NA
Cord blood				
PAH-DNA adducts in cord blood (adducts per 10^8 nucleotides)	288	0.29	0.28 (0.26–0.30)	0.42 (0.000)
α-Tocopherol in cord blood (μg/dL)	261	274.20	325.30 (295.19–355.42)	0.16 (0.012)
Carotenoid composite in cord blood (μg/dL)	260	0.36	0.42 (0.34–0.50)	0.51 (0.000)
Retinol in cord blood (μg/dL)	261	20.70	20.74 (20.01–21.47)	0.12 (0.053)
γ-Tocopherol in cord blood (μg/dL)	259	11.30	13.71 (11.43–15.99)	0.303 (0.000)
Maternal blood				
PAH-DNA adducts in maternal blood (adducts per 10^8 nucleotides)	319	0.29	0.28 (0.26–0.30)	
α-Tocopherol in maternal blood (μg/dL)	264	1,654.05	1,656.31 (1,600.44–1,712.18)	
Carotenoids in maternal blood (μg/dL)	263	3.00	2.92 (2.85–2.99)	
Retinol in maternal blood (μg/dL)	264	37.00	38.01 (36.53–39.50)	
γ-Tocopherol in maternal blood (μg/dL)	264	79.70	85.28 (80.42–90.14)	

Table 3

Linear regression models examining the association of PAH-DNA adducts in cord blood sample with prenatal environmental PAH exposure and concentration of micronutrients in cord and maternal blood sample (all natural log-transformed), adjusted for ETS exposure, consumption of smoked foods, and baby's gender (all dichotomized)

Adducts in cord blood as outcome	Total sample				High micronutrient*			Low micronutrient*		
	Sample size	β for main effect	*P*-value for main effect	*P*-value for interaction term	Sample size	β for main effect	*P*-value for main effect	Sample size	β for main effect	*P*-value for main effect
Model 1										
Environmental PAH exposure	235			0.005	119	0.05	0.165	116	0.15	0.001
α-Tocopherol in cord blood										
Model 2										
Environmental PAH exposure	235			0.004	122	0.06	0.111	113	0.16	0.000
Carotenoids in cord blood										
Model 3										
Environmental PAH exposure	235	0.10	0.001	0.207	114	0.08	0.041	121	0.13	0.001
Retinol in cord blood		0.25	0.038							
Model 4										
Environmental PAH exposure	234	0.09	0.002	0.103	123	0.07	0.070	111	0.10	0.017
γ-Tocopherol in cord blood		0.06	0.257							
Model 5										
Environmental PAH exposure	232	0.11	0.000	0.872	121	0.08	0.034	111	0.15	0.001
α-Tocopherol in maternal blood		−0.12	0.201							
Model 6										
Environmental PAH exposure	232	0.11	0.000	0.895	118	0.09	0.020	114	0.13	0.001
Carotenoids in maternal blood		−0.08	0.140							
Model 7										
Environmental PAH exposure	232	0.11	0.000	0.698	109	0.10	0.013	123	0.13	0.002
Retinol in maternal blood		0.16	0.122							
Model 8										
Environmental PAH exposure	232	0.11	0.000	0.405	121	0.12	0.001	111	0.10	0.017
γ-Tocopherol in maternal blood		−0.06	0.377							

* Micronutrients dichotomized at the median. In cord blood: high retinol, >20.9 μg/dL; high α-tocopherol, >273.6 μg/dL; high carotenoids, >0.35 μg/dL; high γ-tocopherol, >11.20 μg/dL. In maternal blood: high retinol, >37.2 μg/dL; high α-tocopherol, >1631.35 μg/dL; high carotenoids, >3.0 μg/dL; high γ-tocopherol, >79.00 μg/dL. Potential confounders were dichotomized as follows: ETS exposure (ever/never), frequency of consumption of PAH-containing foods [low (<xx per week), high (≥xx per week)], and gender (male/female).

Table 4

Adjusted logistic regression models examining the association of PAH-DNA adducts in cord blood sample as a dichotomous variable (detectable or not) with environmental PAH exposure (dichotomized at the 3rd quartile as >56ng/m^3) and micronutrient concentrations in cord or maternal blood sample (dichotomized at the median) while controlling for ETS exposure, frequency of PAH consumption in food, and infant gender

Detectable adducts in cord blood as outcome	Total sample				High micronutrient*			Low micronutrient*		
	Sample size	OR (95% CI)	*P*	*P*-value for interaction term	Sample size	OR (95% CI)	*P*	Sample size	OR (95% CI)	*P*
Model 1										
Environmental PAH >56 ng/m^3	235	2.49 (1.21–5.11)	0.013	0.330	119	1.86 (0.73–4.78)	0.196	116	3.96 (1.19–13.21)	0.025
α-Tocopherol in cord blood >273.6 μg/dL		2.09 (1.14–3.83)	0.017							
Model 2										
Environmental PAH >56 ng/m^3	235	2.80 (1.37–5.73)	0.005	0.108	122	1.81 (0.76–4.34)	0.184	113	7.89 (1.71–36.53)	0.008
Carotenoids in cord blood >0.35 μg/dL		1.26 (0.64–2.08)	0.627							
Model 3										
Environmental PAH >56 ng/m^3	235	2.85 (1.40–5.78)	0.004	0.951	114	2.80 (1.01–7.80)	0.048	121	2.89 (1.07–7.80)	0.036
Retinol in cord blood >20.9 μg/dL		1.25 (0.70–2.23)	0.450							
Model 4										
Environmental PAH >56 ng/m^3	234	2.44 (1.18–5.03)	0.016	0.705	123	2.81 (1.08–7.32)	0.035	111	2.22 (0.71–6.97)	0.172
γ-Tocopherol in cord blood >11.20 μg/dL		1.62 (0.89–2.93)	0.113							
Model 5										
Environmental PAH >56 ng/m^3	232	3.02 (1.45–6.28)	0.003	0.250	121	1.95 (0.71–5.35)	0.196	111	4.57 (1.58–13.26)	0.005
α-Tocopherol in maternal blood >1,631.35 μg/dL		1.73 (0.97–3.09)	0.063							
Model 6										
Environmental PAH >56 ng/m^3	232	2.99 (1.44–6.20)	0.003	0.091	118	1.82 (0.75–4.44)	0.189	114	8.90 (1.93–40.96)	0.005
Carotenoids in maternal blood >3.0 μg/dL		0.84 (0.47–1.51)	0.560							
Model 7										
Environmental PAH >56 ng/m^3	232	2.96 (1.42–6.14)	0.004	0.920	109	3.37 (1.02–11.12)	0.046	123	2.93 (1.15–7.46)	0.025
Retinol in maternal blood >37.2 μg/dL		1.78 (0.99–3.19)	0.054							
Model 8										
Environmental PAH >56 ng/m^3	232	2.95 (1.43–6.10)	0.004	0.267	121	5.05 (1.61–15.79)	0.005	111	2.02 (0.75–5.49)	0.167

Detectable adducts in cord blood as outcome	Total sample				High micronutrient*			Low micronutrient*		
	Sample size	OR (95% CI)	*P*	*P*-value for interaction term	Sample size	OR (95% CI)	*P*	Sample size	OR (95% CI)	*P*
γ-Tocopherol in cord blood >79.00 μg/dL		1.10 (0.62–1.96)	0.740							

*Micronutrients dichotomized at the median. In cord blood: high retinol, >20.9 μg/dL; high α-tocopherol, >273.6 μg/dL; high carotenoids, >0.35 μg/dL; high γ-tocopherol, >11.20 μg/dL. In maternal blood: high retinol, >37.2 μg/dL; high α-tocopherol, >1631.35 μg/dL; high carotenoids, >3.0 μg/dL; high γ-tocopherol, >79.00 μg/dL. Potential confounders were dichotomized as follows: ETS exposure (ever/never), frequency of consumption of PAH-containing foods [low (<xx per week), high (≥xx per week)], and gender (male/female).