Molecular Evidence of an Interaction Between Prenatal Environmental Exposures and Birth Outcomes in a Multiethnic Population

Frederica P. Perera,1 Virginia Rauh,1 Robin M. Whyatt,1 Wei-Yann Tsai,1 John T. Bernert,2 Yi-Hsuan Tu,1 Howard Andrews,1 Judyth Ramirez,1 Lirong Qu,1 and Deliang Tang1

1Columbia Center for Children’s Environmental Health, Mailman School of Public Health, Columbia University, New York, USA; 2Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Atlanta, Georgia, USA

Abstract

Inner-city, minority populations are at increased risk for adverse birth outcomes and are also more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), benz[a]pyrene (BaP), and other polycyclic aromatic hydrocarbons (PAHs) found in urban air. In a sample of non-smoking African-American and Dominican women, we examined the effects on birth outcomes of prenatal exposure to ETS, using questionnaire data and plasma cotinine as a biomarker of exposure, and environmental PAHs using BaP-DNA adducts as a molecular dosimeter. We previously reported that among African Americans, high prenatal exposure to PAHs estimated by prenatal personal air monitoring was associated with lower birth weight (p = 0.003) and smaller head circumference (p = 0.01) after adjusting for potential confounders. In the present analysis, self-reported ETS was associated with decreased head circumference (p = 0.04). BaP-DNA adducts were not correlated with ETS or dietary PAHs. There was no main effect of BaP-DNA adducts on birth outcomes. However, there was a significant interaction between the two pollutants such that the combined exposure to high ETS and high adducts had a significant multiplicative effect on birth weight (p = 0.04) and head circumference (p = 0.01) after adjusting for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age. This study provides evidence that combined exposure to environmental pollutants at levels currently encountered in New York City adversely affects fetal development. Key words: adducts, birth outcomes, development, environmental, ETS, PAHs, pollutants, prenatal. Environ Health Perspect 112:626–630 (2004). doi:10.1289/ehp.6617 available via http://dx.doi.org/ [Online 14 January 2004]

The impact of environmental toxicants on children’s health is increasingly being recognized as significant [Fausman 2000; Greater Boston Physicians for Social Responsibility (GBPSR) 2000; Landrigan et al. 1999; Perera et al. 1999; U.S. Environmental Protection Agency (EPA) 1996]. However, only limited information is available on the effects of environmental toxicants on fetal growth and development [reviewed in Nieves 1992]. However, only limited information is available on the effects of environmental toxicants on fetal growth and development. Etiologic studies have largely been ecologic in nature, lacking individual-level data on exposure.

In the present study, we evaluated the effects of prenatal exposure to common urban pollutants: environmental PAHs estimated by DNA adducts in white blood cells (WBCs) formed by benz[a]pyrene (BaP), a representative PAH, and ETS estimated by questionnaire data and plasma concentrations of cotinine. In addition to being genotoxic and carcinogenic, PAHs such as BaP are endocrine disruptors [Bosstrom et al. 2002; Bui et al. 1986; Davis et al. 1993]. Prior laboratory and two human studies in Central Europe indicate that transplacental exposure to PAHs at relatively high concentrations (annual average airborne concentrations of 7–17 ng/m3 BaP in the human studies) is associated with adverse birth outcomes [Barbieri et al. 1986; Bui et al. 1986; Dejmek et al. 2000; Legrand et al. 1984; Perera et al. 1998]. We recently reported that prenatal PAH exposure estimated by personal air monitoring was associated with reduced birth weight and head circumference among African Americans in the present New York City, New York, cohort (Perera et al. 2002). ETS is a complex mixture of > 4,000 chemicals, including PAHs and carbon monoxide (Leikauf et al. 1995). ETS measured by self-report or by biomarkers such as cotinine has been shown in many studies to adversely affect fetal growth as well as child growth and development (reviewed in Eskenazi et al. 1995; Etzel 1997; National Research Council 1986). Adverse effects include deficits in birth weight, birth length, and cognitive functioning at age 3 (Janerich et al. 1990; Martinez et al. 1994; Schuster-Kolbe and Ludvig 1994; Sexton et al. 1990). Likely mechanisms underlying the adverse effects of fetal exposure to ETS include anti-estrogenic effects, induction of P450 enzymes, DNA damage resulting in activation of apoptotic pathways, binding to receptors for placental growth factors resulting in decreased exchange of oxygen and nutrients, and direct effects of carbon monoxide.

Here we tested the hypothesis that prenatal exposure to environmental pollutants alone and/or in combination is negatively associated with birth weight, length, and head circumference, after controlling for the effects of known physical, biologic, and toxic determinants of fetal growth. As reported previously, the study cohort has substantial exposure to multiple contaminants during pregnancy (Perera et al. 2002; Whyatt et al. 2002, 2003). Specifically, analysis of PAHs in air samples from the first 250 subjects showed that all samples had detectable levels of one or more carcinogenic PAHs, ranging across 4 orders of magnitude (Perera et al. 2002). Almost half of the mothers and infants initially enrolled had cotinine levels indicative of ETS exposure (> 0.05–25 ng/mL). Maternal and newborn plasma cotinine levels were significantly higher for mothers who reported smoking by others in the household than for mothers who reported no smoking in the home (p < 0.001).

Address correspondence to F.P. Perera, Columbia Center for Children’s Environmental Health, Mailman School of Public Health, Columbia University, New York, New York 10032 USA. Telephone: (212) 544-1943. Fax: (212) 544-1943. E-mail: Fpp1@columbia.edu

We thank D. Holmes, M. Borjas, A. Reyes, J. Ramirez, L. Cruz, L. Qu, Y. Cosme, S. Illman, L. Needham, R. Jackson, Harlem Hospital, Allen Pavillion, and New York-Presbyterian Hospital.

Support was provided by the National Institute of Environmental Health Sciences (grants P50 ES09609, P50 ES0077, RO1 ES11158, RO1 ES012468, RO1 ES10165), the U.S. Environmental Protection Agency (grants R827027, 826001, and NCER STAR Program), Irving General Clinical Research Center (grant RR00645), Bauman Family Foundation, Gladys & Roland Harriman Foundation, New York Community Trust, Educational Foundation of America, and the Horace W. Goldsmith Foundation.

The authors declare they have no competing financial interests.

Received 29 July 2003; accepted 14 January 2004.

http://dx.doi.org/10.1289/ehp.6617
**Materials and Methods**

**Study subjects.** Study subjects are Dominican and African-American women residing in Washington Heights, Central Harlem, and the South Bronx, New York, who delivered at New York Presbyterian Medical Center (NYPMC), Harlem Hospital (HH), or their satellite clinics (Perera et al. 2002; Whyatt et al. 2002); Table 1 presents demographic and exposure characteristics of the population. Ethnicity was self-identified. Women were eligible if they were nonsmokers; were 18–35 years of age; were registered at the obstetrics and gynecology clinics at NYPMC and HH by the 20th week of pregnancy; were free of diabetes, hypertension, or known HIV; and had resided in the area for at least 1 year. The mean gestational age at enrollment was 39.5 weeks. Two hundred ninety-eight women were considered to be fully enrolled prenatally during the third trimester using a personal air monitor and had delivered, and a maternal and/or umbilical cord blood sample had been collected.

The 214 subjects included in the present analysis are those with adduct measurements in umbilical cord blood samples (in some cases the amount of blood collected was inadequate for the assay), and complete questionnaire and medical record data were used as covariates in the multiregression models. Fully enrolled subjects with missing data for any of these data points (48) were excluded from the analysis. Only nonsmokers were included. Nonsmokers were initially defined as having answered “no” to the question “presently, does a household member or regular visitor to your home smoke cigarettes, pipes, marijuana, or cigars in your home” and as having plasma cotinine concentrations ≤ 15 ng/mL. Cotinine data were available for approximately 90% of the subjects. Three subjects with plasma cotinine concentrations > 15 ng/mL were excluded to rule out active smoking. There were no significant differences in sociodemographic characteristics or levels of exposure between the present subset and fully enrolled subjects with missing data required in the present analysis.

**Personal interview.** A 45-min questionnaire was administered by a trained bilingual interviewer during the last trimester of pregnancy. The questionnaire included demographic information, lifetime residential history (country of birth, location, and duration of residence), travel outside the current area of residence during the past year, history of active and passive smoking (including number of household members who smoke), alcohol use during each trimester of pregnancy, and consumption of PAH-containing meat (frequency of eating fried, broiled, or barbecued meat during the last 2 weeks). Socioeconomic information related to income and education was also collected. The questionnaire was based on that used in a prior study of women and newborns and adapted for the New York City population (Perera et al. 1998).

**Biologic sample collection and analysis.** Maternal blood (30–35 mL) was collected within 1 day postpartum, and umbilical cord blood (30–60 mL) was collected at delivery. Samples were transported to the laboratory immediately. The buffy coat, packed red blood cells, and plasma samples were separated and stored at −70°C. A portion of each sample was shipped to the Centers for Disease Control and Prevention (CDC) for analysis of cotinine (2 mL) and pesticides (10 mL). Plasma cotinine was analyzed by the CDC using high-performance liquid chromatography atmospheric-pressure ionization tandem mass spectrometry as described previously (Berner et al. 1997, 2000). The limit of detection for cotinine was 0.05 ng/mL.

**DNA adducts.** BaP-DNA adducts in extracted WBC DNA from maternal and cord blood were analyzed by the HPLC/fluorescence method of Alexandrov et al. (1992), which uses an HPLC method to detect BaP tetramers. This assay is a sensitive and specific method for measuring BaP-DNA adducts in WBCs from individuals exposed to BaP (Bartsch 1996). The method has a coefficient of variation of 12%. Samples from mother–child pairs were run in the same batch.

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The median of all samples as the cutpoint (0.0435 ng/mL).

The relationships between the exposure variables and the birth outcomes were analyzed by multiple regression, adjusting for known or potential confounders. In addition to cord blood adducts dichotomized as high/low and self-reported ETS (yes/no smokers in the home), the final regression model included covariates representing known or suspected risk factors that were associated with birth outcomes ($p \leq 0.1$ by linear regression). Birth outcomes were log transformed to provide a better fit to the data and/or to approximate the normal distribution and stabilize the variance. Models 1, 2, and 3 evaluated the main effects of self-reported ETS, high/low cotinine, and high/low BaP-DNA, adjusting for potential confounders, including ethnicity, body mass index, gestational age, dietary PAHs, infant sex, and cesarean delivery (a predictor of head circumference). Income ($< 10,000 or \geq 10,000$), parity (0 or \geq 1 live birth), social adversity (a composite score based on marital status, income, education, and whether currently on assistance), and alcohol consumption (yes/no) were not significant predictors of outcomes ($p > 0.1$) and were not included. The other variables, including dietary PAHs, were included as covariates. The final models tested the interaction between adducts and ETS (or cotinine) using appropriate interaction terms, adducts, and ETS (or cotinine).

### Models

**Model 1:**
- **Beta ($\beta$)**: ETS
  - $\beta = 0.02$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: high/low BaP-DNA
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
- **Beta ($\beta$)**: high/low cotinine
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
- **Beta ($\beta$)**: income ($< 10,000 \geq 10,000$)
  - $\beta = 0.01$, $p = 0.05$, $t = 1.94$
- **Beta ($\beta$)**: parity (0 or \geq 1)
  - $\beta = -0.01$, $p = 0.05$, $t = -1.94$
- **Beta ($\beta$)**: ethnicity
  - $\beta = 0.04$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: sex
  - $\beta = 0.01$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: body mass index
  - $\beta = 0.05$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: dietary PAHs
  - $\beta = -0.06$, $p = 0.01$, $t = -2.07$
- **Beta ($\beta$)**: gestational age
  - $\beta = 1.416$, $p = 0.05$, $t = 2.07$

**Model 2:**
- **Beta ($\beta$)**: ETS
  - $\beta = 0.02$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: high/low BaP-DNA
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
- **Beta ($\beta$)**: high/low cotinine
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
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- **Beta ($\beta$)**: gestational age
  - $\beta = 1.416$, $p = 0.05$, $t = 2.07$

**Model 3:**
- **Beta ($\beta$)**: ETS
  - $\beta = 0.02$, $p = 0.01$, $t = 2.07$
- **Beta ($\beta$)**: high/low BaP-DNA
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
- **Beta ($\beta$)**: high/low cotinine
  - $\beta = -0.01$, $p = 0.001$, $t = -3.07$
- **Beta ($\beta$)**: income ($< 10,000 \geq 10,000$)
  - $\beta = 0.01$, $p = 0.05$, $t = 1.94$
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- **Beta ($\beta$)**: gestational age
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### Results

Demographic and exposure characteristics for the subjects included in the present analysis are provided in Table 1 together with summary data on cord blood BaP-DNA and cotinine. The subset did not differ from the overall cohort in terms of demographic variables. Among subjects in the present analysis, 46% of mothers and 49% of newborns had cotinine levels > 0.05 and < 15 ng/mL, indicative of ETS exposure. Self-reported ETS and plasma cotinine differed by ethnicity, with African Americans being significantly more likely to report ETS exposure ($p = 0.05$) and to have a higher rate of detectable cotinine (78.1 vs. 31.9%; $p = 0.01$). Sixty-two percent of cord and 61% of maternal blood samples had nondetectable levels of BaP-DNA (< 0.25 adducts/10^-8 nucleotides).

The mean birth weight was 3445.6 g (SD = 475.3). Mean head circumference was 34.2 cm (SD = 1.4). Mean birth weight and head circumference were lower, and there was greater variability in these outcomes, among African-American than among Dominican infants. The differences between individual outcomes were not significant by $t$-test; however, by multivariate Hotelling’s $t$-test, at least one of these outcomes (weight, length, head circumference) was significantly lower in African Americans ($p < 0.01$). Reflecting the fact that all women had reached their third trimester of pregnancy, only 3% percent of infants were preterm (< 37 weeks of gestation). African-American infants had a significantly lower mean gestational age than did Dominican infants (39.2 vs. 39.6 weeks, $p < 0.01$).

As shown in Table 2 by linear regression, ETS exposure was associated with smaller head circumference ($\beta = -0.01$, $p = 0.04$) after adjusting for potential confounders (model 1). Cotinine was significantly associated with birth length ($\beta = -0.01$, $p = 0.05$). By Spearman’s test, BaP-DNA adducts were not significantly correlated with ETS, cotinine, or dietary PAHs. BaP-DNA alone (either as a continuous or dichotomous variable) was not significantly associated with birth outcomes. However, the interaction between high/low adducts and ETS was significant using either adduct variable. For example, as shown in Table 3, the interaction effect of BaP-DNA and ETS was significant on birth weight ($p = 0.05$) and head circumference ($p = 0.01$). Figure 1 shows the effect of this interaction on birth weight and head circumference. There was a 232-g (6.8%) reduction of birth weight and 1-cm (2.9%) reduction of head circumference in the ETS-positive group with high BaP-DNA levels, compared with the ETS-negative group with low BaP-DNA levels. The interaction effect of BaP-DNA and cotinine was not significant.

### Discussion

In this study we found an association between ETS, with or without BaP-DNA adducts, and two birth outcomes, namely decreased birth weight and smaller head circumference, after adjusting for potential confounders. This association is of potential concern because, although the literature is inconsistent, several factors.
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