

Relation between prenatal lipid-soluble micronutrient status, environmental pollutant exposure, and birth outcomes¹⁻³

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ABSTRACT

Background: Adverse postnatal health effects have been associated with compromised fetal growth, which makes it essential to understand its determinants. Significant effects of environmental pollutants on birth outcomes have been observed in our study population, and nutritional status may be an additional factor influencing fetal development and effects of environmental toxins.

Objective: The objective of the study was to examine the relations between birth outcomes and lipid-soluble plasma micronutrient concentrations and to explore interactions between micronutrients and environmental pollutant exposure in newborns in Krakow, Poland.

Design: In this prospective cohort study, retinol, α -tocopherol, and carotenoids were measured in maternal and cord blood samples obtained at delivery (251 maternal-newborn pairs), and birth weight, birth length, head circumference (HC), and gestational age were evaluated. Linear regression analysis was used to estimate the effects of micronutrients while covariates were controlled for. Interaction terms assessed whether the effects of polycyclic aromatic hydrocarbons (PAHs), common environmental pollutants, varied by nutrient status.

Results: Infants whose mothers had low plasma α -tocopherol concentrations (below the median) weighed 92.9 g less and had 0.41-cm smaller HCs than did infants whose mothers had high α -tocopherol concentrations. Infants with low plasma retinol (below the median) weighed 125.9 g less and had 0.31-cm smaller HCs. There was no evidence of an interaction between PAHs and micronutrients, although power was limited.

Conclusion: Maternal α -tocopherol and cord retinol concentrations were significantly and positively associated with BW and HC. These micronutrients may have direct effects or may be markers for other underlying determinants of these pregnancy outcomes. *Am J Clin Nutr* 2007;86:1139-45.

KEY WORDS Micronutrients, pregnancy, birth outcomes, fetal growth, cord blood, polycyclic aromatic hydrocarbons, Poland

INTRODUCTION

Birth weight is associated with infant mortality (1) and cognitive development (2), and reduced fetal growth may be related to risk of diseases later in life (3). Therefore, identifying the dietary and other environmental factors involved in determining fetal size is of public health importance. In a study of pregnant women and their newborns from Krakow, Poland, we investigated whether circulating concentrations of several lipid-soluble

micronutrients were associated with fetal growth. Few studies have examined the effects of micronutrient status on birth outcomes in pregnant women from developed countries where malnutrition is infrequent. In a relatively well-nourished population, it may be that fetal growth is affected by an imbalance of nutrients more often than by a true deficiency (4). The existing evidence is inconclusive concerning the association between maternal or fetal nutrient status and birth outcomes.

The current study is part of ongoing research evaluating the effects of environmental pollutants, including polycyclic aromatic hydrocarbons (PAHs) and environmental tobacco smoke (ETS), on fetal growth. In this study we analyzed a panel of lipid-soluble micronutrients in cord and maternal blood and examined whether maternal or cord blood concentrations of retinol (vitamin A), α -tocopherol (vitamin E), and several carotenoids were associated with gestational age (GA), birth weight (BW), birth length (BL), and head circumference (HC). We further tested whether the effects of PAHs could be altered by micronutrient status. Given that nutritional status is modifiable through improved dietary intake or supplementation, this knowledge could potentially positively affect birth outcomes.

SUBJECTS AND METHODS

Enrollment

This study is part of an ongoing, longitudinal investigation of the health effects of prenatal exposure to outdoor and indoor air pollution on infants and children in Krakow, Poland (5). The

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ethics committee of the Jagiellonian University approved the study. Women attending prenatal healthcare clinics in the first and second trimesters of pregnancy were eligible. Only non-smoking women with singleton pregnancies who were 18–35 y of age and free of chronic diseases were enrolled. 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 occupational exposure to developmental toxicants (including coke oven, chemical, or rubber workers; workers in environments with dust, fumes, or solvents; toll collectors; and bus, tram, and taxi drivers), diabetes, or hypertension. Eligible women were given a full description of the study and the requirements for participation and were then invited to participate in the study. Informed consent was obtained from all participants. A detailed questionnaire was administered to study participants on entry into the study and during the third trimester to obtain information on demographic and household characteristics, date of last menstrual period (LMP), medical and reproductive history, occupational hazards, alcohol consumption, and smoking habits of other persons present in the home. After the participants gave birth, maternal and infant hospital records were reviewed to obtain data on the pregnancy and delivery. BW, BL, HC, GA, and Apgar score at 1 and 5 min were recorded. GA obtained from the infant's medical record was estimated on the basis of the LMP. We previously reported a high degree of reliability between this GA estimate and independently derived LMP- and sonogram-based GA estimates (6). Full enrollment into the study required participants to complete prenatal monitoring and interviews and to provide a blood sample (maternal blood, cord blood, or both).

Personal ambient air monitoring

The 48-h personal air monitoring was undertaken during the second 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 to collect semivolatile vapors and aerosols on a polyurethane foam cartridge. During the morning of the second day, the air monitoring staff person and interviewer visited the woman's home to change the battery pack and administer a full questionnaire. The polyurethane foam cartridges were analyzed at Southwest Research Institute for concentrations of pyrene and 8 carcinogenic PAHs: (benz(a)anthracene, chrysene/iso-chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene), as previously described (7).

Biological sample collection

In the operating room after delivery, a cord blood sample was collected from the umbilical cord vein immediately after the umbilical cord was cut. A venous blood sample was obtained from each woman within 1 d of delivery. Plasma was separated at the Jagiellonian University Clinical Laboratory in Krakow (supervised by Danuta Fedak), stored at -70°C , and then shipped to Columbia University in New York City.

Micronutrient analyses

Plasma concentrations of micronutrients were measured in maternal and cord blood samples at the Centers for Disease Control and Prevention Nutrition Laboratory by using isocratic HPLC and multiwavelength detection. The method involved minor modifications of a published method (8). Briefly, a 100- μL aliquot of plasma precipitated with a mixture of 2 internal standards (nonapreno- β -carotene and retinyl butyrate) dissolved in ethanol was extracted, dried, redissolved in equal parts ethanol and acetonitrile, and filtered to remove any insoluble material. An aliquot of the filtrate was injected onto a C_{18} reversed-phase column (Phenomenex Ultracarb 4.6 \times 150 mm; 3 μm particle size; Phenomenex, Torrance, CA) 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. Vitamins A and E were compared with retinyl butyrate at 325 and 300 nm, respectively. Carotenoids were compared with nonapreno- β -carotene at 450 nm.

Statistical analyses

SPSS (version 11.5) for WINDOWS (SPSS Inc, Chicago, IL) was used for the calculation of descriptive statistics and correlation coefficients and for linear regression analysis. Statistical significance was set at a P value < 0.05 . Women whose cotinine values were ≥ 25 ng/mL were excluded to eliminate active smokers from the analysis. Subjects were included if both maternal and cord blood micronutrient values were available. Women who completed < 37 wk of gestation and newborns with major birth defects were excluded from the analyses, which resulted in a final sample of 251 mother-newborn pairs. Nondetectable values were included in the analyses as half the limit of detection (LOD/2). Overall, 220 of the 251 mother-newborn pairs included at least one nondetectable micronutrient value. However, in the case of retinol and α -tocopherol, none of the subjects included in the analyses had nondetectable values. Nonparametric statistics were used because of the distributional properties of the micronutrients. The Kolmogorov-Smirnov test assessing the normality of the data generally rejected the assumption of normality. To be consistent we therefore used nonparametric methods throughout. Means and SDs for micronutrient concentrations in paired maternal and cord blood samples were determined and compared by paired Wilcoxon's signed-rank test. Spearman correlations between micronutrient concentrations in paired maternal and cord blood samples and between individual micronutrients within a blood sample were determined.

We used multiple linear regression to assess the associations of micronutrients and GA, BW, BL, and HC. Each micronutrient variable was dichotomized into high or low categories on the basis of median maternal or cord plasma concentrations of the micronutrient. The carotenoid variable was computed as the average of the plasma β -carotene, α -carotene, β -cryptoxanthin, lutein/zeaxanthin, and lycopene concentrations. Sex of the newborn and maternal education, prepregnancy weight, height, and parity were included as covariates in all analyses. GA was included as a covariate in models with BW, BL, or HC as dependent variables, and cesarean delivery was included in models with HC. BW, BL, HC, and GA were natural



TABLE 1

Characteristics of the study population¹

	Subjects included in analysis (n = 251) ²	Subjects not included in analysis (n = 138) ³
Maternal characteristics		
Age (y)	28.26 ± 3.72 ⁴	27.52 ± 3.43 ⁵
Education beyond high school (%)	64.14	65.22
ETS exposure (% reporting smoker in the home)	21.51	26.09
Alcohol consumption during pregnancy (%)	66.93	52.17 ⁶
Height (cm)	164.70 ± 5.67	165.3 ± 5.56
Prepregnancy weight (kg)	58.06 ± 8.87	58.62 ± 9.11
White race (%)	100	100
Prenatal PAH exposure (ng/m ³)	42.65 ± 49.81 ⁷	25.52 ± 33.92 ^{5,8}
Newborn characteristics		
Gestational age (wk)	39.61 ± 1.20	39.54 ± 1.10
Birth weight (g)	3485.2 ± 427.3	3418.0 ± 466.5
Birth length (cm)	54.82 ± 2.48	54.65 ± 2.90
Head circumference (cm)	34.03 ± 1.40	33.86 ± 1.40
Sex (% female)	52.19	42.75
Low birth weight (%) ⁹	0.72	0.80

¹ PAH, polycyclic aromatic hydrocarbons; ETS, environmental tobacco smoke.² Subjects had both maternal and cord blood micronutrient concentration data, had a cotinine concentration <25 ng/mL, had a gestational age ≥37 wk, and their children had no major malformations.³ Subjects either did not have micronutrients analyzed, had a cotinine concentration ≥25 ng/mL, had a gestational age <37 wk, or had a child with a major malformation. Twenty-one newborns who were delivered preterm were excluded from the study population.⁴ $\bar{x} \pm SD$ (all such values).⁵ Significantly different from those included in the analysis $P < 0.05$ (Wilcoxon's rank-sum test, 2-tailed).⁶ Significantly different from those included in the analysis, $P < 0.05$ (Fisher's exact test, 2-tailed).⁷ $n = 243$.⁸ $n = 84$.⁹ Defined as a weight <2500 g.

log (ln) transformed to provide a better fit to the data or to approximate the normal distribution and stabilize the variance. The skewed distribution of measures of size at birth was due to the eligibility criteria of the study (which precluded enrollment of women with risk factors for preterm birth or low birth weight) and the exclusion of infants with short gestation (<37 wk) from our analysis. Interactions between maternal and cord blood micronutrient concentrations were also tested by including an interaction term in the regression models. Finally, a variable for total PAHs (defined as the sum of the air concentrations of the 8 carcinogenic PAHs measured from personal air samples and dichotomized at the median) was included.

RESULTS

Description of the sample

Characteristics of the women and newborns enrolled in the study are shown in **Table 1**. The mean age for mothers included in the analysis was 28 y. On average, the mothers were 165 cm tall and weighed 58 kg before pregnancy. The mean BW, BL, HC, and GA of the newborns were 3485 g, 54.8 cm, 34.0 cm, and 39.6 wk, respectively. Women included in the analysis were not significantly different from women who were not included with respect to all demographic characteristics except alcohol consumption, age, and prenatal PAH exposure; subjects included in the analysis had values for these 3 variables that were significantly higher than those of the persons not included.

Concentrations of micronutrients in maternal and cord blood

Mean maternal and cord plasma concentrations of the micronutrients for paired samples are presented in **Table 2**. The mean concentrations of all micronutrients were significantly higher ($P < 0.001$) in maternal plasma than in cord plasma. Cord plasma concentrations were 55% of maternal concentrations for retinol, 19% for α -tocopherol, and between 6% and 19% for the carotenoids. Inadequate vitamin A and E status were uncommon in this population; only 3.98% of the women had retinol concentrations <20 $\mu\text{g}/\text{dL}$, and 1.99% had α -tocopherol concentrations <500 $\mu\text{g}/\text{dL}$, which indicated below normal plasma concentrations for these micronutrients (9, 10).

Micronutrient correlations

By Spearman's rank test, maternal and cord blood micronutrient concentrations were significantly correlated ($P < 0.05$) for α -tocopherol, retinol, and the carotenoids. The maternal-cord correlations for α -tocopherol ($r = 0.19$, $P = 0.02$) and retinol ($r = 0.13$, $P = 0.038$) were relatively weak, whereas a moderate correlation was observed for the carotenoids ($r = 0.52$, $P < 0.001$). Similarly, there were statistically significant correlations for maternal ($r_{\text{retinol-}\alpha\text{-tocopherol}} = 0.15$, $P < 0.02$; $r_{\text{carotenoids-retinol}} = 0.15$, $P < 0.02$; $r_{\text{carotenoids-}\alpha\text{-tocopherol}} = 0.44$, $P < 0.001$) and cord ($r_{\text{retinol-}\alpha\text{-tocopherol}} = 0.27$, $P < 0.001$; $r_{\text{carotenoids-}\alpha\text{-tocopherol}} = 0.65$,

TABLE 2

Maternal and cord plasma lipid-soluble micronutrient concentrations ($n = 251$)¹

Micronutrient	Maternal plasma concentration		Cord plasma concentration	
			$\mu\text{g/dL}$	
α -Tocopherol	1684.07 \pm 444.61	(1038–2368)	315.57 \pm 216.27 ²	(165.0–514.4)
Retinol	37.97 \pm 13.37	(21.50–58.05)	20.86 \pm 5.71 ²	(12.35–30.25)
α -Carotene	17.72 \pm 12.44	(3.70–41.04)	1.51 \pm 2.57 ²	(0.35–4.60)
β -Carotene	46.60 \pm 31.88	(10.35–105.6)	3.38 \pm 5.99 ²	(0.40–9.14)
β -Cryptoxanthin	17.72 \pm 12.44	(4.60–42.24)	2.30 \pm 3.20 ²	(0.43–5.93)
Lutein/zeaxanthin	28.66 \pm 11.66	(13.20–50.80)	5.39 \pm 4.31 ²	(2.60–9.65)
Lycopene	16.17 \pm 8.35	(4.20–32.10)	1.03 \pm 3.44 ²	(0.38–2.10)

¹ All values are $\bar{x} \pm \text{SD}$; 5th–95th percentile range in parentheses.² Significantly different from maternal plasma concentration, $P < 0.001$ (Wilcoxon's signed-rank test).

$P < 0.001$) micronutrients; however, none were highly correlated. Because there were no highly correlated variables and, therefore, no evidence of multicollinearity, we included the variables together in the following regression models.

Regression analysis: relation of maternal and cord micronutrient concentrations to birth outcomes

Multiple linear regression models were used to assess the relation of these lipid-soluble micronutrients to birth outcomes with adjustment for confounders and covariates. None of the micronutrients under study was significantly associated with GA. The influence of retinol, α -tocopherol, and carotenoids on BW, BL, and HC was examined, after adjustment for covariates. With each micronutrient in a separate model, we found that the carotenoid variable was not significantly associated with any of the birth outcomes; therefore, it was not included in the final models. The full models that included both retinol and α -tocopherol along with other covariates are shown in **Table 3** and **Table 4**. Results for separate models with the covariates and either retinol or α -tocopherol were similar to those for the vitamins combined in one model; therefore, we only presented the combined models here. Birth outcomes were log (ln) transformed to approximate the normal distribution, and retinol and α -tocopherol were dichotomized at the median. Results from the

analyses with dichotomous micronutrients are presented here because they are less influenced by extreme, outlying values; however, when the micronutrients were treated as continuous variables, all results were in the same direction.

These models showed that maternal α -tocopherol was significantly positively associated with ln HC ($\beta = 0.012$, $P = 0.01$) and borderline significantly associated with ln BW ($\beta = 0.025$, $P = 0.06$). This translates into BWs and HCs that were on average 92.9 g and 0.41 cm lower, respectively, in the group with low maternal α -tocopherol (α -tocopherol below median) than in those with the high α -tocopherol group. Cord retinol was positively associated with ln BW ($\beta = 0.036$, $P < 0.01$) and ln HC ($\beta = 0.009$, $P = 0.04$). On average, the BW was 125.9 g lower and the HC was 0.31 cm smaller in the low-retinol group (retinol below the median) than in the high-retinol group.

The model including both maternal and cord retinol and α -tocopherol is shown in **Table 5**. The results are generally consistent with the individual nutrient models, which suggests that control for maternal retinol and α -tocopherol does not significantly alter the relation between cord micronutrients and birth outcomes and vice versa. We previously reported that exposure to PAHs had a significant, negative association with BW, BL, and HC in this cohort (6). However, in this smaller sample, the

TABLE 3

Multiple linear regression models testing associations of maternal plasma retinol and α -tocopherol concentrations and body weight (BW), birth length (BL), and head circumference (HC) ($n = 251$)¹

	ln BW			ln BL			ln HC		
	β	95% CI	P	β	95% CI	P	β	95% CI	P
Retinol ²	-0.02	(-0.045, 0.006)	0.12	0.001	(-0.009, 0.011)	0.81	-0.006	(-0.015, 0.003)	0.21
α -Tocopherol ³	0.025	(-0.001, 0.051)	0.06	0.009	(-0.001, 0.019)	0.08	0.012	(0.003, 0.022)	0.01
Sex ⁴	-0.065	(-0.091, -0.040)	< 0.01	-0.026	(-0.036, -0.016)	< 0.01	-0.026	(-0.035, -0.017)	< 0.01
Maternal prepregnancy weight (kg)	0.002	(0.001, 0.004)	< 0.01	0.001	(-0.0001, 0.001)	0.09	0.001	(0.0001, 0.0012)	0.03
Maternal height (cm)	0.004	(0.001, 0.006)	< 0.01	0.001	(0.0003, 0.002)	< 0.01	0.001	(0.0003, 0.002)	< 0.01
Parity	0.058	(0.031, 0.085)	< 0.01	0.013	(0.002, 0.023)	0.02	0.02	(0.011, 0.030)	< 0.01
Log gestational age (log wk)	1.591	(1.171, 2.011)	< 0.01	0.482	(0.318, 0.645)	< 0.01	0.239	(0.091, 0.387)	< 0.01
Cesarean delivery	—	—	—	—	—	—	0.013	(0.001, 0.026)	0.03

¹ Analysis by multiple linear regression with adjustment for the covariates shown in the table: sex, maternal prepregnancy weight, maternal height, parity, gestational age, and cesarean delivery (for HC only). The carotenoid variable was not significantly associated with any of the birth outcomes; therefore, it was not included in the final models.

² Dichotomized by using the median value in maternal plasma samples: 1 = all values $>37.2 \mu\text{g/dL}$ (high), 0 = all values $\leq 37.2 \mu\text{g/dL}$ (low).

³ Dichotomized by using the median value in maternal plasma samples: 1 = all values $>1631.35 \mu\text{g/dL}$ (high), 0 = all values $\leq 1631.35 \mu\text{g/dL}$ (low).

⁴ 0 = male, 1 = female.

TABLE 4

Multiple linear regression models testing associations of cord plasma retinol and α -tocopherol concentrations and body weight (BW), birth length (BL), and head circumference (HC) ($n = 251$)¹

	ln BW			ln BL			ln HC		
	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>
Retinol ²	0.036	(0.010, 0.062)	< 0.01	0.001	(-0.009, 0.011)	0.88	0.009	(0.0002, 0.019)	0.04
α -Tocopherol ³	-0.014	(-0.040, 0.013)	0.31	-0.005	(-0.016, 0.005)	0.3	-0.005	(-0.014, 0.005)	0.34
Sex ⁴	-0.068	(-0.094, -0.043)	< 0.01	-0.026	(-0.036, -0.016)	< 0.01	-0.027	(-0.037, -0.018)	< 0.01
Maternal prepregnancy weight (kg)	0.003	(0.001, 0.004)	< 0.01	0.001	(-0.00003, 0.0012)	0.06	0.001	(0.0001, 0.0012)	0.02
Maternal height (cm)	0.003	(0.001, 0.006)	< 0.01	0.001	(0.0004, 0.002)	< 0.01	0.001	(0.0002, 0.002)	0.02
Parity	0.048	(0.021, 0.075)	< 0.01	0.01	(-0.0003, 0.021)	0.06	0.016	(0.007, 0.026)	< 0.01
Log gestational age (log wk)	1.581	(1.162, 1.999)	< 0.01	0.482	(0.317, 0.647)	< 0.01	0.237	(0.088, 0.387)	< 0.01
Cesarean delivery	—	—	—	—	—	—	0.01	(-0.002, 0.023)	0.1

¹ Analysis by multiple linear regression with adjustment for the covariates shown in the table: sex, maternal prepregnancy weight, maternal height, parity, gestational age, and cesarean delivery (for HC only).

² Dichotomized by using the median value in cord plasma samples: 1 = all values >20.9 $\mu\text{g}/\text{dL}$ (high), 0 = all values \leq 20.9 $\mu\text{g}/\text{dL}$ (low).

³ Dichotomized by using the median value in cord plasma samples: 1 = all values >273.6 $\mu\text{g}/\text{dL}$ (high), 0 = all values \leq 273.6 $\mu\text{g}/\text{dL}$ (low).

⁴ 0 = male, 1 = female.

association between PAHs and birth outcomes was not significant. Post hoc power analysis was carried out to determine the difference in birth outcomes we would have been likely to detect, given this sample size and observed SD. There was a probability of 0.95 that the test would be significant if the true differences in birth outcome between low-exposed and high-exposed groups were large (ie, 160 g for BW, 0.99 cm for BL, and 0.56 cm for HC). However, the power of the study was far lower (ie, < 0.50) if the true differences in birth outcomes were small (ie, 68.75 g for BW, 0.48 cm for BL, and 0.21 cm for HC). There was no evidence that these micronutrients modify the association between PAH exposure and birth outcomes in this analysis; the interaction terms (PAH \times each maternal and cord micronutrient)

were not statistically significant for any birth outcomes. However, our power was very limited to detect the interactions.

DISCUSSION

This study provides evidence of an association between maternal and fetal nutrition and birth outcomes. Our regression analyses suggest that higher maternal concentrations of α -tocopherol and cord retinol are associated with greater BW and HC. Although statistically significant, the correlations of maternal and cord micronutrients were relatively weak. Compared with cord micronutrient concentrations, maternal concentrations were significantly higher, as other studies have shown (11–18),

TABLE 5

Multiple linear regression models testing associations of maternal and cord plasma retinol and α -tocopherol micronutrient concentrations and body weight (BW), birth length (BL), and head circumference (HC) ($n = 251$)¹

	ln BW			ln BL			ln HC		
	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>
Maternal retinol ²	-0.024	(-0.050, 0.0008)	0.06	0.001	(-0.009, 0.011)	0.9	-0.007	(-0.016, 0.002)	0.12
Maternal α -tocopherol ³	0.027	(0.001, 0.053)	0.04	0.01	(-0.0001, 0.020)	0.05	0.013	(0.004, 0.022)	< 0.01
Cord retinol ²	0.039	(0.013, 0.065)	< 0.01	0.001	(-0.009, 0.011)	0.88	0.01	(0.001, 0.019)	0.03
Cord α -tocopherol ³	-0.019	(-0.046, 0.007)	0.15	-0.007	(-0.017, 0.004)	0.2	-0.006	(-0.016, 0.002)	0.15
Sex ⁴	-0.066	(-0.092, -0.041)	< 0.01	-0.025	(-0.035, -0.015)	< 0.01	-0.026	(-0.036, -0.017)	< 0.01
Maternal prepregnancy weight (kg)	0.002	(0.0009, 0.004)	< 0.01	0.001	(-0.0001, 0.0011)	0.09	0.0006	(0.00004, 0.001)	0.03
Maternal height (cm)	0.003	(0.0009, 0.006)	< 0.01	0.001	(0.0003, 0.002)	< 0.01	0.001	(0.0002, 0.002)	0.02
Parity	0.052	(0.024, 0.078)	< 0.01	0.011	(0.001, 0.023)	0.03	0.018	(0.009, 0.028)	< 0.01
Log gestational age (log wk)	1.553	(1.138, 1.968)	< 0.01	0.474	(0.310, 0.638)	< 0.01	0.226	(0.079, 0.374)	< 0.01
Cesarean delivery	—	—	—	—	—	—	0.012	(0.0001, 0.025)	0.05

¹ Analysis by multiple linear regression with adjustment for covariates shown in the table: sex, maternal prepregnancy weight, maternal height, parity, gestational age, and cesarean delivery (for HC only).

² Dichotomized by using the median value of the micronutrient in maternal or cord plasma samples. The maternal retinol variable was coded as 1 = all values >37.2 $\mu\text{g}/\text{dL}$ (high) and 0 = all values \leq 37.2 $\mu\text{g}/\text{dL}$ (low). The cord retinol variable was coded as 1 = all values >20.9 $\mu\text{g}/\text{dL}$ (high) and 0 = all values \leq 20.9 $\mu\text{g}/\text{dL}$ (low).

³ Dichotomized by using the median value of the micronutrient in maternal or cord plasma samples. The maternal α -tocopherol variable was coded as 1 = all values >1631.35 $\mu\text{g}/\text{dL}$ (high) and 0 = all values \leq 1631.35 $\mu\text{g}/\text{dL}$ (low). The cord α -tocopherol variable was coded as 1 = all values >273.6 $\mu\text{g}/\text{dL}$ (high) and 0 = all values \leq 273.6 $\mu\text{g}/\text{dL}$ (low).

⁴ 0 = male, 1 = female.

and maternal and cord nutrient concentrations were differently related to birth outcomes, which may reflect the limited placental transfer of these lipid-soluble micronutrients (19). Although we observed greater birth size with higher concentrations of retinol and α -tocopherol at the ranges in this study, it is important to note that very high micronutrient levels, particularly for vitamin A, have been shown to be associated with toxicity (20).

Other studies relating retinol and α -tocopherol with birth outcomes in well-nourished populations have produced inconclusive results. Some studies in healthy women have found no evidence of an association between maternal retinol or α -tocopherol and birth outcomes (13, 21–23), whereas others have (24–26). Several features of the current study distinguish it from existing literature, including its analysis of several micronutrients in both cord and maternal plasma and its ability to control for potential confounding variables. The prospective design and relatively large sample size are additional strengths. Although many studies use dietary data to assess maternal nutritional status, we incorporated nutritional biomarkers, thereby minimizing problems associated with obtaining accurate information.

Several studies have concluded that cord retinol concentrations are positively associated with birth outcomes (13, 16, 27, 28). Our models also showed a consistently positive, significant association for cord retinol and BW, and suggested a negative relation between maternal retinol and BW. A similar negative association was reported in a study that measured nutrients in maternal blood samples at 16 and 28 wk of gestation (24). Although these findings may reflect a direct negative relation, it is possible that they indicate a condition such as low plasma volume expansion.

In our analyses of plasma α -tocopherol, we observed statistically significant associations with birth outcomes for maternal but not cord blood concentrations. The explanation for the different relations for retinol and α -tocopherol is unclear; however, the metabolism and placental transfer of these micronutrients are different, and while maternal concentrations of α -tocopherol increase during pregnancy, retinol concentrations decrease (18). A small number of studies have examined the relation of birth outcomes and α -tocopherol in maternal-newborn pairs and have shown different results. Dison et al (13) concluded that maternal α -tocopherol is not significantly correlated with BW, whereas a significant, negative relation exists between cord α -tocopherol and BW. However, unlike our multivariate analysis, the study by Dison et al used a correlation analysis and did not control for potential confounding variables. On the other hand, a small study by von Mandach et al (29) compared vitamin E concentrations of maternal-newborn pairs between a group with normal pregnancies and those with low-birth-weight infants. Consistent with our results, vitamin E concentrations in women with low-birth-weight babies were significantly lower than those with normal pregnancies, whereas cord blood concentrations were not significantly different.

Although little research involving analysis of α -tocopherol in pairs of mothers and newborns is available, studies examining cord or maternal vitamin E alone have been conducted. Other studies have similarly not observed a significant association between cord α -tocopherol and birth outcomes (28, 30). Interestingly, Ghebremeskel et al (28) examined several micronutrients in cord blood and, consistent with our results, determined that whereas cord blood vitamin A was significantly associated with birth outcomes, cord vitamin E was not. Whereas some studies have found no evidence of a significant association between maternal α -tocopherol and birth outcomes (21, 22, 24), others

have suggested a relation similar to that seen in our study (25, 26, 31). A study of >1200 pregnant women concluded that maternal plasma α -tocopherol concentrations at study entry and at week 28 of gestation were associated with increased BW (26). Another study that measured intakes of multiple micronutrients determined that maternal vitamin E intake had a significant, positive association with BW after control for relevant covariates (25). Finally, an examination of maternal serum vitamins E and C showed a positive trend between maternal vitamin E and birth outcomes, but the association was not significant (31).

The results of our analyses showed an association between individual lipid-soluble micronutrients (α -tocopherol and retinol) and birth outcomes (BW and HC). The mechanisms by which these micronutrients might affect fetal growth are not well understood; however, it is known that vitamin A (retinol) plays an important role in cell proliferation and differentiation in embryonic development (32). Through interactions with nuclear receptors, retinoic acid, the biologically active form of vitamin A, can alter gene transcription (19). Therefore, we hypothesized that vitamin A affects the birth-outcome measures included in this study. We had also hypothesized that carotenoids could affect fetal growth, because several (eg, α -carotene, β -carotene, and β -cryptoxanthin) are precursors to retinol (33); however, we did not observe an association between these compounds and birth outcomes. Additionally, vitamin A, the carotenoids, and vitamin E possess antioxidant activity and protect tissues and cells by reacting with oxygen free radicals (14). Animal studies have shown that reactive oxygen species and oxidative stress are associated with poor fetal growth (34, 35), and evidence from studies of pregnant women suggests that oxidative stress plays a role in low birth weight (36, 37). Compounds with antioxidant activity may help to protect the developing fetus and could therefore play a role in fetal growth and development. For vitamin E, a further suggested mechanism relates to the ability of vitamin E to increase vasodilation through its influence on prostacyclin release, which leads to increased blood flow to the fetus (26). Increased blood flow and nutrient supply could affect birth outcomes and may explain a relation between vitamin E and fetal growth. However, in addition to these potential mechanisms, it is also important to consider that these micronutrients may be markers for another underlying factor, even another dietary factor, affecting birth outcomes. These findings may import to development and health in later life because it has been shown that low birth weights, even those within the normal range (≥ 2500 g), are associated with poor outcomes (1, 3).

Exploration of the factors that influence interindividual susceptibility to environmental exposures, such as nutrition, is an important area of research. It was our original hypothesis that nutritional status would modify the association between environmental pollutants and birth outcomes. We hypothesized that this interaction may be related to the ability of these lipid-soluble micronutrients to detoxify PAHs. It has been shown that metabolites of PAHs can generate reactive oxygen species and are associated with oxidative DNA damage (38–41). Because vitamins A and E and carotenoids have antioxidant activity and can interact with reactive oxygen species, these micronutrients may be able to offset the fetal toxicity of PAHs. However, in the present study we found no evidence that vitamin A or E status modifies the association between PAH exposure and birth outcomes, probably because the small sample size limited our power. In the future, this issue should be pursued further in a larger sample to enable the detection of this potential interaction. The results of the current analysis, however, suggest that retinol



and α -tocopherol concentrations and exposure to PAHs may be independent predictors of birth outcomes.

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REFERENCES

- Arias E, MacDorman MF, Strobino DM, Guyer B. Annual summary of vital statistics—2002. *Pediatrics* 2003;112:1215–30.
- Richards M, Hardy R, Kuh D, Wadsworth M. Birthweight, postnatal growth and cognitive function in a national UK birth cohort. *Int J Epidemiol* 2002;31:342–8.
- Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341:938–41.
- Metcoff J, Costiloe JP, Crosby W, et al. Maternal nutrition and fetal outcome. *Am J Clin Nutr* 1981;34:708–21.
- Jedrychowski W, Bendkowska I, Flak E, et al. Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland. *Environ Health Perspect* 2004;112:1398–402.
- Choi H, Jedrychowski W, Spengler J, et al. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. *Environ Health Perspect* 2006;114:1744–50.
- Tonne C, Whyatt R, Camann D, Perera F, Kinney P. Predictors of personal polycyclic aromatic hydrocarbon exposures among pregnant minority women in New York City. *Environ Health Perspect* 2004;112:754–9.
- Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, alpha-tocopherol, lutein/zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwave-length detection. *Clin Chem* 1994;40:411–6.
- Ross AC. Vitamin A and retinoids. In: Shiels ME, Olson JA, Shike M, Ross AC, eds. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Williams & Wilkins, 1999:305–27.
- Traber MG. Vitamin E. In: Shiels ME, Olson JA, Shike M, Ross AC, eds. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Williams & Wilkins, 1999:347–62.
- Yeum KJ, Ferland G, Patry J, Russell RM. Relationship of plasma carotenoids, retinol and tocopherols in mothers and newborn infants. *J Am Coll Nutr* 1998;17:442–7.
- Kiely M, Cogan PF, Kearney PJ, Morrissey PA. Concentrations of tocopherols and carotenoids in maternal and cord blood plasma. *Eur J Clin Nutr* 1999;53:711–5.
- Dison PJ, Lockitch G, Halstead AC, Pendray MR, Macnab A, Wittmann BK. Influence of maternal factors on cord and neonatal plasma micronutrient levels. *Am J Perinatol* 1993;10:30–5.
- Baydas G, Karatas F, Gursu MF, et al. Antioxidant vitamin levels in term and preterm infants and their relation to maternal vitamin status. *Arch Med Res* 2002;33:276–80.
- Dejmek J, Ginter E, Solansky I, et al. Vitamin C, E and A levels in maternal and fetal blood for Czech and Gypsy ethnic groups in the Czech Republic. *Int J Vitam Nutr Res* 2002;72:183–90.
- Gazala E, Sarov B, Hershkovitz E, et al. Retinol concentration in maternal and cord serum: its relation to birth weight in healthy mother-infant pairs. *Early Hum Dev* 2003;71:19–28.
- Herrera E, Ortega H, Alvino G, Giovannini N, Amusquivar E, Cetin I. Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutr* 2004;58:1231–8.
- Scaife AR, McNeill G, Campbell DM, Martindale S, Devereux G, Seaton A. Maternal intake of antioxidant vitamins in pregnancy in relation to maternal and fetal plasma levels at delivery. *Br J Nutr* 2006;95:771–8.
- Debiec C, Larondelle Y. Vitamins A and E: metabolism, roles and transfer to offspring. *Br J Nutr* 2005;93:153–74.
- van den Broek N, Kulier R, Gulmezoglu AM, Villlar J. Vitamin A supplementation during pregnancy. *Cochrane Database Syst Rev* 2002: CD001996.
- Tamura T, Goldenberg RL, Johnston KE, Cliver SP, Hoffman HJ. Serum concentrations of zinc, folate, vitamins A and E, and proteins, and their relationships to pregnancy outcome. *Acta Obstet Gynecol Scand Suppl* 1997;165:63–70.
- Jagadeesan V, Prema K. Plasma tocopherol and lipid levels in mother and umbilical cord; influence on birth weight. *Br J Obstet Gynaecol* 1980;87:908–10.
- Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. *BMJ* 1999;319:339–43.
- Mathews F, Youngman L, Neil A. Maternal circulating nutrient concentrations in pregnancy: implications for birth and placental weights of term infants. *Am J Clin Nutr* 2004;79:103–10.
- Lagiou P, Tamimi RM, Mucci LA, Adami HO, Hsieh CC, Trichopoulos D. Diet during pregnancy in relation to maternal weight gain and birth size. *Eur J Clin Nutr* 2004;58:231–7.
- Scholl TO, Chen X, Sims M, Stein TP. Vitamin E: maternal concentrations are associated with fetal growth. *Am J Clin Nutr* 2006;84:1442–8.
- Rondo PH, Abbott R, Tomkins AM. Vitamin A and neonatal anthropometry. *J Trop Pediatr* 2001;47:307–10.
- Ghebremeskel K, Burns L, Burden TJ, et al. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. *Early Hum Dev* 1994;39:177–88.
- von Mandach U, Huch R, Huch A. Maternal and cord serum vitamin E levels in normal and abnormal pregnancy. *Int J Vitam Nutr Res* 1994;64:26–32.
- Chan DK, Lim MS, Choo SH, Tan IK. Vitamin E status of infants at birth. *J Perinat Med* 1999;27:395–8.
- Lee BE, Hong YC, Lee KH, et al. Influence of maternal serum levels of vitamins C and E during the second trimester on birth weight and length. *Eur J Clin Nutr* 2004;58:1365–71.
- Clagett-Dame M, DeLuca HF. The role of vitamin A in mammalian reproduction and embryonic development. *Annu Rev Nutr* 2002;22:347–81.
- Stahl W, Sies H. Bioactivity and protective effects of natural carotenoids. *Biochim Biophys Acta* 2005;1740:101–7.
- Ishimoto H, Natori M, Tanaka M, Miyazaki T, Kobayashi T, Yoshimura Y. Role of oxygen-derived free radicals in free growth retardation induced by ischemia-reperfusion in rats. *Am J Physiol* 1997;272:H701–5.
- Saito K, Maeda M, Yoshihara H, Amano K, Nishijima M, Nakamura K. Effect of SOD-mimetic Fe-chlorine e6-Na on the level of brain lipid peroxide of rat fetal brains exposed to reactive oxygen species leading to intrauterine growth retardation. *Biol Neonate* 2000;77:109–14.
- Karowicz-Bilinska A, Suzin J, Sieroszewski P. Evaluation of oxidative stress indices during treatment in pregnant women with intrauterine growth retardation. *Med Sci Monit* 2002;8:CR211–6.
- Scholl TO, Stein TP. Oxidant damage to DNA and pregnancy outcome. *J Matern Fetal Med* 2001;10:182–5.
- Burdick AD, Davis JW, Liu KJ, et al. Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. *Cancer Res* 2003;63:7825–33.
- Wu MT, Pan CH, Huang YL, Tsai PJ, Chen CJ, Wu TN. Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. *Environ Mol Mutagen* 2003;42:98–105.
- Yu D, Berlin JA, Penning TM, Field J. Reactive oxygen species generated by PAH o-quinones cause change-in-function mutations in p53. *Chem Res Toxicol* 2002;15:832–42.
- Seike K, Murata M, Oikawa S, Hiraku Y, Hirakawa K, Kawanishi S. Oxidative DNA damage induced by benz[a]anthracene metabolites via redox cycles of quinone and unique non-quinone. *Chem Res Toxicol* 2003;16:1470–6.