Associations of pregnancy characteristics with maternal and cord steroid hormones, angiogenic factors, and insulin-like growth factor axis

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Abstract

Background The objective of this study was to comprehensively profile biological factors in pregnancy that have been postulated to be important components of the in utero environment and may also have relevance to later susceptibility to cancer and other chronic diseases.

Methods Steroid sex hormones, IGFs, and angiogenic factors were measured in maternal and cord serum from term normotensive pregnancies. Spearman correlations and linear regression estimated relationships among the biological factors and clinical characteristics.

Results The analytes were generally not correlated between maternal and fetal circulations. However, significant correlations were demonstrated among several analytes within maternal or cord samples. A few analytes were associated with clinical characteristics (e.g., maternal IGF-1 and IGFBP-3 were inversely correlated with offspring birth weight, while maternal leptin and cord testosterone were positively correlated with this characteristic). Maternal androgens were higher in African-Americans than whites, and maternal PlGF and soluble fms-like tyrosine kinase-1 (sFlt-1) were higher in male than female offspring.

Conclusions There were significant correlations among analytes, but the patterns differed depending on whether they were measured in the maternal or fetal circulation. The number and magnitude of correlations among analytes, however, should affect the design and interpretation of future studies.

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Introduction

Alterations in steroid sex hormones, the insulin-like growth factor axis, and angiogenic proteins have been associated with pregnancy outcomes such as small-for-gestation-age infants [1] and preeclampsia [2–4]. For example, prior studies have shown that women who develop preeclampsia have highly elevated circulating levels of the anti-angiogenic proteins, soluble fms-like kinase 1 (sFlt-1) and soluble endoglin (sEng), both prior to and at clinical diagnosis of the disease [5–13]. Androgens (androstenedione and

testosterone) also have been shown to be higher in the maternal circulation in women with preeclampsia [2, 4, 14]. These endogenous in utero exposures have been associated with later disease susceptibility [15, 16], including cardiovascular health and cancer risk [17] in both the mother and the offspring [18].

Most studies that have examined endogenous in utero exposures with the aim of developing biological hypotheses to explain epidemiological associations of pregnancy complications with later risk of cancer have focused exclusively on steroid sex hormones [17, 19, 20] without examining the relationships of the steroid sex hormones to other analytes. Laboratory studies show estrogen can promote expression of vascular endothelial growth factor (VEGF), a potent pro-angiogenic factor, in female endometrium and breast tissue [21–26]. Therefore, concentrations of steroid sex hormones may be associated with angiogenic balance. In this report, we sought to examine a comprehensive set of biological factors that have been shown to be important in the maintenance and progression of pregnancy and also are associated with incidence of one or more types of cancer [3, 17, 27–30] to determine whether there are discernable patterns of association between these analytes in normal pregnancy. We also hypothesized that maternal and neonatal characteristics will correlate with the levels of these analytes during pregnancy, and thereby provide a more expansive profile from which to examine the in utero environment and its influence on subsequent risk of cancer or other adult health outcomes.

Methods

Study design and population

Participants included in the present analysis were controls from a case–control study of preeclampsia that was conducted at Louisiana State University Health Sciences Center at Shreveport, LA [31]. Controls in the original study were singleton pregnancies that remained normotensive and without proteinuria. Initially, only nulliparous women were recruited, but this criterion was relaxed later in the study. Exclusion criteria consisted of conceptions via any fertility assistance, chronic hypertension, preexisting or gestational diabetes mellitus, and chronic renal disease. The original study was approved by the Institutional Review Boards (IRB) of Louisiana State University Health Sciences Center and The Eunice Kennedy Shriver National Institute of Child Health and Human Development. The analyses presented here were from anonymized samples, and therefore were deemed exempt from IRB review by the NIH Office of Human Subject Research.

Data collection

Medical and demographic information for both mother and offspring was obtained from an in-person interview and medical chart review. A blood sample was collected when the woman was admitted to the labor and delivery unit. After delivery, mixed arterial and venous umbilical cord serum (referred to as "cord") samples were collected. Samples were allowed to clot, then centrifuged, and serum stored at −80°C.

Laboratory assays

Maternal and cord androstenedione (A4), testosterone (T), estradiol (E2), estriol (E3), progesterone (P), and prolactin (PRL) were measured in the Reproductive Endocrine Research Laboratory, (University of Southern California Keck School of Medicine) by sensitive and specific immunoassays. A4, P, T, and E_2 were measured by radioimmunoassays (RIAs) following their extraction with ethyl acetate/hexane (3:2) and separation by Celite column partition chromatography [32–35]. E_3 was quantified by RIA after a dual extraction using different concentrations of ethyl acetate in hexane [36]. Prolactin was measured by direct chemiluminescent immunoassay on the Immulite analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA). Coefficients of variation (CVs) were 13.3, 6.9, 9.5, 15.4, 10.7, and 4.5% for maternal measures and 14.6, 12.0, 16.3, 8.3, 7.6, and 3.4% for cord measures of A4, T, E2, E3, P, and PRL, respectively. Placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) were measured in the laboratory of Dr. S. Ananth Karumanchi (Beth Israel Hospital, Harvard Medical School) using commercially available ELISAs (R&D Systems; Minneapolis, MN). For the maternal measurements, the interassay CVs were 5% for sFlt-1, 10% for PLGF, and 29% for endoglin. The interassay CV for cord sFlt-1 was 15%. PlGF was undetectable in 70% of cord samples; therefore, it was not included in the analyses. Cord s-Eng also was excluded from the analyses, because of its high CV (42%). IGF-1, IGF-2, IGFBP-3, C-peptide, and leptin were measured in the Pollak Research Laboratory using ELISA methods and reagents from DSL (Webster, TX). The CVs were 9.6, 6.4, 5.7, 8.1, and 4.3% for maternal measures and 9.4, 7.5, 5.7, 8.0, and 3.6% for cord measures of IGF-1, IGF-2, IGFBP-3, C-peptide, and leptin, respectively.

Because of limited sera, cord measurements were prioritized based on assay volume requirements to achieve the maximum results from each sample. Thus, the sample sizes vary for each of the cord analytes. For most of the hormones, approximately 70% of the population had both cord and maternal serum measurements: for E3, IGF-1, and

sFlt-1, approximately 58% had both; for IGF-2, 52% had both measurements; and for leptin, 46% had both, whereas only 25% had both measures for C-peptide. Maternal, prenatal, and neonatal characteristics did not differ between pregnancies, in which all analytes were measured and those for which levels of one or more analytes were not able to be determined.

Statistics

Because the analyte values tended to be non-normally distributed, Spearman correlations were used to examine the associations among the hormones and proteins (1) for the same analyte between the maternal and cord circulation; (2) among the analytes separately for the maternal and cord circulations; and (3) between maternal and cord measurements and continuous values of maternal and neonatal characteristics. Linear regression models estimated the associations of hormone and protein measures with maternal race or offspring gender after adjustment for gestational age and maternal or neonatal factors significantly associated with the specific hormone or protein concentrations in the univariate analysis reported in Table 6. Analyses were completed with SAS (version 9.0, SAS Institute, Inc., Cary, NC), and statistical significance was defined as two sided $p < 0.05$.

Results

Demographics of study population and median concentrations of analytes in maternal and cord samples

The maternal, gestational, and neonatal characteristics of the study population are reported in Table 1. A total of 49 women with term pregnancies were included in this study. Of these, 38 were African-American, three were Hispanic, and eight were Caucasian, non-Hispanic. Approximately

65% of the women had vaginal deliveries, while 32% were delivered by cesarean section. The majority of the women, per design of the parent study, were nulliparous (90%) and, on average, young (mean age 20.5 years) with a self-reported pre-pregnancy body mass index (BMI) of mean 25.9 kg/m^2. Mean gestational age at delivery was 39 weeks. Maternal and neonatal characteristics did not differ by whether the delivery was vaginal or by cesarean section (data not shown).

Analyte concentrations in maternal and cord samples

Medians for the sex steroids and proteins measured in both maternal and cord samples are presented in Table 2. For most steroid and protein measures, maternal concentrations were equivalent to or higher than cord concentrations, with the exception of A4, E3, P, and PRL, which were higher in the fetal circulation. The hormone and protein measures were analyzed by the method of delivery (vaginal vs. cesarean), and no significant differences were detected, except for higher cord E2 concentrations associated with vaginal deliveries (data not shown).

Correlation between the same analyte in the maternal and cord circulation

Overall, the only analyte that showed moderate correlation between the maternal and cord circulations was T ($r = 0.47$, $p = 0.006$). A4, IGF-1, C-peptide, and leptin were weakly correlated between maternal and cord samples; all other hormones and proteins analyzed exhibited no correlation (Table 3).

Patterns of correlation among analytes in the maternal and fetal circulation

The results in Tables 4 and 5 demonstrate multiple, positive correlations between hormones and protein analytes in

Table 1 Descriptive characteristics of the study sample

Characteristics	n	Mean	Range	SD
Gestational age (weeks)	49	39.0	34.0–41.3	1.5
Birth weight (g)	49	3,209	2,385–4,110	445.2
Birth length (cm)	48	46.4	20.0–54.6	5.8
Head circumference (cm)	48	33.5	30.5–37.0	1.3
Maternal age (years)	46	20.5	15.0–32.0	3.4
Maternal pre-pregnancy weight (lbs)	46	151	95–250	43.7
Maternal height (in)	49	64.0	59.0–70.0	2.6
Maternal pre-pregnancy body mass index (wt(kg)/ht (m)^2)	45	25.9	16.8–43.1	6.9
Maternal weight gain (lbs)	45	34	−18 to + 114	25.6

Table 2 Median concentrations of hormones and proteins in maternal and mixed cord serum samples from uncomplicated pregnancies

Analyte	*n*	Median	Range	SD
Androstenedione (ng/ml)				
Maternal	48	5.3	1.9–19.8	3.4
Cord	34	8.0	3.3–92.3	15.0
Testosterone (ng/dl)				
Maternal	48	230	73.2–835	184
Cord	34	37.6	9.5–631	125
Estradiol (ng/ml)				
Maternal	48	25.4	0.2–65.8	19.0
Cord	34	16.6	1.2–182	40.3
Estriol (ng/ml)				
Maternal	48	14.7	0.5–31.5	9.1
Cord	29	276	0.2–831	203
Progesterone (ng/ml)				
Maternal	48	145	17–308	74
Cord	32	1,706	202–4,007	934
PRL (ng/ml)				
Maternal	48	144	44.3–338	67.8
Cord	34	337	48.6–904	191
IGF-1 (ng/ml)				
Maternal	47	161	22–399	86
Cord	28	67	41–311	52
IGF-2 (ng/ml)				
Maternal	48	1,748	620–3,039	479
Cord	25	795	459–1,356	257
IGFBP-3 (ug/ml)				
Maternal	48	5.2	2.4–8.2	1.4
Cord	34	1.5	0.7–5.1	0.9
C-peptide (ng/ml)				
Maternal	35	0.7	0.3–2.6	0.6
Cord	12	0.5	0.3–1.0	0.2
Leptin (ng/ml)				
Maternal	46	32.4	3.8–109	22.6
Cord	22	11.6	2.0–76	17.7
sFlt-1 (pg/ml)				
Maternal	48	7,478	1,620–20,348	5,050
Cord	28	1,100	20–366,923	85,627
PLGF (pg/ml)				
Maternal	48	113	1–688	141
Endoglin (ng/ml)				
Maternal	48	10.8	2.0–33.6	8.6

the maternal and fetal circulations, respectively. When comparing correlations among maternal and cord measures, several consistent patterns were evident. For the most part, the steroid sex hormones were moderately to highly correlated with each other. IGF-1, IGF-2, and IGFBP-3 were also correlated with each other ($r = 0.42–0.81$) and, in general, with the estrogens.

There also were patterns that differed between the maternal and cord measures. Surprisingly, cord A4 was significantly correlated with all other cord measures ($r = 0.38–0.66$), except PRL, IGF-2, and C-peptide. P and PRL were correlated with members of the IGF axis in maternal samples, but not at all in cord samples. Among angiogenic factors, maternal PLGF and sFlt-1 were

Table 3 Spearman correlations of maternal and mixed cord serum hormone and protein concentrations in uncomplicated pregnancies

Analyte	n	r_s	p value
Androstenedione	33	0.30	0.09
Testosterone	33	0.47	0.006
Estradiol	33	−0.11	0.55
Estriol	28	−0.06	0.76
Progesterone	31	−0.05	0.79
Prolactin	33	0.006	0.97
Insulin-like growth factor-1	27	0.21	0.28
Insulin-like growth factor-2	24	−0.07	0.74
IGF-binding protein-3	33	0.07	0.68
C-peptide	10	0.30	0.40
Leptin	21	0.34	0.13
sFlt-1	27	−0.04	0.74

Table 4 Spearman correlations among hormone and protein concentrations in maternal serum from uncomplicated pregnancies

Analyte	A4	T	E2	E3	Prog	PRl	IGF-1	IGF-2	IGFBP-3	C-pep	Leptin	sFlt-1	PlGF	Endog
A4	–													
Testosterone	0.81*	–												
Estradiol	0.35*	0.12	–											
Estriol	0.27	0.07	0.82*	–										
Progesterone	0.23	−0.06	0.73*	0.73*	–									
Prolactin	0.11	0.01	0.18	0.24	0.07	–								
IGF-1	0.01	−0.12	0.31*	0.36*	0.39*	0.50*	–							
IGF-2	−0.12	−0.09	0.14	0.15	0.18	0.33*	0.44*	–						
IGFBP-3	−0.02	−0.07	0.25	0.23	0.38*	0.40*	0.77*	0.81*	–					
C-peptide	0.09	0.01	−0.04	0.13	−0.09	−0.03	0.01	−0.21	−0.19	–				
Leptin	−0.004	−0.03	0.19	0.24	0.19	0.07	0.07	0.05	−0.08	−0.17	–			
sFlt-1	0.22	0.10	0.38*	0.27	0.50*	0.03	0.29*	0.14	0.26	0.22	0.05	–		
PlGF	0.01	−0.12	0.46*	0.53*	0.52*	0.03	0.09	0.17	0.22	−0.05	0.21	−0.03	–	
Endoglin	0.18	0.10	0.01	−0.04	−0.003	0.26	0.17	−0.01	−0.01	0.20	0.16	0.40*	−0.17	–

* $p < 0.05$

associated with the maternal sex steroid hormones E2 and P ($r = 0.38$–0.52), while in the cord, sFlt-1 correlated with cord sex steroid hormones A4 and T ($r = 0.53$–0.72).

Associations of pregnancy characteristics with maternal and cord analytes

Univariate analysis demonstrated that only a few maternal or neonatal characteristics were independently associated with any of the hormones or angiogenic factors (Table 6). Birth weight was positively correlated with cord T ($r = 0.44$) and inversely correlated with maternal IGF-1 ($r = -0.32$) and IGFBP-3 ($r = -0.31$). Maternal pre-pregnancy weight ($r = 0.36$), weight gain ($r = 0.39$), and offspring birth weight ($r = 0.30$) were correlated with maternal leptin. Maternal age was positively correlated with maternal IGF-2 ($r = 0.29$), but inversely correlated with maternal C-peptide ($r = -0.38$). Placental weight was inversely correlated with maternal IGFBP-3 ($r = -0.42$), and birth length was positively correlated with maternal PlGF ($r = 0.29$). When these same associations were examined in linear regression models including gestational age and additional maternal or neonatal characteristics associated with the analyte in univariate analyses, the only associations that remained significant were the inverse associations of maternal IGFBP-3 with placental weight and IGF-1 with birth weight, as well as the positive associations of maternal leptin with maternal weight and weight gain.

Associations with offspring gender also were evaluated in models including gestational age and maternal or neonatal characteristics that were significantly associated with

Table 5 Spearman correlations among hormone and protein concentrations in mixed cord serum from uncomplicated pregnancies

Analyte	A4	T	E2	E3	Prog	PRL	IGF-1	IGF-2	IGFBP-3	C-pep	Leptin	sFlt-1
A4	–											
Testosterone	0.66*	–										
Estradiol	0.46*	0.22	–									
Estriol	0.47*	0.31	0.33	–								
Progesterone	0.53*	0.06	0.47*	0.50*	–							
Prolactin	0.009	−0.26	0.08	0.18	0.28	–						
IGF-1	0.38*	0.32	0.18	0.39*	0.03	0.09	–					
IGF-2	0.24	0.40*	0.005	0.34	0.06	0.23	0.42*	–				
IGFBP-3	0.44*	0.51*	0.12	0.33	0.10	0.03	0.65*	0.52*	–			
C-peptide	−0.15	0.10	−0.41	−0.13	−0.75*	−0.50	−0.06	−0.62*	0.05	–		
Leptin	0.56*	0.50*	0.07	0.24	0.20	−0.01	0.62*	−0.02	0.48*	0.17	–	
sFlt-1	0.53*	0.72*	0.14	0.39	0.17	−0.13	0.20	0.63*	0.35	−0.45	0.29	–

* $p < 0.05$

the analyte in univariate analyses. Maternal A4 ($p = 0.07$), E3 ($p = 0.07$), P ($p = 0.01$), sFlt-1 ($p = 0.04$), and PlGF ($p = 0.03$), as well as cord T ($p = 0.03$), were all higher in pregnancies with men than women. For steroid hormones, maternal A4, E3, P, and cord T were approximately 21, 84, 68, and 126% higher, respectively, in pregnancies with male compared to female offspring. Among angiogenic factors, maternal sFlt-1 and PlGF were 46 and 161% higher, respectively, in pregnancies with male compared to female offspring.

Although there was little diversity in the study population with respect to race, we conducted exploratory analyses of potential differences in hormone and protein concentrations between African-Americans and Caucasians, given prior findings that have shown racial differences particularly with steroid hormones. As with offspring gender, analyses including race were adjusted for gestational age and maternal or neonatal characteristics that were significantly associated with the analyte in univariate analysis. In the present data, maternal T ($p = 0.04$) and A4 ($p = 0.04$) were higher in African-Americans than Caucasians. Cord IGF-1 ($p = 0.06$) was higher in Caucasians than in African-Americans. The few Hispanic individuals in this analysis had maternal A4 and T concentrations similar to those in Caucasians, but cord IGF-1 levels were more similar to those in African-Americans. There were no differences in angiogenic factor measurements by race/ethnicity in this population.

Discussion

To our knowledge, these data represent the most comprehensive profile of steroid hormones and proteins measured at delivery in matched maternal and cord samples in a largely African-American population. In particular, these are the first data correlating steroid sex hormones with angiogenic factors in pregnancy. There also was little or no correlation between most maternal and cord measures of the same analyte, which has been seen in prior studies [37]. When examining maternal or cord measures alone, there was a high degree of correlation among analytes in each sample, and some of the correlation patterns among analytes differed depending on whether they are measured in the maternal or cord circulation. These results suggest that cord as well as maternal measurements should be included in studies seeking to characterize pregnancy biomarker concentrations in relation to pregnancy characteristics, as inferences regarding the in utero environment will not be accurate if based on maternal samples alone.

Few hormone or protein measures, from either maternal or cord samples, were associated with maternal or neonatal characteristics. Since the majority (>90%) of the women in this study were nulliparous, associations with parity were not evaluated. Notable associations with maternal and neonatal characteristics include those with offspring gender and maternal race. Interestingly, we found that maternal concentrations of several sex steroid hormones and the angiogenic factors PlGF and sFlt-1 were higher in pregnancies with male offspring. Replication of these findings is warranted.

The results regarding higher maternal T and A4 concentrations and lower cord IGF-1 levels in African-American compared to Caucasians are consistent with prior studies [19, 38, 39]. These prior studies have suggested that the variation in pregnancy androgens by race may result in an in utero environment that could contribute to later differences in adult cancer susceptibility between African-Americans and Caucasians. Specifically, higher pregnancy androgen levels have been hypothesized to be protective

Table 6 Spearman correlations of maternal and mixed cord serum hormone and protein concentrations with maternal, neonatal, and gestational characteristics in uncomplicated pregnancies

Analyte	Gestational age	Birth weight	Birth length	Head circle	Maternal age	Maternal pre-pregnancy weight	Maternal weight gain	Maternal height	Placental weight
Androstenedione									
Maternal	0.16	0.17	0.05	0.11	−0.03	0.16	−0.06	0.04	0.17
Cord	0.11	0.16	0.04	0.17	0.005	−0.15	−0.16	−0.02	0.05
Testosterone									
Maternal	0.22	0.20	0.07	0.10	0.00005	0.21	−0.06	0.18	0.08
Cord	0.21	0.44*	0.24	0.32	0.07	0.01	0.03	0.01	0.18
Estradiol									
Maternal	0.01	0.06	0.13	0.26	0.01	0.17	−0.07	−0.04	−0.11
Cord	−0.16	0.01	0.01	−0.001	0.04	0.10	0.01	0.11	0.13
Estriol									
Maternal	−0.18	0.007	0.06	0.15	0.05	0.22	−0.05	−0.05	0.05
Cord	0.14	0.26	−0.31	0.02	0.24	−0.30	0.14	−0.10	−0.11
Progesterone									
Maternal	−0.11	0.02	0.13	0.15	−0.05	−0.08	0.05	−0.23	−0.12
Cord	−0.04	−0.10	−0.12	−0.11	0.09	−0.24	0.06	0.07	−0.24
Prolactin									
Maternal	0.04	−0.15	0.06	−0.11	0.10	0.09	−0.18	0.16	−0.14
Cord	0.31	−0.16	−0.12	−0.25	−0.25	−0.34*	−0.01	−0.42*	−0.33
IGF-1									
Maternal	−0.25	−0.32*	−0.05	−0.19	−0.17	−0.03	−0.12	0.14	−0.27
Cord	−0.18	0.13	0.03	0.07	0.06	−0.06	0.07	0.20	0.12
IGF-2									
Maternal	−0.11	−0.12	0.08	−0.02	0.29*	0.10	−0.007	0.04	−0.18
Cord	0.27	0.21	−0.11	0.04	0.22	−0.01	0.07	−0.02	0.26
IGFBP-3									
Maternal	−0.14	−0.31*	0.006	−0.23	0.02	−0.03	−0.14	−0.02	−0.42*
Cord	0.02	0.26	−0.08	0.12	0.13	0.07	0.03	0.01	0.06
C-peptide									
Maternal	0.09	−0.07	−0.24	−0.02	−0.38*	−0.24	0.05	0.07	0.28
Cord	−0.15	−0.01	0.12	0.02	0.38	0.34	−0.19	0.21	0.07
Leptin									
Maternal	−0.09	0.31*	0.27	0.21	0.19	0.36*	0.39*	0.17	0.15
Cord	−0.02	0.31	0.30	0.29	−0.30	−0.28	0.25	0.02	0.21
sFlt-1									
Maternal	0.01	0.17	0.05	0.12	−0.08	−0.09	0.23	−0.09	−0.27
Cord	0.37	0.30	0.07	0.11	0.12	−0.04	−0.01	−0.19	0.07
PlGF									
Maternal	−0.07	0.0004	0.25*	0.09	0.10	0.03	0.02	−0.17	0.05
Endoglin									
Maternal	−0.03	0.06	0.20	−0.10	0.05	0.05	0.14	0.19	0.11

* $p < 0.05$

with regard to breast [17] and testicular cancer [19], but a potential risk factor for prostate cancer [19, 38]. While we did see differences by race in maternal samples, no variation in cord androgen concentrations was detected. This suggests that caution should be used when extrapolating hypotheses based on maternal variation by race to the in utero environment of the offspring.

We also observed positive associations of cord T and maternal leptin and inverse associations of maternal IGF-1 and IGFBP-3 with birth weight. Maternal PLGF was

associated with birth length. Birth weight is consistently associated with increased breast cancer risk in numerous studies [17, 40–42] and also with childhood leukemia [43]. In a meta-analysis by dos Santos Silva et al. [41], birth length also was independently (of birth weight) associated with breast cancer risk.

The study presented here included a relatively small sample size, which may have limited the ability to detect statistically significant associations. Also, the large number of comparisons could have resulted in chance associations. As in many studies, maternal samples were collected when the woman was admitted to the labor and delivery unit. We cannot exclude that factors associated with delivery, including stress, might influence the associations of maternal analytes with maternal and neonatal characteristics. However, this effect is at best minimal, because the mode of delivery was not related to mean concentrations of cord or maternal hormones or proteins in our data.

The associations of hormone and/or protein measures with maternal and neonatal characteristics have been central to current hypotheses that suggest the in utero milieu may be associated with subsequent cancer risk either directly or indirectly [17, 19, 20, 39, 44]. Within this population of healthy, term pregnancies in a predominantly African-American population, we saw few associations of maternal and/or neonatal characteristics with the proteins or hormones measured, which may in part be due to the limitations above. However, the significant contributions of this current study include the wide range of analytes measured and demonstration of intercorrelation among many pregnancy hormones and growth factors. These data imply that caution must be applied when evaluating early life/in utero exposures and attributing causal associations with chronic disease outcomes to only one measure. These results also highlight the importance of casting a wide net when determining which analytes to measure, although clearly assay costs and volume requirements are a major limitation in epidemiologic studies. One possible approach is to assess a panel of markers to determine a hormonal and/or protein profile in relation to subsequent outcome.

In conclusion, our results demonstrate the need for studies of pregnancy biomarkers to be more inclusive, to evaluate the correlations among analytes, and to include both maternal and cord samples. Larger studies to confirm the associations reported here are needed as the studies are aimed at understanding the biological mechanisms (including the hormone and/or protein profile) underlying these associations. Future studies also should be designed to permit analysis of these associations across racial/ethnic subgroups as the in utero environment may be part of the explanation for variation in cancer incidence by race/ethnicity.

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