Biomarkers in Maternal and Newborn Blood Indicate Heightened Fetal Susceptibility to Procarcinogenic DNA Damage

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Polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene (BaP) are widespread air contaminants released by transportation vehicles, power generation, and other combustion sources. Experimental evidence indicates that the developing fetus is more susceptible than the adult to carcinogenic effects of PAHs, although laboratory studies in rodents suggest that the dose to fetal tissues is an order of magnitude lower than that to maternal tissues. To assess fetal versus adult susceptibility to PAHs and environmental tobacco smoke (ETS), we compared carcinogen-DNA adducts (a biomarker associated with increased cancer risk) and cotinine (a biomarker of tobacco smoke exposure) in paired blood samples collected from mothers and newborns in New York City. We enrolled 265 nonsmoker African-American and Latina mother–newborn pairs in New York City between 1997 and 2001 (estimated average ambient air BaP concentrations < 0.5 ng/m^3). Despite the estimated 10-fold lower fetal dose, mean levels of BaP-DNA adducts as determined by high-performance liquid chromatography–fluorescence were comparable in paired New York City newborn and maternal samples (0.24 adducts per 10^8 nucleotides, 45% of newborns with detectable adducts vs. 0.22 per 10^8 nucleotides, 41% of mothers with detectable adducts). However, by the Wilcoxon signed-rank test, the levels in newborns were higher (p = 0.02). Mean cotinine was higher in newborns than in mothers (1.7 ng/mL, 47% detectable vs. 1.28 ng/mL, 44% detectable). Consistent with our prior study in a Caucasian Polish population, these results indicate increased susceptibility of the fetus to DNA damage and reduced ability to clear ETS constituents. The findings have implications for risk assessment, given the need to protect children as a sensitive subset of the population. *Key words:* **cancer, DNA adducts, fetus, polycyclic aromatic hydrocarbons, susceptibility.** *Environ Health Perspect* **112:1133–1136 (2004). doi:10.1289/ehp.6833 available via *http://dx.doi.org/* [Online 22 March 2004]**

As previously discussed (Whyatt et al. 2001), experimental and human evidence indicates that the developing fetus and neonate have heightened susceptibility to certain chemical carcinogens compared with the adult (reviewed in Anderson et al. 2000; National Research Council 1993). Factors that may increase fetal susceptibility include higher rates of cell proliferation, the greater number of target cells at risk, lower immunologic competence, and decreased capacity to activate and detoxify carcinogens as well as to repair DNA (Anderson et al. 2000; National Research Council 1993).

Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants commonly found in ambient air, as well as workplace air, food, and drinking water [International Agency for Research on Cancer (IARC) 1983]. Incomplete combustion of organic material is the major source of PAHs. Airborne PAHs result mainly from combustion of fossil fuels, tobacco products, and other organic materials. Generally, emissions from motor vehicles and residential heating are the major source of PAHs in outdoor urban air, whereas environmental tobacco smoke (ETS) is a major indoor source (Donnenfeld et al. 1993; Lewtas 1994). A number of PAHs, of which benzo[*a*]pyrene (BaP) is a representative member, are transplacental carcinogens in experimental bioassays, producing tumors in the liver, lung, lymphatic tissues, and nervous system of the offspring (Bulay and Wattenberg 1971; Rice and Ward 1982; Vesselinovitch et al. 1975). Experimental animal studies have demonstrated that the fetus and infant are more susceptible to PAH-induced carcinogenesis than are adults (Rice and Ward 1982; Soyka 1980; Toth et al. 1963; Vesselinovitch et al. 1975; Walters 1966). No comparable human data are available on age-related susceptibility to PAH carcinogenesis (Anderson et al. 2000). However, epidemiologic studies have shown the human fetus to be more sensitive than the adult to the carcinogenic effects of drugs such as diethylstilbestrol (Fraumeni 1974).

Biomarkers are biochemical or molecular alterations that can document variation in susceptibility to carcinogens among human populations (Bearer 1998; Perera 2000; Whyatt et al. 2001). We have compared levels of biomarkers in paired maternal and newborn blood samples from a cohort of mothers and newborns in New York City. Biomarkers were BaP-DNA adducts in white blood cells (WBCs) measured by high-performance liquid chromatography (HPLC)–fluorescence and plasma cotinine (an internal dosimeter of cigarette smoke).

As an indicator of DNA damage, carcinogen-DNA adducts represent a critical step in the carcinogenic pathway and are an informative biomarker of age-related susceptibility and potential risk of cancer. The biologic basis for measuring DNA adducts derives from extensive experimental data supporting their role in the initiation and possibly in the progression of cancer (Perera 2000). An association between PAH-DNA adduct levels and cancer risk has been seen previously in both experimental and epidemiologic research (Bartsch and Hietanen 1996; Poirier and Beland 1992; Stowers and Anderson 1985; Tang et al. 1995, 2001; Veglia et al. 2003).

PAHs cross the placenta. However, experimental studies in laboratory animals using radiolabeled PAHs indicate that the dose to the fetus is generally at least an order of magnitude lower than the dose to paired maternal tissues (Neubert and Tapken 1988; Srivastava et al. 1986; Withey et al. 1993). In a number of rodent bioassays, fetal levels of PAH-DNA adducts have generally been higher than expected, given the lower estimated transplacental dose of PAHs (Lu et al. 1986; Lu and Wang 1990; Wang and Lu 1990). Similarly, levels of micronuclei formation and DNA single-strand breaks after transplacental PAH exposure have been shown experimentally to be higher in fetal than in paired maternal tissues (Bolognesi et al. 1985; Harper et al. 1989; Wang and Lu 1990). A previous study of a small number of maternal and cord blood

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samples from a Chinese population found comparable levels of PAH-DNA adducts measured by competitive enzyme-linked immunosorbent assay (ELISA) (Mumford et al. 1993).

Cotinine is the major proximal metabolite of nicotine but has a longer half-life in serum (15–40 hr vs. 1–3 hr for nicotine). Cotinine has been widely used as a specific marker of exposure to tobacco smoke (Benowitz 1996), which contains a myriad of carcinogens, including PAHs and the nicotine-derived carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N′*-nitrosonornicotine (Brunnemann et al. 1996; Hoffmann et al. 1985; U.S. Public Health Service 1990). Like nicotine, cotinine readily crosses the placenta (Haddow et al. 1988; Lambers and Clark 1996; Sorsa and Husgafvel-Pursiainen 1988; Ueda et al. 1989). The few studies comparing cotinine levels in paired maternal–fetal samples have given conflicting results (Donnenfeld et al. 1993; Jauniaux et al. 1999; Luck et al. 1985; Mercelina-Roumans et al. 1996; Nafstad et al. 1995).

We previously reported a detailed comparison of maternal–fetal biomarkers in a cohort of 320 mothers and newborns, including 70 mother–newborn pairs from Krakow, Poland (an industrial city with elevated ambient air pollution, including PAHs from coal burned for industrial purposes and residential heating) and 90 mother–newborn pairs from Limanowa, Poland (a town located 70 km southeast of Krakow with lower ambient pollution levels but 2-fold more frequent use of coal stoves for indoor home heating) (Whyatt et al. 2001). That study reported that PAH-DNA adducts measured by immunoassay were comparable in mothers and their newborns and that PAH-aromatic adducts measured by ^{32}P-postlabeling were significantly higher in newborns than in mothers (Whyatt et al. 2001). Here we present results of analysis of biomarkers in 530 New York City mothers and newborns (265 pairs). The two populations (U.S. and Polish) represent an estimated 10- to 30-fold range of exposure to PAHs.

Materials and Methods

Study populations and biomarkers. Study subjects are nonsmoking 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, Harlem Hospital, or their satellite clinics since February 1998, as previously described (Perera et al. 2003) (Table 1). Subjects signed a consent form approved by the Columbia University institutional review board. Race/ethnicity was self-identified. Subjects were interviewed using a questionnaire to elicit environmental and health histories, including exposure to tobacco smoke at home or work and dietary ingestion of PAHs via smoked, fried, broiled, barbecued, and grilled foods. Subjects included in the present analysis are those with available PAH-DNA adduct data for both mother and child. Not all subjects in the ongoing parent study (n = 474) had adduct data available because assays are ongoing and in some cases the amount of DNA was inadequate for analysis. However, the present subset is representative of the larger study cohort in that there were no significant differences between the present subset and the overall cohort with respect to any of the demographic and exposure variables shown in Table 1. Maternal blood (30–35 mL) was collected within 1 day postpartum, and umbilical cord blood (30–60 mL) was collected at delivery (Perera et al. 2003). Samples were transported to the laboratory immediately after collection. The buffy coat, packed red blood cells, and plasma were separated and stored at –70°C. BaP-DNA adducts in extracted WBC DNA were analyzed using the HPLC–fluorescence method of Alexandrov et al. (1992), which detects BaP tetraols. The assay gives zero values when unexposed calf thymus DNA is tested (D. Tang, personal communication). The method has a coefficient of variation of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides. HPLC analysis of DNA samples for BaP-DNA adducts was performed in batches, with 18-paired maternal and newborn samples in the same batch. A portion of each sample was shipped to the Centers for Disease Control and Prevention for analysis of cotinine using HPLC atmospheric-pressure ionization tandem mass spectrometry, as previously described (Bernert et al. 2000). Current data on annual average ambient concentrations of BaP in New York City are not available. However, outdoor 24-hr average BaP concentrations measured in U.S. urban areas have generally been in the range of 0.5–4 ng/m^3 in recent years (U.S. EPA 1990). Personal air monitoring of the New York City mothers for 48 hr during pregnancy provided data on PAHs, including BaP (Perera et al. 2003). For the first 344 subjects analyzed, the average personal BaP concentration was 0.5 ng/m^3 (range, 0.02–6.44). This may be an overestimate of mean ambient exposure because personal monitoring can also reflect indoor sources such as ETS. We estimate that the ambient BaP exposure of the New York City cohort, and by inference their exposure to other related PAHs, was at least 6- to 30-fold lower than that of the Polish cohort (0.5 vs. 3–15 ng/m^3).

Statistical analyses. Statistical analyses used nonparametric methods because of the distributional properties of the biomarkers and because many of the samples were below the limit of detection. As in prior studies (Whyatt et al. 2001), we assigned nondetectable samples a value midway between the limit of detection and zero. We used nonparametric methods because the variables were not normally distributed. Spearman's rank test was used to test correlations between biomarkers in paired maternal and newborn samples, whereas differences between biomarker levels in paired maternal versus newborn samples were assessed by the Wilcoxon signed-rank test. The variable for ETS exposure was based on the presence of smoker(s) in the home, and that for dietary PAHs on frequency of consumption of fried, broiled, or barbecued food. Associations were considered significant at $p \le 0.05$.

Results

The demographic characteristics of the 265 mother–newborn pairs from the New York City cohort study are provided in Table 1. The mothers were all nonsmokers. Detectable adduct levels were found in 42% of mothers and 45% of newborns. Detectable adducts were found in 53 (34%) newborns whose mothers had nondetectable levels of adducts; 43 (30%) mothers with detectable adducts had newborns with nondetectable levels of adducts (see Table 2). Levels of adducts in paired newborn–maternal samples were modestly but significantly correlated (r = 0.28, $p < 0.001$), as were cotinine levels (r = 0.31, $p < 0.001$). DNA adducts were not significantly correlated with cotinine in either maternal (r = 0.07, p = 0.36) or newborn (r = 0.05, p = 0.54) samples. ETS was significantly correlated with cotinine in both maternal (r = 0.4, $p < 0.001$) and newborn (r = 0.4, $p < 0.001$) samples. Dietary PAHs and ETS were not significantly associated with adducts ($p > 0.05$), nor were adducts correlated with PAHs in air monitored during pregnancy (mothers: r = 0.01, p = 0.9; newborns: r = 0.02, p = 0.8). Mean cotinine levels in mothers and newborns were higher in African Americans than in Dominicans, as was ETS exposure ($p < 0.05$). However, mean adduct levels did not differ between the two ethnic groups.

As shown in Table 3, the mean levels of BaP-DNA adducts (per 10^8 nucleotides) were comparable in New York City newborns and mothers (0.24, 45% detectable in newborns vs. 0.22, 41% detectable in mothers). However, by the Wilcoxon signed-rank test,

Table 1. New York City cohort: number of mother–newborn pairs,[a] maternal age, race/ethnicity, and ETS exposure of mothers.

Characteristics	Values
No. of mother–newborn pairs	265
Mother's age [years (mean ± SD)]	24.5 ± 4.8
Race/ethnicity [no. of pairs (%)]	
African American	102 (37.7)
Dominican	163 (62.3)
Self-reported ETS [no. of pairs (%)]	
ETS exposure	100 (37.7)
No ETS exposure	165 (62.3)

[a]Number of pairs with adduct data.

the concentrations in newborns were significantly higher than those in the mothers ($p = 0.02$).

Cotinine levels were significantly higher in newborns (1.7 per 10^8 nucleotides, 47% detectable vs. 1.28 per 10^8 nucleotides, 44% detectable; $p < 0.001$). Table 4 provides details on the cotinine data.

Discussion

In a cohort of mothers and newborns from an inner-city minority population in New York City, given the lower estimated dose of PAHs to the fetus, the levels of DNA damage from PAHs in WBC were higher than expected in newborns compared with paired mothers. The results are consistent with our prior finding in a Polish cohort of 160 paired Caucasian mothers and newborns (Whyatt et al. 2001) that PAH-DNA adducts measured by ELISA and PAH–aromatic adducts measured by ^{32}P-postlabeling were significantly higher in the newborns. Although the methods of adduct analysis differed between the two studies, they all reflect DNA damage from PAHs and related aromatic compounds. Comparing the three assays with respect to specificity to exposure, the postlabeling method detects the broadest spectrum of adducts (multiple PAHs and other aromatic/hydrophobic compounds bound to DNA); the ELISA detects BaP diol-epoxide (DE)-DNA adducts and structurally related PAH-DNA adducts; and the HPLC–fluorescence method is the most specific (BaP-DNA only). The results point to increased susceptibility of the developing fetus to DNA damage from this class of carcinogens. The lack of a correlation between adducts and ETS, monitored PAHs in air, or dietary PAHs probably reflects the fact that adducts capture both individual exposure and susceptibility.

There are no data in humans on maternal versus fetal dose of PAHs. However, experimental studies in rodents using radiolabeled PAHs indicate that the dose to the embryo/fetus is generally an order of magnitude or more lower than the dose to paired maternal tissues (Neubert and Tapken 1988; Srivastava et al. 1986; Withey et al. 1993; reviewed in Whyatt et al. 2001). Because humans and rodents may not metabolize PAHs in the same way, the data suggest, but do not prove, that the human fetus also has differential exposure. With this caveat, our findings suggest that the amount of DNA damage per unit dose of PAHs may be on the order of 10-fold higher in the fetus relative to the mother. Increased susceptibility could result from reduced detoxification capabilities or decreased DNA repair capacity during fetal development and may be an important factor in the observed greater carcinogenic impact of PAHs when administered to experimental animals prenatally or neonatally compared with later in life (Rice and Ward 1982; Soyka 1980; Toth et al. 1963; Vesselinovitch et al. 1975; Walters 1966).

Against the backdrop of the Polish study, our present finding indicates heightened susceptibility of the fetus to PAH-DNA damage over a wide range of BaP exposure (30-fold range, from 0.5 to 15 ng/m^3). This is of concern in light of the association seen previously in molecular epidemiologic research between PAH-DNA adduct levels and cancer risk (Tang et al. 2001). In a prior study of Polish newborns, an association has also been observed between PAH-DNA adducts in cord blood and adverse birth outcomes (Perera et al. 1998).

In the New York City cohort, as in the Polish cohort, cotinine levels were significantly higher in newborn plasma compared with paired maternal plasma. Few prior studies have compared cotinine levels in mother–newborn pairs and results have been conflicting (Donnenfeld et al. 1993; Jauniaux et al. 1999; Luck et al. 1985; Mercelina-Roumans et al. 1996; Nafstad et al. 1995). Our present results in nonsmoking mothers and newborns suggest that, like nicotine, cotinine concentrates in the fetus (Lambers and Clark 1996; Luck et al. 1985). However, some of the differences we observed between fetal and maternal samples may have occurred because the maternal blood

Table 2. Adduct data for 265 mother–newborn pairs.

	Mothers' adduct levels			
Newborns' adduct levels	Nondetectable	Low detectable	High detectable	Total
Nondetectable				
No.	103	25	18	146
Percent of newborns	70.5	17.1	12.3	100.0
Percent of mothers	66.0	53.2	29.0	55.1
Low detectable[a]				
No.	25	15	15	55
Percent of newborns	45.5	27.3	27.3	100.0
Percent of mothers	16.0	31.9	24.2	20.8
High detectable[a]				
No.	28	7	29	64
Percent of newborns	43.8	10.9	45.3	100.0
Percent of mothers	17.9	14.9	46.8	24.2
Total				
No.	156	47	62	265
Percent of newborns	58.9	17.7	23.4	100.0
Percent of mothers	100.0	100.0	100.0	100.0

[a]The cut point of low/high detectable was the median of the detectable level of adducts for mothers and newborns, respectively.

Table 3. New York City cohort: biomarkers in paired maternal and newborn blood samples.

	Mean ± SD (% detectable)		
	Maternal	Newborn	*p*-Value
WBC BaP-DNA adduct levels by HPLC–fluorescence (per 10^8 nucleotides)			
Total cohort (265 pairs)	0.22 ± 0.14 (41)	0.24 ± 0.15 (45)	0.02[a]
ETS exposure (100 pairs)	0.23 ± 0.16 (42)	0.24 ± 0.15 (44)	0.31
No ETS exposure (165 pairs)	0.21 ± 0.12 (41)	0.24 ± 0.15 (45)	0.08
Plasma cotinine (ng/mL)			
Total cohort (160 pairs)	1.28 ± 9.77 (44)	1.7 ± 10.50 (47)	< 0.001
ETS exposure (65 pairs)	3.04 ± 15.22 (69)	4.02 ± 16.27 (69)	< 0.001
No ETS exposure (95 pairs)	0.08 ± 0.24 (26)	0.12 ± 0.48 (32)	0.001

[a]Wilcoxon signed-rank test for paired samples comparing maternal and newborn levels.

Table 4. Cotinine data for 160 mother–newborn pairs.

	Mothers' cotinine levels			
Newborns' cotinine level	Nondetectable	Low detectable	High detectable	Total
Nondetectable				
No.	81	3	1	85
Percent of newborns	95.3	3.5	1.2	100.0
Percent of mothers	90.0	8.3	2.9	53.1
Low detectable[a]				
No.	9	26	2	37
Percent of newborns	24.3	70.3	5.4	100.0
Percent of mothers	10.0	72.2	5.9	23.1
High detectable[a]				
No.	0	7	31	38
Percent of newborns	0	18.4	81.6	100.0
Percent of mothers	0	19.4	91.2	23.8
Total				
No.	90	36	34	160
Percent of newborns	56.3	22.5	21.3	100.0
Percent of mothers	100.0	100.0	100.0	100.0

[a]The cut point of low/high detectable was the median of the detectable level of adducts for mothers and newborns, respectively.

samples were collected on the day after delivery, whereas the newborn samples were collected at delivery. We note, however, that among 80 Polish mother and newborn pairs whose blood samples were collected within 1 hr of each other the cotinine was significantly higher in the newborns ($p < 0.05$) (Whyatt et al. 2001). Cotinine has low toxicity relative to nicotine (Jordanov 1990; Lambers and Clark 1996) but is a good internal dosimeter for nicotine (Benowitz 1996). Concentration of nicotine and cotinine in the fetus appears to be caused by reduced clearance during fetal development and by the fact that, in addition to transfer from the maternal circulation, the fetus is exposed from gastrointestinal reabsorption of the chemicals in swallowed amniotic fluid (Jauniaux et al. 1999). The adverse effects of nicotine on fetal growth are well documented (Lambers and Clark 1996). In addition, like BaP, the nicotine-derived nitrosamine NNK binds to DNA to form adducts (Hecht 1999) and is a potent transplacental carcinogen in experimental bioassays (Jordanov 1990).

These findings highlight the need for pollution and smoking prevention programs to protect women of childbearing age and their children from PAHs and ETS. Individuals are generally exposed throughout most of their life to low levels of carcinogens; if, in addition, they experience prenatal exposure to carcinogens, the possibility that they will develop cancer over their lifetime may be disproportionately increased (Bulay and Wattenberg 1971). There are several possible factors involved. First, as shown in our research, prenatal exposures apparently result in differentially higher levels of procarcinogenic DNA damage. Second, there is a higher probability that low-level/long-latency effects will be manifested as cancer during the individual's life span if these effects are initiated *in utero* rather than later in life. Therefore, the results presented here have implications for risk assessment and environmental health policy and highlight the need to protect pregnant women and especially their children as a sensitive subset of the population.

Conclusion

Consistent with a prior study in a Caucasian Polish population, these results in a New York City minority population indicate increased susceptibility of the fetus to DNA damage and reduced ability to clear ETS constituents. The findings have implications for risk assessment, given the need to protect children as a sensitive subset of the population.

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