

## Thyroid and Pregnancy

# Maternal, Infant, and Delivery Factors Associated with Neonatal Thyroid Hormone Status

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**Background:** Thyroid function is dynamic during the perinatal period with many factors potentially influencing maternal, fetal and neonatal TSH and thyroid hormone levels. We sought to identify the impact of numerous maternal, fetal and delivery attributes on thyroid parameters in newborns.

**Methods:** This was a cross sectional study of 300 newborns. Detailed information was obtained from medical records and multiple characteristics from the record were tested as predictors of cord blood serum total T<sub>4</sub>, free T<sub>4</sub> and TSH and infant T<sub>4</sub> levels from the Maryland newborn screening program.

**Main outcome:** Outcomes are levels of thyroid stimulating hormone (TSH), thyroxine (T<sub>4</sub>), and free T<sub>4</sub> in newborn cord serum and total T<sub>4</sub> in postnatal heelstick bloodspot samples.

**Results:** Multivariate models identified a number of variables that are independently associated with thyroid hormone levels: higher birth order (lower cord TSH); older maternal age (lower cord total T<sub>4</sub>); pregnancy-induced hypertension and/or preeclampsia (lower cord total T<sub>4</sub> and free T<sub>4</sub>); gestational diabetes (higher cord free T<sub>4</sub>); sexually transmitted disease during pregnancy (lower cord TSH); alcohol use during pregnancy (lower cord TSH); thyroid condition/medications (higher bloodspot total T<sub>4</sub>, both neonatal and subsequent); Asian ancestry (higher cord TSH); male sex (higher TSH and lower neonatal bloodspot total T<sub>4</sub>); and C-section (lower cord TSH). Gestational age was independently associated with lower cord TSH, higher cord total T<sub>4</sub>, and higher neonatal and subsequent bloodspot total T<sub>4</sub>.

**Conclusions:** Fetal and newborn thyroid hormone levels during the perinatal period are dynamic and influenced by several biological and delivery related factors. Efforts to identify fetal thyroid disruptors in late gestation must carefully consider these factors.

### Introduction

THE CONCERN ABOUT CHEMICALS IN THE environment that may impact thyroid hormones is largely centered around the potential for adverse impacts on the health of children. Among the many exposures that may be of concern, there are a number of chemicals classified by toxicologists as having the potential to disrupt normal endocrine function. Dozens of chemicals have been found in animal studies to have some kind of thyroid-modulating effect (1). Prenatal exposure to naturally occurring and anthropogenic xenobiotics may impact thyroid function by altering hormone transport, metabolism, or other aspects of normal function (2). Toxicological models and limited epidemiological studies have indicated that many

organohalogen aromatic compounds, including dioxins, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), may be classified as "endocrine disruptors," as they have the potential to alter normal thyroid function (2–4). During development, these subtle changes may have more deleterious and permanent consequences as compared with similar exposures in adults (5).

Multiple targets and mechanisms have been proposed to evaluate the impact of exposure to thyroid-disrupting compounds in the environment. In addition to the challenge of identifying the most sensitive targets and most relevant biological mechanisms, studying the impact of endocrine-disrupting chemicals in children is complicated by their rapid growth and development. This results in "windows of

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vulnerability," which are certain critical points during development when children may be more sensitive to adverse effects. For example, thyroid hormone is essential for proper development of the central nervous system. During brain development, thyroid hormones assist in migration, proliferation, and differentiation of neuronal cells (1,6). Even subtle decreases in thyroid hormone level may affect the normal development of the brain if they occur during critical developmental windows (5,7). Therefore, both the presence and the timing of alterations in the concentration of thyroid-related hormones may be associated with overt neurological deficiencies (8).

Children, particularly disadvantaged children, can suffer disproportionately from multiple threats. Too often, exposures and other adverse conditions occur together. The same children who are in more contaminated environments may also be in environments where there is more substance abuse, more risks of injury, more poverty, inadequate nutrition, and more exposure to tobacco smoke. Because environmental exposures rarely occur singly, it is often difficult to disentangle a single exposure from other co-occurring environmental exposures and conditions that may confer risks upon children. Therefore, there is often the potential for confounding, in which both the exposure and the outcome under investigation are related to a third factor that may distort the observed exposure-disease relationship (9). In epidemiologic studies, it is critically important to identify, measure, and control for such confounders.

Many studies have explored the impact of maternal, fetal, and delivery characteristics on thyroid hormones at birth, mostly in the context of the design and implementation of newborn screening programs for congenital hypothyroidism (10) and in the context of public health programs to provide iodine supplementation (11–13). To assist in the design and conduct of an environmental epidemiology study to examine the relationship between exposures to PCBs, PBDEs, and neonatal thyroid hormone levels, we examined potential confounding factors by exploring the relationships between thyroid hormone levels and a variety of maternal, delivery, and infant-related factors.

To improve understanding derived from past studies, we conducted multivariate analyses to simultaneously examine the relationship between neonatal thyroid hormone measures and an array of maternal, delivery, and infant factors that have been explored in prior studies. This identifies factors that would need to be measured and addressed in studies of environmental thyroid hormone disruptors.

## Methods

### *Study population*

We conducted a cross-sectional study (the Baltimore THREE Study) of newborn deliveries at the Johns Hopkins Hospital in Baltimore, MD. The THREE Study was designed to examine the associations with exposures to a number of toxic chemicals, thyroid hormone levels, and birth outcomes. (This paper presents determinants of thyroid hormone levels and subsequent papers will examine associations with chemical exposures.) Umbilical cord blood samples were collected from the Johns Hopkins Hospital between November 26, 2004, and March 16, 2005. Over the course of the study

period, 609 live births occurred at the Johns Hopkins Hospital, of which 597 were singleton births. We obtained cord blood specimens from 341 of these, of which 41 had insufficient volume for laboratory analyses and were excluded from this study. We conducted a brief survey of hospital personnel to understand the major reasons for missed specimen collection. The most common explanations for missed collection included: complications during delivery, premature birth and/or small size of the infant resulting in small quantity of available cord blood, and logistical factors such as understaffing. The newborns who were not included had somewhat lower gestational ages and birth weights. In addition, factors associated with insufficient blood volumes collected were more likely to be born preterm, first born, and births of younger mothers.

### *Data collection*

The Baltimore THREE study has the approval of the Maternal and Fetal Research Committee in the Department of Gynecology and Obstetrics and the Institutional Review Board (IRB) at the Johns Hopkins University School of Medicine and a Health Insurance Portability and Accountability (HIPAA) waiver. The requirement for informed consent was waived by the IRB because drawing blood from the placenta involves no more than minimal risk to the subjects and biosamples collected would otherwise have been discarded. Strict safeguards preserving patient confidentiality were established. Members of a community advisory committee, who were selected for their specific knowledge and expertise, as well as their focus on important child health concerns in Maryland, had the opportunity to learn about and comment on this study and will be informed about the results.

Nurses or physicians who routinely collect cord blood specimens for clinical purposes obtained cord blood from the umbilical vein using a syringe following newborn delivery but prior to the delivery of the placenta. The cord blood was transferred from the syringe to silicon-coated (contaminant screened) Vacutainer® tubes that were temporarily stored for a maximum of 2 hours at 4°C. The tubes were centrifuged to attain a force of 1000 g and the serum was transferred by pipette into smaller tubes for storage at –80°C. They were shipped on dry ice to a commercial clinical laboratory for analysis.

For 300 study participants from whom we had obtained at least 5.2 mL of cord serum, we attempted to measure three thyroid hormone parameters in serum from cord blood samples: thyroid stimulating hormone (TSH), total thyroxine (T<sub>4</sub>), and free T<sub>4</sub> in cord blood. The cord blood hormone indices were measured by Quest Diagnostics (Baltimore, MD). TSH was measured using the ADVIA Centaur TSH assay; total T<sub>4</sub> was analyzed using the Microgenics/CEDIA® immunoassay; and free T<sub>4</sub> was quantified using the Centaur/Competitive Chemiluminescent® immunoassay. Three TSH samples and two total and free T<sub>4</sub> samples were lost during sample preparation, leaving 297 and 298 available for cord blood TSH and T<sub>4</sub> analyses, respectively.

With approval from the Maryland Department of Health and Mental Hygiene (MdDHMH) Institutional Review Board, we were able to obtain the results of total T<sub>4</sub> concentrations measured in bloodspots from heelstick capillary blood samples that were collected on dried filter paper as a

part of the Maryland newborn screening program. Maryland is a primary  $T_4$  screening program and TSH levels are not routinely determined unless  $T_4$  levels are abnormal. These neonatal bloodspot samples are collected by hospital staff, just prior to hospital discharge. During the time of the study, total  $T_4$  was measured using a radioimmunoassay system. Initial postnatal total  $T_4$  results were available for 275 of the 300 samples that were sent for cord blood analyses. According to MdDHMH protocol, it is recommended that newborns have an additional total  $T_4$  bloodspot measurement during the first routine pediatric visit. These results are also stored at the MdDHMH and this additional "follow-up" measurement was abstracted when it was available ( $n = 143$ ).

Two study investigators abstracted demographic information as well as relevant biological measures from the maternal medical records. We abstracted similar data from infants' records. Cross-tabulations were conducted and outliers were identified and reassessed, as part of a quality assurance/quality control protocol. Study clinicians re-reviewed a 10% random sample of the data. We derived gestational age from the best obstetric estimate. Maternal race was categorized into three categories: white, Asian, and African American. Four Hispanic babies were grouped with whites. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. We categorized BMI into four groupings, as specified by current U.S. Centers for Disease Control and Prevention (CDC) guidelines: underweight (BMI <18.5), normal (BMI 18.5–24.9), overweight (BMI 25.0–29.9), and obese (BMI  $\geq$ 30.0) (14). We determined smoking status using self-reported smoking during pregnancy on the medical record and supplemented with cotinine measurements. Women with cotinine measures greater than 10.0 ng/mL were coded as active smokers even if they were identified as nonsmokers (15). We dichotomized the number of previous live births as zero compared to one or more. A number of medical conditions were abstracted from the chart. Prob-

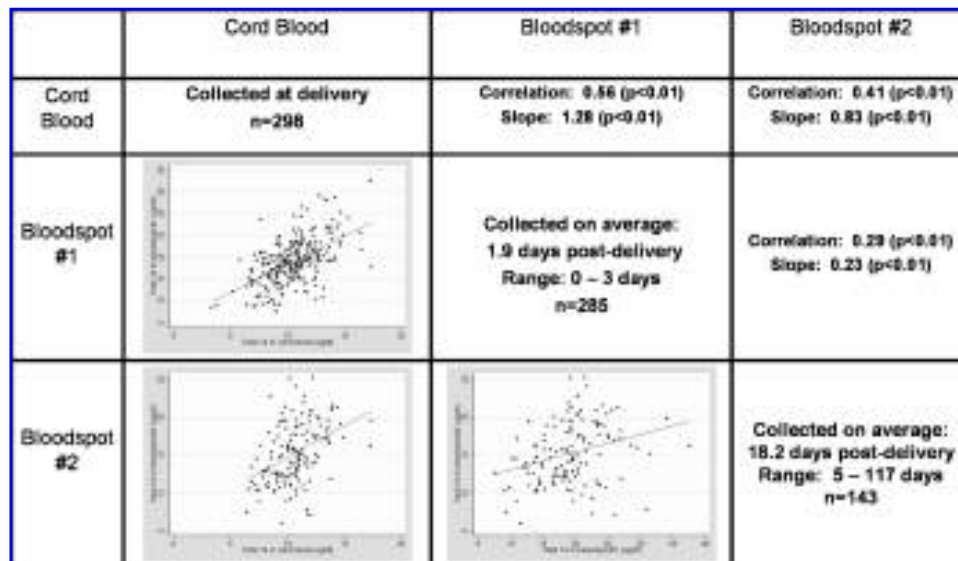
lem lists and medications recorded on the electronic record were abstracted to ascertain diagnoses of maternal hypertension, gestational diabetes, and thyroid disorders; recreational use of drugs and alcohol also were abstracted from the electronic record.

#### Statistical analysis

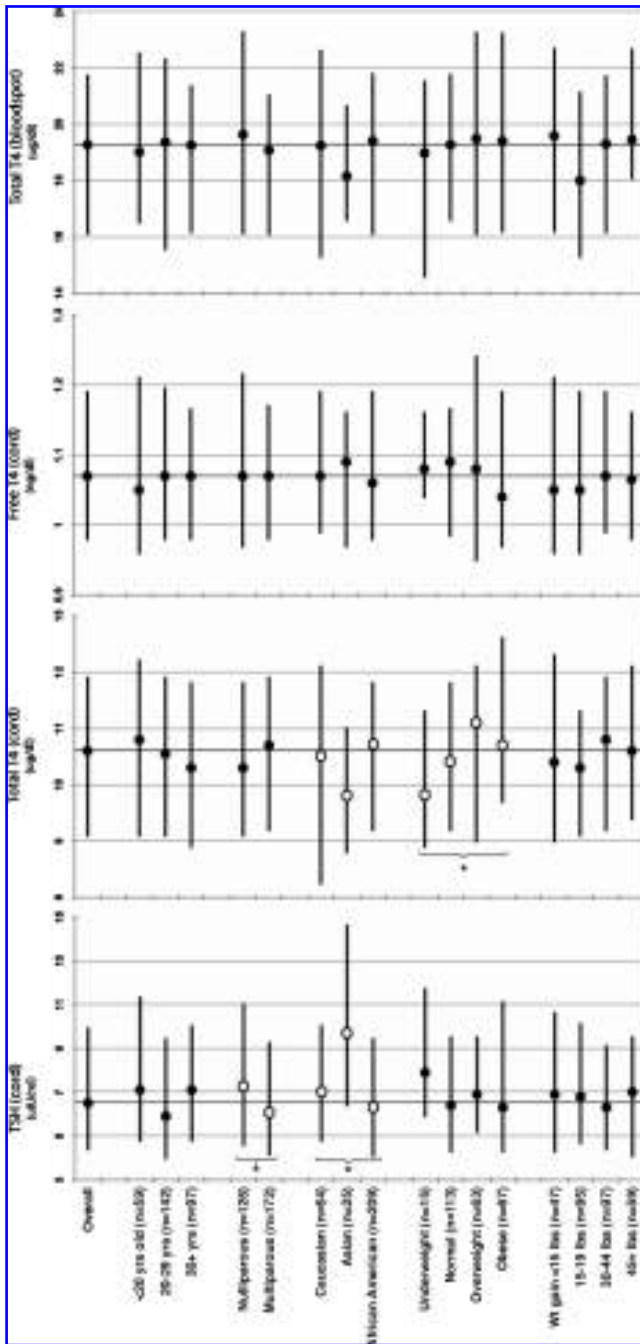
We used Pearson's correlation and simple linear regression to evaluate the relationship between total  $T_4$  measurements. The Kruskal Wallis test was used to detect difference in median and analysis of variance (ANOVA) in mean hormone levels by groups defined by perinatal factors. Using simple and multivariate linear regression, each thyroid measurement was modeled using the parameters described in Figures 1 through 5. Variables were excluded only if they were co-linear with another covariate, based on the variance inflation factor; in this case, the factor that described more of the variation (as indicated by the  $R^2$  value) in the outcome measure was retained in the final model. TSH was log-adjusted to comply with assumptions of normality of a dependent variable. Multivariate analyses were conducted to examine the simultaneous impact of maternal, delivery, and infant factors on neonatal TSH and thyroid hormone levels. The predictors were first examined separately in two strata: maternal and delivery/infant combined (models not shown) to select candidates for the final models. Likelihood ratio tests guided the selection of the best fitting models. Regression coefficients and 95% confidence intervals (95% CI, corresponding to  $\alpha = 0.05$ ) are presented.

#### Results

The median TSH measurement from cord blood in this population was 6.5  $\mu$ IU/mL (range: 1.3–37.5) and TSH levels were log-normally distributed. The median free  $T_4$  measurement in cord blood was 1.07 ng/dL (range: 0.61–1.70).

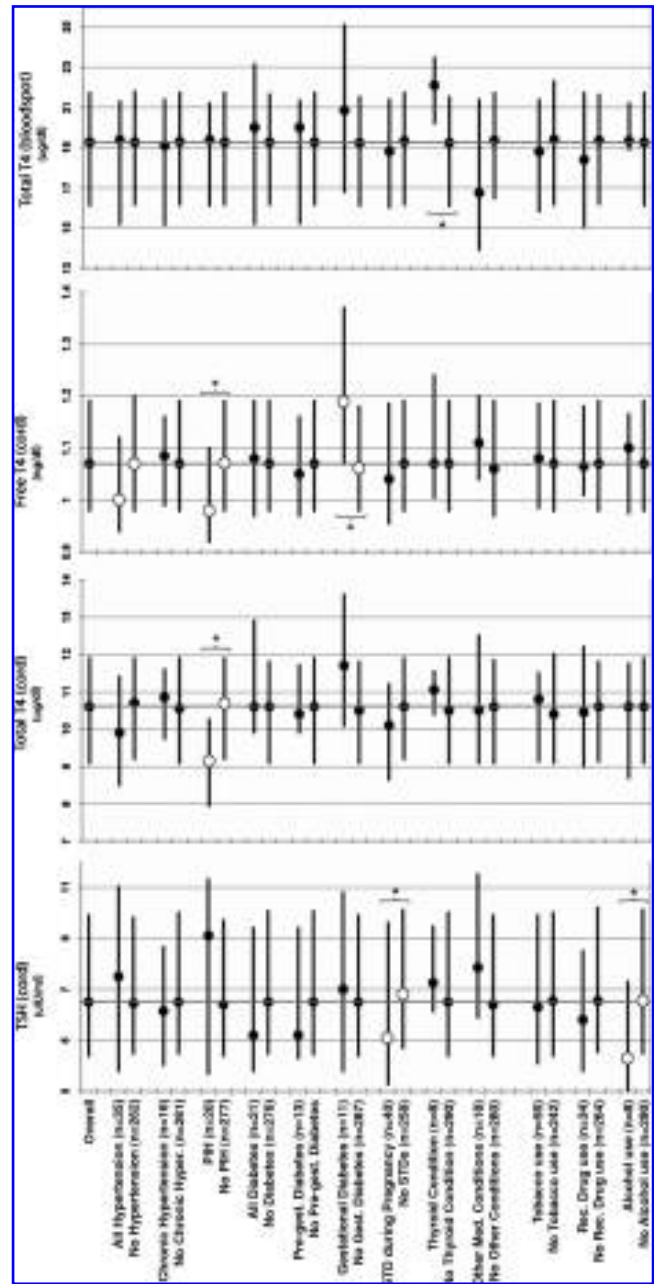


**FIG. 1.** Baltimore THREE Study (2004–2005) neonatal total thyroxine ( $T_4$ ) measurements collected at delivery (cord blood) and postdelivery initial (bloodspot #1) and follow-up (bloodspot #2) levels. Data and bivariate linear regression lines are plotted and linear coefficients and slopes (and associated  $p$  values) are presented.



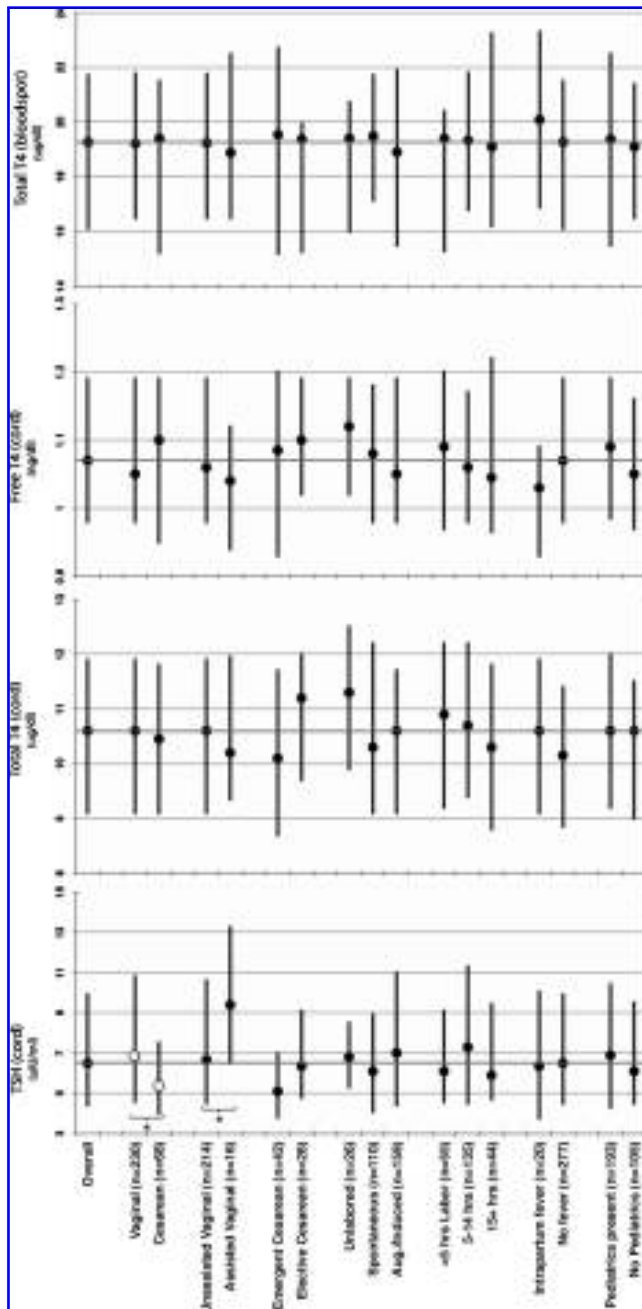
**FIG. 2.** Baltimore THREE Study (2004–2005) neonatal thyroid measurements and maternal demographics. Unadjusted, medians, and IQRs are presented, where IQR = interquartile range, the range from the 25th to 75th percentile. Red lines indicate overall median values. Hollow circles indicate where means among categories are different from one another at  $p < 0.05$  using ANOVA. Asterisks where medians among categories are different from one another at  $p < 0.05$  using the Kruskal Wallis test.

We measured total  $T_4$  twice for the majority of this population (once in cord blood and once in the neonatal bloodspot sample); for a subset, we have an additional subsequent bloodspot total  $T_4$  measurement. Figure 1 presents the total  $T_4$  summary statistics, timing of collection, and relationships



**FIG. 3.** Baltimore THREE Study (2004–2005) neonatal thyroid measurements and maternal medical conditions and substance use. Unadjusted, medians, and IQRs are presented, where IQR = interquartile range, the range from the 25th to 75th percentile. Red lines indicate overall median values. Hollow circles indicate where means among categories are different from one another at  $p < 0.05$  using ANOVA. Asterisks where medians among categories are different from one another at  $p < 0.05$  using the Kruskal Wallis test.

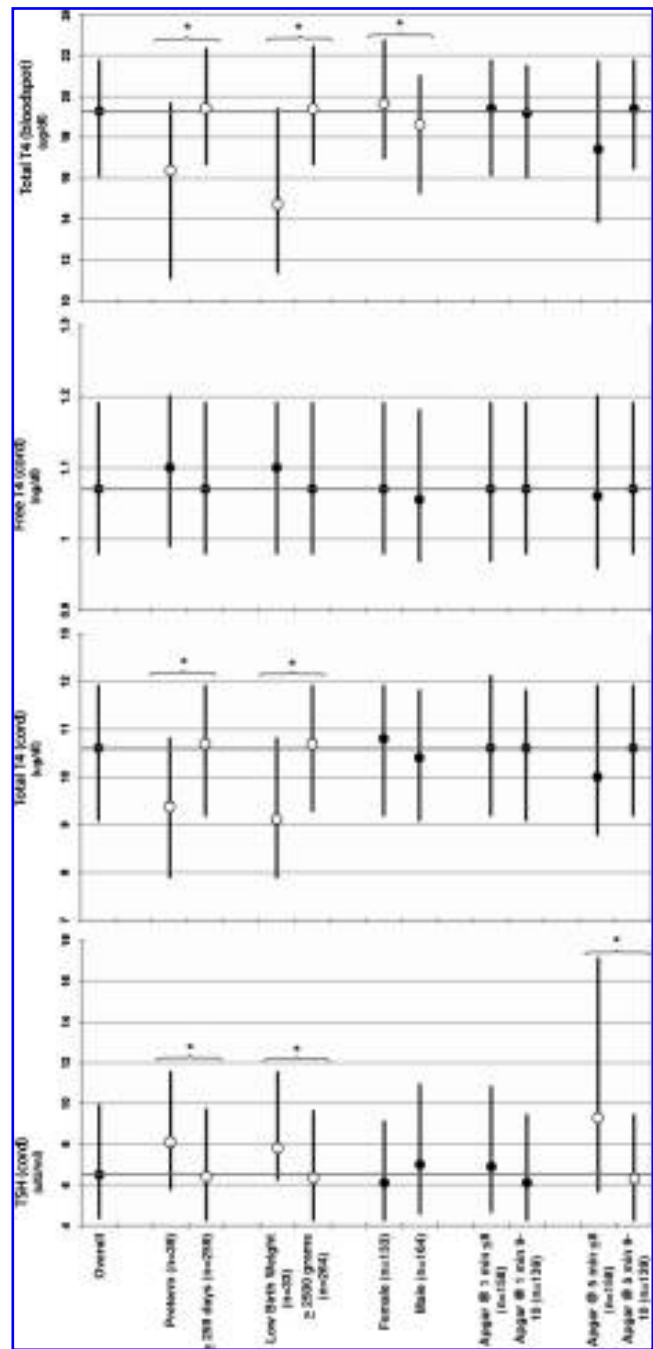
between the measurements. All total  $T_4$  measurements were positively correlated with one another. The average increase in  $T_4$  from cord to bloodspot 1 was  $8.66 \mu\text{g/dL}$  ( $\text{SD} = 4.16 \mu\text{g/dL}$ ). Because the interval between the collection of the first and second bloodspots was variable, the difference between these samples is not reported.



**FIG. 4.** Baltimore THREE Study (2004–2005) neonatal thyroid measurements and delivery factors. Unadjusted, medians, and IQRs are presented, where IQR = interquartile range, the range from the 25th to 75th percentile. Red lines indicate overall median values. Hollow circles indicate where means among categories are different from one another at  $p < 0.05$  using ANOVA. Asterisks where medians among categories are different from one another at  $p < 0.05$  using the Kruskal Wallis test.

*Maternal factors*

Figure 2 presents the distribution (median and interquartile range) of thyroid hormone levels by maternal demographic characteristics using univariate analyses. Umbilical cord serum levels of TSH and total and free  $T_4$  were not associated



**FIG. 5.** Baltimore THREE Study (2004–2005) neonatal thyroid measurements and infant factors. Unadjusted, medians, and IQRs are presented, where IQR = interquartile range, the range from the 25th to 75th percentile. Red lines indicate overall median values. Hollow circles indicate where means among categories are different from one another at  $p < 0.05$  using ANOVA. Asterisks where medians among categories are different from one another at  $p < 0.05$  using the Kruskal Wallis test.

with maternal age (modeled both continuously and categorically) or weight gain during pregnancy. Infants of mothers delivering their first child had higher TSH levels as compared to mothers who were multiparous. Infants of Asian mothers had higher TSH levels and lower mean total cord  $T_4$  levels as

compared to whites and African Americans. Babies whose mothers had low BMI (underweight) had lower total  $T_4$  in cord blood. There were no detectable differences in free  $T_4$  levels by maternal demographic characteristics.

Figure 3 presents median thyroid hormone levels in newborns by the presence and absence of a number of maternal medical conditions and maternal substance use using univariate analyses. Umbilical cord serum levels of TSH and total and free  $T_4$  were not associated with maternal diagnosis of pregestational hypertension, pregestational diabetes, "other" medical conditions, tobacco use, and reported recreational drug use. Infants of mothers with pregnancy-induced hypertension and/or preeclampsia had a significant decrease in total and free  $T_4$  in umbilical cord blood. Among mothers with gestational diabetes, there was a significant increase in cord serum free  $T_4$  levels. Infants of mothers who reported a diagnosis of a sexually transmitted disease (STD) during pregnancy had lower TSH levels in cord blood. There was an increase in median bloodspot total  $T_4$  among infants born to mothers with histories of thyroid conditions and/or use of thyroid medications, although the number of participants in this category is low ( $n = 8$ ) and clinical confirmation was not possible. Alcohol use during pregnancy was related to a significant reduction in cord serum TSH but not with any changes in total or free  $T_4$ .

#### *Delivery factors*

Figure 4 presents median thyroid hormone levels by delivery factors using univariate analyses. Umbilical cord serum levels of TSH and total and free  $T_4$  were not associated with most delivery factors that were examined, including, whether there was labor, duration of labor, detection of intrapartum fever, and whether a pediatrician was called to the delivery (a possible measure of stress). However, babies born via vaginal delivery had higher levels of TSH in cord blood. Moreover, within the group of infants born via vaginal delivery, those with assisted deliveries had higher median TSH levels as compared with unassisted deliveries. The group of infants born via elective C-sections had higher TSH levels of borderline statistical significance ( $p = 0.095$  for means and  $p = 0.055$  for medians).

#### *Infant factors*

Figure 5 presents the impact of infant factors on thyroid hormone levels in the current study population using univariate analyses. Infants born preterm had higher levels of TSH and lower total  $T_4$  (as measured both in cord blood and bloodspots). These relationships were consistent when gestational age was examined on a continuous scale (data not shown). Likewise, infants who were born with low birth weight (<2500 g) also had higher TSH and lower total  $T_4$  values at both time points. When birth weight was adjusted for gestational age, it was not independently related to TSH but was associated with a smaller increase in total  $T_4$  in cord blood (data not shown). Males had lower total  $T_4$  levels (in bloodspots). Apgar scores at 1 minute were not related to neonatal thyroid hormone levels. However, infants with lower Apgar scores at 5 minutes had higher TSH values. Additionally, there were significant interactions between infant sex and Apgar at 5 minutes with total and free  $T_4$ , as well as with total  $T_4$  measured in bloodspots, such that among girls only, Apgar scores

at 5 minutes were positively associated with total and free  $T_4$  in cord blood and total  $T_4$  in bloodspots (data not shown).

#### *Multivariate analyses*

The results of the multivariate models are shown in Table 1. In the best fitting model for TSH, the inclusion of all predictive covariates from the maternal and delivery/infant models explained 15.9% of the total variation. Multiparity, reporting an STD during pregnancy, alcohol use during pregnancy, and birth by cesarean section were independently associated with having lower cord blood TSH levels; Asian race, birth by elective cesarean (as compared to emergent), male sex of the baby, and preterm delivery were independently associated with higher cord blood TSH levels.

For total  $T_4$  in cord blood, 10.3% of the variation was explained by the best fitting multivariate model. Preeclampsia/pregnancy-induced hypertension, spontaneous or induced labor (as compared to unlabored deliveries), earlier gestational age, and older maternal age were independently associated with lower umbilical cord total  $T_4$  levels. Based on the covariates we measured, there were very few predictors that had any influence on free  $T_4$  levels in a multivariate model. Among the significant predictors, preeclampsia/pregnancy-induced hypertension was associated with lower free  $T_4$  and gestational diabetes was associated with higher free  $T_4$ .

The multivariate model explained 15.3% of the variation in total  $T_4$  levels in neonatal bloodspots; newborns with mothers having a current or historical thyroid condition and/or use of thyroid medications had higher (although not statistically significant) total  $T_4$  levels in bloodspots; those with shorter gestational age, boys, and those who were not first born had lower levels. Total  $T_4$  measured in initial neonatal bloodspot samples increased as the time between heelstick and birth increased. In the subsequent bloodspot samples taken at the first routine pediatric visits, longer gestation and maternal historical or current thyroid conditions were associated with increased total  $T_4$  levels. In these pediatric bloodspot samples, the time between birth and sample collection was associated with decreased total  $T_4$ .

## **Discussion**

#### *Maternal factors*

Compared to many of the other potential influences on neonatal thyroid function, maternal demographic factors have received very little attention. This is of particular concern for environmental health studies because exposures may vary greatly by demographic characteristics, establishing the potential for confounding if these factors are not properly identified, assessed, and controlled. In both univariate and multivariate models, maternal race was associated with TSH levels in cord blood higher for infants of Asian mothers, although the reason for this association is unclear and one earlier study found no association with maternal race (16). The finding that TSH levels were higher among first born children was reported in one prior publication (17). This may be a potential confounding factor for environmental exposures as some persistent chemicals have been found to be present in higher levels in first born children than in subsequent births (18).

A number of maternal conditions, including hypertension, preeclampsia, cardiac, renal, hepatic, respiratory, immuno-

TABLE 1. FACTORS ASSOCIATED WITH NEONATAL THYROID LEVELS (MULTIVARIATE MODELS) (N=298), BALTIMORE THREE STUDY, 2004–2005<sup>a</sup>

	Reference group	$\beta$	95% CI
TSH (log transformed, $\mu\text{IU/mL}$ ) in cord blood ( $R^2 = 15.9$ )			
Multiparous women	Nulliparous women	-0.15	-0.29 to -0.01
STD during pregnancy	No STD during pregnancy	-0.17	-0.37 to 0.03
Asian maternal race	Caucasian race	0.29	0.01 to 0.56
Alcohol use during pregnancy	No alcohol use during pregnancy	-0.45	-0.89 to -0.01
Cesarean section	Vaginal birth	-0.34	-0.54 to -0.15
Elective cesarean	Emergent cesarean	0.29	-0.01 to 0.58
Preterm birth	$\geq 259$ days gestation	0.22	0.02 to 0.42
Boys	Girls	0.14	0.01 to 0.27
Apgar of $\leq 8$ at 5 minutes	Apgar of 9–10 at 5 minutes	0.30	0.11 to 0.50
Total $T_4$ ( $\mu\text{g/dL}$ ) in cord blood ( $R^2 = 10.3$ )			
Preeclampsia or PIH	No preeclampsia or PIH	-0.85	-1.86 to 0.16
Maternal age	(continuous; per year of age)	-0.03	-0.07 to 0.00
Spontaneous labor	Unlabored	-0.95	-1.92 to 0.02
Induced labor	Unlabored	-1.30	-2.2 to -0.35
Gestational age	(continuous; per day of gestation)	0.04	0.02 to 0.06
Pediatrics at delivery	No pediatrics at delivery	0.38	-0.14 to 0.90
Free $T_4$ (ng/dL) in cord blood ( $R^2 = 3.0$ )			
Preeclampsia or PIH	No preeclampsia or PIH	-0.08	-0.16 to -0.01
Gestational diabetes	No gestational diabetes	0.10	0.00 to 0.20
Total $T_4$ ( $\mu\text{g/dL}$ ) in neonatal bloodspots ( $R^2 = 15.3$ )			
Time between bloodspot collection and birth	(continuous; per day since birth)	1.13	0.02 to 2.25
Multiparous women	Nulliparous women	-0.87	-2.0 to 0.26
Current or history of thyroid condition	No current or history of thyroid condition	2.76	-0.40 to 5.92
Gestational age	(continuous; per day of gestation)	0.10	0.06 to 0.14
Boys	Girls	-1.59	-2.65 to -0.53
Total $T_4$ ( $\mu\text{g/dL}$ ) in subsequent bloodspots ( $R^2 = 10.1$ )			
Time between bloodspot collection and birth	(continuous; per day since birth)	-0.04	(-0.08 to 0.00)
Gestational age	(continuous; per day of gestation)	0.07	(0.02 to 0.12)
Current or history of thyroid condition	No current or history of thyroid condition	3.23	(-0.63 to 7.10)

<sup>a</sup>TSH = thyrotropin; STD = sexually transmitted disease;  $T_4$  = thyroxine; PIH = pregnancy induced hypertension. Italics represent 95% CIs that do not include the null value (1)

logic conditions, and HIV have not been found to be associated with neonatal thyroid hormone status either in this or in prior studies (16,19–21). We found evidence for an association between hypertension of pregnancy and/or preeclampsia and lower cord  $T_4$  levels (both total and free). However, prior studies have not examined this relationship with the exception of a paper in which authors hypothesized that maternal diseases that impact placental dynamics, like preeclampsia, might influence neonatal  $T_4$  measurements (20).

Many studies have examined the impact of maternal diabetes or gestational diabetes on neonatal thyroid status. Most of these have found no association (10,16,20,22). However, few have controlled for other potentially confounding factors that may distort the observed association. Chan *et al.* (19) found that babies of diabetic mothers had higher cord blood TSH levels in multivariate but not univariate models. Studies that specifically examined gestational diabetes reported associations with higher TSH levels in cord blood (21,23). There

was speculation that this elevation might be a function of increased stress among newborns of mothers with gestational diabetes (23). In the current study, gestational diabetes was associated with higher free  $T_4$  levels in both univariate and multivariate models but not with changes in cord blood TSH or total  $T_4$ , although the number of mothers with maternal diabetes in this sample was small ( $n = 11$ ). We did not identify any other reports about gestational diabetes and free  $T_4$ , indicating that this issue needs further study.

We did not observe a relationship between maternal history of diabetes and infants' thyroid hormone levels. However, it should be noted that in our study, such past diagnoses could not be verified and were probably less accurate than more proximate illnesses such as gestational diabetes. Additionally, we were not able to capture medical conditions that were not recorded in the patient's records, leading to possible misclassification. Past findings regarding chronic diabetes have been equivocal. A number of studies have not found any relationship

between diabetes and cord TSH levels (20,22,24). A limitation of these studies is that the analyses were conducted without adjustment for potential confounding factors. This is illustrated by *Chan et al.* (19) who found increased cord blood TSH levels in infants of diabetic mothers in multivariate, but not in univariate, models. There also have been inconsistent results regarding the impact of diabetes on  $T_4$  measurements, such that *Wilker et al.* (22) found lower  $T_4$  levels measured at various points during the postnatal period (cord blood, 2 hours, 12 hours, and 72 hours after delivery) among infants of diabetics. An early study by *Erenberg* (10) found no difference between  $T_4$  measured in cord blood among infants of diabetic and nondiabetic mothers.

In our population, women with STDs during pregnancy had lower TSH levels, an association that persisted in both univariate and multivariate models. This relationship has not been examined in other study populations and it is unclear why TSH levels would be lower among these women.

Maternal behavioral factors including nutrition and substance use/abuse may also impact thyroid function. Iodine intake is among the most important nutritional factors influencing thyroid hormone level, as it is required for thyroid hormone synthesis (25). When dietary iodine is insufficient, compensatory mechanisms enable the thyroid gland to hoard more of the available iodine and more efficiently reuse iodide that is released when  $T_4$  is converted to triiodothyronine ( $T_3$ ) (26). In spite of this compensation, thyroid hormone concentrations are affected by iodine deficiency, such that total and free  $T_4$  are decreased and both  $T_3$  and TSH are normal or increased (26). During pregnancy, iodine availability is especially important due to the increased demand placed on the maternal thyroid system (25). It has been shown that maternal iodine deficiency is related to higher TSH concentrations in the newborn, such that, at a population level, the cumulative TSH shifts to the right as the severity of iodine deficiency increases (11,17). Iodine may also be associated with environmental exposures since there is geographic variability in iodine in the environment which, at least in some parts of the world, leads to variability in iodine intake. Although it is controversial how important this factor is in the United States, it is nonetheless a limitation of this study that no iodine measures were available.

This study and several previous studies have reported no relationship between smoking during pregnancy and TSH, total  $T_4$ , or free  $T_4$  as measured in either cord blood or neonatal bloodspots (27–29). A study examining the effect of drug abuse (marijuana and/or cocaine) during pregnancy on neonatal thyroid parameters found no associations (30). Consistent with our findings, they reported that alcohol use during pregnancy was related to a reduction in TSH (30). An additional study found no relationship with alcohol use and neonatal  $T_4$  measured in bloodspots, which also is consistent with our results (29). The possible relationship between TSH and alcohol intake is potentially important to the design of environmental studies since alcohol can affect metabolism, and therefore levels of exposure to many xenobiotic substances.

#### Delivery factors

The majority of studies, including ours, have found that vaginal deliveries result in higher average TSH cord levels (16,23,31–33). This may be related to the observation that

stress during delivery seems to be associated with elevated TSH in cord blood (34,35). However, not all studies that have examined delivery mode have produced consistent findings. Some report that TSH in cord blood is not influenced by mode of delivery (10,28,36) and one concluded that deliveries via cesarean section result in higher TSH measured in neonatal bloodspots (37). In a few studies, the researchers were able to classify the deliveries further, dividing cesarean section into elective C-section and emergent C-section and dividing vaginal deliveries into spontaneous vaginal deliveries and instrumental vaginal deliveries. Consistent with our findings, one study concluded that babies born via elective C-sections had higher average cord TSH levels (34). Two other studies reported that instrumental vaginal deliveries had higher cord blood TSH values than spontaneous vaginal deliveries (31,33). In our study, the highest average cord blood TSH levels were among “assisted” vaginal births, consistent with the notion that stress during vaginal delivery may lead to higher TSH levels in cord blood.

In our study, we found a slight decrease in total  $T_4$  in cord blood among women who underwent labor as compared to unlabored deliveries. Labor can be spontaneous or augmented and either can result in a vaginal or cesarean delivery. Women who deliver by elective C-section generally do not go into labor at all. Other studies have found that cesarean sections with and without labor have similar cord TSH levels, which is not consistent with our findings (19,38). The only study to explicitly study labor augmentation found that oxytocin use was not associated with either TSH or  $T_4$  levels measured in cord blood (36). Prior studies examining the duration of labor have reported inconsistent results; one found no association between labor duration and cord TSH or free  $T_4$  (36) and others found that longer duration of labor (particularly the second stage) was associated with increased cord TSH levels (31,39). We did not detect a significant association between labor duration and neonatal hormone levels.

Finally, other factors marking a stressful delivery have been linked to higher cord TSH levels, including malpresentation (32) and intrapartum fever (19). In our study, we found no relationship between TSH and intrapartum fever, but did detect a suggested reduction in total and free cord  $T_4$  in this subgroup.

It is possible that factors such as delivery mode, labor, augmentation of labor, and labor duration are potential confounding factors for thyroid hormone status and environmental exposures. However, there are no clear examples of this in the published literature. Of these factors, delivery mode (C-section vs. vaginal; elective C-section vs. emergent C-section) has a strong and consistent relationship to thyroid hormone status and should be considered a potential confounder in environmental studies.

#### Infant factors

We found that preterm babies had significantly lower total  $T_4$  levels in both cord blood and bloodspots, and a slight increase in TSH levels. It is known that as gestational age increases, the fetus increases the synthesis of both  $T_4$  and TSH (40). A study by *Klein et al.* (41) found a constant positive relationship between TSH and  $T_4$  until approximately 34 weeks gestation, at which time, none of the thyroid parameters varied with increasing age. Among infants who are

born preterm, the hypothalamic–pituitary axis development is attenuated and therefore the TSH surge for preterm births is smaller and later (42,43). At birth, term newborns typically experience a surge in TSH, peaking at around 30 minutes after delivery and followed by a gradual rise in  $T_4$  over the first 24 hours of life (44). In our study, there was a significant positive correlation between cord blood and both bloodspot levels of total  $T_4$ . It is known that there is a negative change in TSH and a positive change in  $T_4$  levels going from cord blood levels to neonatal heelstick bloodspot measurements (22). In our study, the timing of bloodspot samples was an important variable because there is an increased  $T_4$  in neonatal bloodspot samples compared to cord blood samples and a decreased  $T_4$  in subsequent bloodspot samples compared to neonatal blood spot samples. These dynamics are important for the design of environmental studies which may involve compounds that increase prematurity and also to understand the importance of timing of blood collection around the perinatal period.

Because birth weight is so highly correlated with gestational age, it is not surprising that birth weight is also positively correlated with TSH and thyroid hormone measures in univariate analyses. Other studies have reported an association between birth weight and TSH levels (45); but not independently of gestational age (19,46). In our study, when birth weight was adjusted for gestational age, there was still a slight positive association between birth weight and total  $T_4$  (in cord blood measurements). Thus, it is important to collect information on and to control for both birth weight and gestational age.

Consistent with our findings, most studies have reported that male infants generally have higher TSH levels, as measured in either cord blood or bloodspots as compared to females, a trend that is consistent with our analyses (17,33). Additionally, the Apgar score at 1 and 5 minutes have been reported to be inversely associated with cord blood and bloodspot TSH (17,23); however, we only observed such an effect for the 5 minute score. Adjusting for the 5 minute Apgar score might be one way to control for the potential confounding effect of intrapartum stress on TSH levels.

## Conclusion

During the perinatal period, TSH and thyroid hormone levels are dynamic. There are many factors that contribute to this variation. However, many are biologically interrelated, which makes meaningful interpretation of these measurements challenging. Many past studies examining these factors have not considered these complexities and instead have examined only one or two variables, which may lead to inferential errors. However, it is admittedly difficult to draw meaningful inferences from multivariate analyses that select covariates from many of the highly correlated maternal, delivery, and infant factors. Despite this limitation, there are trends that were robust to the analytic method in the analyses presented in this study population and among those presented in the literature.

One major limitation of our study is that we lacked measures of iodine nutrition. At a population level, the current prevalence of iodine deficiency in Baltimore is unknown. Despite the fact that table salt (sodium chloride) in the United States has been iodized for over 80 years, it has been

theorized that only approximately 15% of daily intake of salt is from table salt. Salt added to processed foods is a major source of salt in the U.S. diet and usually is not iodized (12). Therefore, we do not know whether iodine deficiency is responsible for any alteration in measured thyroid hormone levels in this population.

However, based on the literature and these analyses, it may be important to take variables such as race, sex, gestational age, birth weight, and mode of delivery under careful consideration when interpreting neonatal thyroid measurements clinically, for congenital hypothyroid screening, to monitor iodine deficiency in a population, and especially in the conduct of epidemiologic research that assesses thyroid hormone status in relationship to environmental exposures.

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