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ORIGINAL ARTICLE

Biological indicators of the *in-utero* environment and their association with birth weight for gestational age

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Birth weight for gestational age (BW/GA) has been associated with a risk of adverse health outcomes. Biological indices of pregnancy complications, maternal mid-pregnancy serum biomarkers and placental pathology may shed light on these associations, but at present, they are most often examined as single entities and offer little insight about overlap. In addition, these indices are typically assessed in relation to the extremes of the BW/GA distribution, leaving open the question of how they relate to the entire BW/GA distribution. Addressing issues such as these may help elucidate why postnatal health outcomes vary across the BW/GA continuum. In this study, we focused on a subset of women who participated in the Pregnancy Outcomes and Community Health Study ($n = 1371$). We examined BW/GA (i.e. gestational age and sex-referenced *z*-scores) in relation to obstetric complications, second trimester maternal serum screening results and histologic evidence of placental pathology along with maternal demographics, anthropometrics and substance use. In adjusted models, mean reductions in BW/GA *z*-scores were associated with preeclampsia ($\beta = -0.70$, 95% CI -1.04, -0.36), high maternal serum alpha fetoprotein ($\beta = -0.28$, 95% CI -0.43, -0.13), unconjugated estriol ($\beta = -0.31$/0.5 multiples of the median decrease, 95% CI -0.41, -0.21) and high levels of maternal obstructive vascular pathology in the placenta ($\beta = -0.46$, 95% CI -0.67, -0.25). The findings were similar when preterm infants, small-for-gestational age or large-for-gestational age infants were excluded. More research is needed to examine how the factors studied here might directly mediate or mark risk when evaluating the associations between BW/GA and postnatal health outcomes.

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Key words: alpha fetoproteins, birth weight, estriol, placenta, pregnancy complications

Introduction

Birth weight for gestational age (BW/GA) has been associated with numerous health outcomes, including the risk of cardiovascular disease and diabetes, low intelligence quotient and psychopathology.[1–4] Such findings are often interpreted within the framework of the developmental origins of adult health and disease hypothesis (DOHaD), which postulates that deleterious influences during sensitive periods (e.g. prenatal period) have long-lasting effects on later development and health. Deleterious influences in the prenatal period are commonly inferred from reductions in the BW/GA continuum and are not limited to those born small-for-gestational age (SGA)[5,6] or preterm.[4,7]

Ideally, investigators should turn their attention to pregnancy-related mediators and markers of BW/GA that may more accurately identify and specify future health risk related to the *in-utero* environment.[1,8] Challenges include selecting relevant mediators/markers to consider and comprehensive modeling to detect overlap. The human and animal literatures have focused considerably on the role of maternal malnutrition and stress in explaining the associations between BW/GA and postnatal health outcomes,[9–11] whereas the role of other factors associated with pregnancy health have received comparatively less attention. To this end, clinical scenarios [e.g. hypertensive disorders, gestational diabetes (GDM)], biomarkers [e.g. maternal serum alpha fetoprotein (MSAFP)] and evidence of placental pathology have been studied most often as separate entities and in relation to BW/GA at the extremes of the distribution.[12–20] Yet, the links between BW/GA and postnatal health outcomes span the BW/GA continuum, suggesting that mediators/markers of the *in-utero* environment should be similarly evaluated across the full distribution of BW/GA.

In this hypothesis-generating investigation, we used data from the Pregnancy Outcomes and Community Health (POUCH) Study to examine the associations between biological indicators of the *in-utero* environment and BW/GA. These data are unique as they bring together commonly studied factors associated with BW/GA (demographics, anthropometrics, substance use and clinical-level diagnosis of pregnancy complications) and biological measures considered in separate studies. The selection of measures was guided by evidence linking particular obstetric complications with

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BW/GA [e.g. preeclampsia (PE), GDM], the wide availability of maternal serum biomarkers used in routine pregnancy health screening [i.e. MSAFP, beta human chorionic gonadotropin (bHCG), unconjugated estriol (uE3)][18,21,22] and an ever-growing appreciation for the role of placental processes in supporting intrauterine growth.[23–25] Our goals were to evaluate: (1) associations between these biological indicators of pathology and BW/GA in a series of adjusted models and (2) whether any observed associations remained following the removal of preterm, small- or large-for-gestational age (LGA) infants.

Methods

Participants

The POUCH Study, designed to examine the etiological pathways leading to premature delivery, enrolled 3019 women between 15 and 27 weeks of gestation from 52 prenatal clinics in five Michigan communities.[26] Eligibility criteria included English proficiency and a singleton pregnancy with no known birth defects, chromosomal anomalies or preexisting diabetes; data reflect one pregnancy per participant. All women exhibiting MSAFP $>$ 2.0 multiples of the median (MoM) were invited to participate in the study (7% of the cohort) and women with MSAFP $<$ 2.0 MoM were stratified by race/ethnicity and sampled into the cohort. For a subset oversampled for African-American race, high MSAFP and preterm delivery, hereafter referred to as the *subcohort* ($n = 1371$), in-depth medical record abstraction was performed and placental samples were collected for later gross and histological examination. This sampling scheme was used to maximize resources when investigating at-risk subgroups,[26] but the use of sampling weights in analyses produces results that reflect the entire cohort. All subcohort women were eligible for inclusion in the present analysis, 1122 of whom had placental samples available and evaluated to date. This study was approved by the Institutional Review Boards of Michigan State University and all participating medical centers.

Measures

Obstetric complications

Evidence of GDM, hypertensive disorders [i.e. chronic hypertension (CH), gestational hypertension (GH), PE] and placental abruption (PA) was abstracted from medical charts by trained staff nurses. A team of clinicians and the principal investigator reviewed this evidence and applied established criteria to assign the diagnostic categories, which included: GDM [failed 3-h glucose tolerance test, failed glucose screening ($>$190 mg/dl) accompanied by a fasting glucose $>$95 mg/dl, or explicit diagnosis in the medical records];[27] CH [diastolic blood pressure (DBP) $>$90 or systolic blood pressure (SBP) $>$140 on at least two occasions before 20 weeks, medical record diagnosis, or use of hypertension medication]; GH (no CH, DBP $>$ 90 or SBP $>$ 140 on at least two occasions after 20 weeks); PE (same criteria as GH plus evidence of proteinuria)[28–30] and PA [documented signs/symptoms of PA (e.g. significant bleeding not attributable to dilation) or retroplacental hematoma visualized on prenatal ultrasound].[31]

Maternal mid-pregnancy serum biomarkers

MSAFP, uE3 and bHCG levels were abstracted from medical records, which were calculated as MoM adjusted for maternal race, weight and gestational age at sampling by the laboratory. Because the POUCH Study oversampled women with MSAFP at $\geqslant$ 2.0 MoM, this value was used to dichotomize women according to this same threshold, whereas the levels of uE3 and bHCG were allowed to vary continuously.

Placental pathology

Placentas were obtained from 88% of the subcohort, and 92% of these have been evaluated to date by the study pathologist, who was blind to all clinical circumstances surrounding delivery. Seven samples (five from the placental disc, two from the membrane roll) were examined per placenta. Microscopic evidence of vascular pathology was evaluated and grouped into five constructs that have been detailed elsewhere.[32] Briefly, these include Maternal Vascular-Obstructive (MV-O; e.g. decidual vessel atherosis), Maternal Vascular-Developmental (e.g. abnormal/incomplete conversion of the uterine spiral arteries), Maternal Vascular-Disturbance of Integrity (e.g. retroplacental hemorrhage and decidual bleeding), Fetal Vascular-Obstructive (e.g. thromboses) and Fetal Vascular-Disturbance of Integrity (e.g. fetal to maternal hemorrhage).[32] Scores for each of the five constructs, assigned on the distribution of findings in term, normal MSAFP deliveries, were dichotomized at approximately the top quintile to describe *high* and *not high* levels of vascular pathology.[32]

Placentas were also examined for evidence of histologic chorioamninoitis (HCA) according to a grading and staging scheme described elsewhere.[33] Evidence of inflammation was described as *none*, *mild*, or *severe*, based on the concentration and location of polymorphonuclear leukocytes (PMNL) and the presence of necrotizing inflammation or PMNL karyorrhexis.

Birth weight for gestational age (BW/GA)

Sex- and gestational age-referenced norms were used to calculate *birth weight for gestational age z-scores (BW/GAz)*, using birth weight, gestational age and sex information abstracted from the participants' medical records.[34] Gestational age was estimated using the last menstrual period unless it was unavailable or differed from the ultrasound estimate (at $<$25 weeks) by more than 2 weeks. In these cases (20% of the subcohort), the ultrasound-based estimate was used in the data analysis. Infants were identified as SGA or LGA using estimates corresponding to the 10th and 90th percentiles, respectively.[34]

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Additional variables

At POUCH Study enrollment, maternal self-reports of race (non-Hispanic white, black), parity (primiparous, multiparous), education (<12, =12, >12 years), Medicaid assistance, age (<20, 20–30, ⩾30 years), height and pre-pregnancy weight were obtained along with their self-reported use of tobacco, alcohol and illicit drugs (marijuana, heroin, cocaine/crack, methamphetamines) during the index pregnancy. All variables were analyzed categorically, except height, which was analyzed continuously. Maternal height and pre-pregnancy weight were used to calculate body mass index (BMI; kg/m^2).

Statistical analyses

To investigate associations among the factors listed above and BW/GA*z*, general linear models (GLMs) were performed. All analyses, both unadjusted and adjusted, were weighted for the oversampling of high MSAFP in the POUCH cohort and oversampling of high MSAFP, African-American race and preterm delivery in the subcohort using PROC SURVEYREG (SAS 9.1).

We first examined associations among maternal demographics, anthropometrics, substance use and BW/GA*z* to identify a base model of covariates. Characteristics associated with BW/GA*z* ($P \leqslant 0.10$ or change in remaining estimates by ⩾10%) were carried forward and compared with each set of obstetric complication indices separately. Using the same criteria, items from each of these analyses were entered into a single GLM to examine the extent to which each was associated with BW/GA*z* after adjusting for the others. This final model was repeated following the removal of preterm (<37 weeks' gestation), SGA or LGA infants to examine whether these groups partially or fully accounted for any findings that were observed. The variable inflation factor was <10 for every variable in every analysis, suggesting that multicollinearity did not jeopardize the validity of the model estimates.

Results

In the POUCH subcohort, 25% self-identified as African-American, 51% had 12 or fewer years of education, 49% received Medicaid and 47% exhibited pre-pregnancy BMIs in the recommended range. Approximately 20% of women reported using tobacco, alcohol or illicit drugs at some point during their pregnancy before enrollment; additionally, 10% of women had hypertensive disorders, 5% had GDM and 1% experienced PA. Approximately 10–40% of placentas exhibited high levels of a particular vascular pathology construct; 51% and 10% had evidence of mild and severe HCA, respectively (weighted percents; Table 1).

Although not the focus of this study, we found that all maternal demographic, anthropometric and substance use variables were significantly associated with BW/GA*z* in their expected directions (e.g. tobacco use, shorter height, lower BMI and lower education were associated with lower BW/GA*z*). Table 2 presents the unadjusted associations between the main study variables and BW/GA*z*; β's for categorical and continuous variables represent the mean differences and slopes, respectively. BW/GA*z* was related to PE ($\beta = -.66$, 95% CI −.95, −.38), GDM ($\beta = 0.62$, 95% CI 0.23, 1.00) and two maternal serum biomarkers (high MSAFP: $\beta = -0.24$, 95% CI −0.39, −0.10; uE3 (per 0.5 MoM decrease): $\beta = -0.29$, 95% CI −0.39, −0.19). Among women with available placental data, high MV-O and the presence of severe HCA were associated with a .63 and .38 mean decrease in BW/GA*z*, respectively.

In adjusted GLMs, all demographic, anthropometric and substance use variables remained significantly associated with BW/GA*z*, except for alcohol and illicit drug use. As a result, these two variables were excluded from subsequent analyses, and those that remained comprised a base model to which the remaining sets of variables were separately compared. For obstetric complications, PE was associated with a decrease in BW/GA*z* ($\beta = -0.68$, 95% CI −1.05, −0.32) and GDM was associated with an increase in BW/GA*z* ($\beta = 0.48$, 95% CI 0.11, 0.84). For maternal serum biomarkers, high MSAFP and lower levels of uE3 remained associated with decreases in BW/GA*z* (MSAFP: $\beta = -0.31$, 95% CI −0.46, −0.15; uE3 (per 0.5 MoM decrease): $\beta = -0.31$, 95% CI −0.41, −0.21). Among the placental pathology constructs, only high MV-O remained significantly associated with decreases in BW/GA*z* (MV-O: $\beta = -0.45$, 95% CI −0.67, −0.24). The associations between severe HCA and BW/GA*z* were attenuated and declined below significant thresholds ($\beta = -0.14$, 95% CI −0.37, 0.01).

Because they were associated with BW/GA*z* in the above-adjusted analyses, PE, GDM, MSAFP, uE3 and MV-O were entered into a simultaneous GLM with the base model to evaluate whether they retained their association with BW/GA*z* (Table 3). With the exception of GDM, the results indicated that they did and that the model accounted for 25% of the variance in BW/GA*z*. The adjusted β's for biological indices approximated or exceeded those for demographics, anthropometrics or substance use (e.g. tobacco use: $\beta = -0.43$) in relation to BW/GA*z*.

The BW/GA*z* associations with PE, MSAFP, uE3 and MV-O persisted and were minimally affected following the exclusion of infants born preterm ($n = 335$) and were slightly attenuated after the exclusion of infants born SGA or LGA (Tables 3 and 4). All models accounted for 19–27% of BW/GA*z* depending on whether preterms, SGAs and LGAs were excluded.

Discussion

We evaluated whether BW/GA was related to maternal serum biomarkers and placental pathology after other known influences on growth were taken into account (e.g. maternal demographics, anthropometrics, substance use). Our findings suggest that beyond these influences and clinical-level

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Table 1. *Maternal characteristics, obstetric complications, maternal mid-pregnancy serum biomarkers and placental pathology*

	POUCH subcohort, $n = 1371$		POUCH subcohort full terms only, $n = 1036$	
	n (%)	Weighted %	*n* (%)	Weighted %
Demographics and anthropometrics				
Race				
White/Other	792 (58)	75	567 (55)	76
African-American	579 (42)	25	469 (45)	23
Parity				
Primiparous	577 (42)	42	433 (42)	41
Multiparous	793 (58)	58	603 (58)	59
Maternal education (years)				
<12	317 (23)	23	240 (23)	18
=12	388 (28)	28	290 (28)	27
>12	666 (49)	49	506 (49)	55
Maternal age (years)				
<20	243 (18)	18	185 (18)	14
20–30	776 (57)	57	589 (57)	57
⩾30	352 (25)	28	262 (25)	28
Medicaid				
No	586 (43)	51	595 (57)	52
Yes	783 (57)	49	440 (43)	48
Pre-pregnancy BMI (kg/m^2)				
<18.5	65 (5)	4)	47 (5)	4
18.5–24.9	609 (44)	47	458 (44)	46
25–29.9	305 (22)	23	238 (23)	23
⩾30	392 (29)	26	293 (28)	26
Substance use				
Tobacco use				
None/quit before pregnancy	1111 (81)	82	841 (81)	83
Yes	260 (19)	18	195 (19)	17
Alcohol use				
None	1126 (83)	82	855 (83)	82
Any	234 (17)	18	173 (17)	18
Illicit drug use				
None	1079 (79)	81	809 (78)	81
Any	288 (21)	19	224 (22)	19
Obstetric complications				
Hypertensive disorders				
None	1222 (89)	90	946 (91)	91
Preeclampsia	44 (3)	3	17 (2)	2
Gestational hypertension	56 (4)	4	42 (4)	3
Chronic hypertension	49 (4)	3	31 (3)	4
Gestational diabetes				
No	1304 (95)	95	989 (94)	95
Yes	67 (5)	5	47 (6)	5
Placental abruption				
No	1328 (97)	99	1023 (99)	99
Yes	43 (3)	1	13 (1)	1
Maternal serum biomarkers				
Unexplained high MSAFP				
Normal (<2.0 MoM)	1161 (85)	97	871 (84)	97
High (⩾2.0 MoM)	210 (5)	3	165 (16)	3
uE3 (MoM)[a]	1.09 (0.01)	1.06 (0.01)	1.07 (0.32)	1.06 (0.01)
bHCG (MoM)[a]	1.25 (0.02)	1.20 (0.02)	1.25 (0.69)	1.20 (0.02)

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Table 1. *Continued*

	POUCH subcohort, $n = 1371$		POUCH subcohort full terms only, $n = 1036$	
	n (%)	Weighted %	*n* (%)	Weighted %
Placental pathology	$n = 1122$[b]		$n = 829$[b]	
Maternal vascular pathology				
Obstructive (e.g. infarcts)				
Not high	993 (88)	89	736 (89)	89
High	129 (12)	11	93 (11)	11
Integrity (e.g. bleeding)				
Not high	873 (78)	79	667 (80)	80
High	249 (22)	21	162 (20)	20
Developmental				
Not high	974 (87)	90	736 (89)	91
High	148 (13)	10	93 (11)	9
Fetal vascular pathology				
Obstructive (e.g. infarcts)				
Not high	882 (79)	77	655 (79)	77
High	240 (21)	23	174 (21)	23
Integrity (e.g. bleeding)				
Not high	659 (59)	60	530 (64)	63
High	463 (41)	40	299 (36)	37
Histologic chorioamnionitis				
None	447 (40)	39	280 (34)	36
Mild	540 (48)	51	456 (55)	54
Severe	135 (12)	10	93 (11)	9

POUCH, Pregnancy Outcomes and Community Health; BMI, body mass index; MSAFP, maternal serum alpha fetoprotein; MOM, multiples of the median; uE3, unconjugated estriol; bHCG, beta human chorionic gonadotropin.

[a] Mean (S.E.).

[b] Sample sizes reflect placental samples evaluated to date.

Note: Missing data from POUCH subcohort: parity ($n = 1$), Medicaid ($n = 3$), alcohol use ($n = 11$), illicit drug use ($n = 4$), bHCG ($n = 68$), uE3 ($n = 67$).

diagnosis of obstetric complications, high MSAFP, lower levels of uE3 and high levels of maternal obstructive vascular pathology were independently and robustly associated with reductions in BW/GA. These findings were maintained irrespective of whether preterms, SGAs or LGAs were excluded from the analysis, suggesting that the factors identified in our analyses are associated with reductions across the BW/GA distribution.

Although originally developed as a non-invasive screen for aneuploidy and other congenital anomalies, studies suggest that even in the absence of these conditions, maternal serum biomarkers are associated with aspects of maternal and fetal health (e.g. intrauterine growth restriction)[18,21] and placental functioning.[35] Of the biomarkers traditionally included in the 'triple screen', high MSAFP and low uE3 have been the most consistently associated with reductions in fetal growth and birth weight.[19,22,36,37] This study not only replicates these findings, but extends them by examining their contribution to BW/GA beyond other indices of pregnancy health (e.g. placental pathology).

The mechanisms underlying high MSAFP in the absence of neural tube defects or ventral wall abnormalities are unclear. One hypothesis is that high MSAFP is a marker for disturbances to the maternal–fetal interface, reflecting the leakage of alpha fetoprotein from the fetal circulation and amniotic fluid into the maternal bloodstream;[18] previous studies have linked unexplained high MSAFP with aspects of placental pathology.[35] Whereas high levels of disruption to fetal vessel integrity (FV-I) were unrelated to birth weight *z*-scores in our study, this may be because the 'high' cutpoint for FV-I was constrained by our sample distribution and captured the upper 40%. MSAFP may be a more specific measure of disturbances to FV-I because its clinical cutpoint of 2.0 MoM applies only to 3–5% of screened populations.

Independent of high MSAFP, lower levels of uE3 were also associated with decreases in BW/GA. uE3 has been interpreted as an indicator of fetal well-being, given that by the second trimester, nearly 90% of circulating maternal serum estriol concentration is derived from the substrate dehydroepiandrosterone sulfate (DHEA-S) produced by the fetal

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Table 2. *Unadjusted associations between pregnancy complication indices and birth weight z-scores*

	Birth weight *z*-score			
	POUCH subcohort, $n = 1371$		POUCH subcohort terms only, $n = 1036$	
	β	95% CI	β	95% CI
Obstetric complications				
Hypertensive disorders				
None (ref)	–	–	–	–
Preeclampsia	−0.66*	−0.95, −0.38	−0.67*	−1.08, −0.26
Gestational hypertension	0.22	−0.12, 0.57	0.25	−0.13, 0.63
Chronic hypertension	−0.13	−0.45, 0.18	−0.11	−0.49, 0.26
Gestational diabetes (ref = none)	0.62*	0.23, 1.00	0.66*	0.23, 1.09
Placental abruption (ref = none)	−0.18	−0.50, 0.15	−0.11	−0.68, 0.46
Maternal serum biomarkers				
Unexplained MSAFP (ref = normal)	−0.24*	−0.39, −0.10	−0.26*	−0.41, −0.10
uE3 (per 0.5 MoM decrease)	−0.29*	−0.39, −0.19	−0.28*	−0.39, −0.16
bHCG (per 0.5 MoM decrease)	0.02	−0.03, 0.07	0.02	−0.04, 0.08
Placental pathology	$n = 1122$[a]		$n = 829$[a]	
Maternal vascular pathology				
Obstructive (e.g. infarcts; ref = not high)	−0.63*	−0.86, −0.41	−0.63*	−0.88, −0.38
Integrity (e.g. bleeding; ref = not high)	0.06	−0.13, 0.24	0.06	−0.15, 0.27
Developmental (ref = not high)	−0.12	−0.39, 0.14	−0.12	−0.44, 0.21
Fetal Vascular Pathology				
Obstructive (e.g. infarcts; ref = not high)	−0.08	−0.28, 0.11	−0.04	−0.25, 0.17
Integrity (e.g. bleeding; ref = not high)	0.11	−0.05, 0.26	0.12	−0.05, 0.30
Histologic chorioamnionitis				
None (ref)	–	–	–	–
Mild	−0.14	−0.30, 0.03	−0.15	−0.32, 0.03
Severe	−0.38*	−0.59, −0.19	−0.40*	−0.63, −0.17

POUCH, Pregnancy Outcomes and Community Health; MSAFP, maternal serum alpha fetoprotein; uE3, unconjugated estriol; MOM, multiples of the median; bHCG, beta human chorionic gonadotropin.

*$P < 0.05$.

[a] Sample sizes reflect placental samples evaluated to date.

adrenals (DHEA-S is converted to androgens and aromatized by placental sulfatases to produce estriol).[18] Low levels of uE3 can thus reflect decreases in fetal production of DHEA-S or placental sulfatase activity.[38,39] However, it is unclear what specific aspect of fetal physiological functioning low concentrations of DHEA-S might reflect (e.g. reduced adrenal sensitivity, decreased central stimulation), whether it is a proxy for less than optimal fetal organ function or whether it directly influences growth through interactions with other hormones.[40] Additionally, a constitutionally smaller fetus might secrete lower amounts of DHEA-S as a function of its smaller size. We do not believe that this explains our findings because women who self-identified as Asian, a population often described as delivering constitutionally smaller babies,[14] exhibited *higher* levels of uE3 than either White or Black women (data not shown). Furthermore, sensitivity to body size would undermine the clinical utility of uE3 as a screening tool.

High MV-O was consistently associated with decreases in BW/GA. Whereas the presence of maternal thrombotic lesions has been reported previously among SGAs,[41] our finding was observed in term deliveries, across the BW/GA distribution and following adjustment for many factors including the presence of other types of vascular pathology and maternal hypertensive conditions. There was minimal overlap across the vascular pathology constructs;[32] thus, we believe that the findings were not affected by multicollinearity. High MV-O likely reflects significant underperfusion of the placenta, which impedes nutrient, waste and gas exchange that supports fetal development and growth.[42]

The POUCH sample is demographically diverse with data on multiple biological indicators of pregnancy health, thus providing a unique opportunity to address our study objectives. However, there are some limitations worth mentioning. First, we used growth standards based on the birth weight

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Table 3. *Adjusted associations between pregnancy complication indices and birth weight z-scores in the POUCH subcohort before and following the removal of SGA or LGA births*[a]

	Birth weight *z*-score					
	POUCH subcohort, $n = 1371$		POUCH subcohort SGAs removed, $n = 1220$		POUCH subcohort LGAs removed, $n = 1229$	
	β	95% CI	β	95% CI	β	95% CI
Obstetric complications						
Hypertensive disorders						
None (ref)	–	–	–	–	–	–
Preeclampsia	−0.70*	−1.04, −0.36	−0.58*	−0.89, −0.26	−0.47*	−0.80, −0.13
Gestational hypertension	0.18	−0.19, 0.54	0.11	−0.26, 0.47	0.14	−0.12, 0.40
Chronic hypertension	−0.30	−0.66, 0.07	−0.44*	−0.79, −0.09	−0.08	−0.37, 0.21
Gestational diabetes (ref = none)	0.30	−0.10, 0.69	0.47*	0.12, 0.82	−0.02	−0.31, 0.27
Maternal serum screening						
High MSAFP (ref = normal)	−0.28*	−0.43, −0.13	−0.21*	−0.35, −0.06	−0.19*	−0.33, −0.05
uE3 (per 0.5 MoM decrease)	−0.31*	−0.41, −0.21	−0.22*	−0.30, −0.12	−0.25*	−0.33, −0.17
Placental pathology	$n = 1122$[b]		$n = 1000$[b]		$n = 1005$[b]	
Maternal vascular pathology						
Obstructive (ref = not high)	−0.46*	−0.67, −0.25	−0.21*	−0.51, −0.10	−0.33*	−0.54, −0.13

POUCH, Pregnancy Outcomes and Community Health; SGA, small-for-gestational age; LGA, large-for-gestational age; MSAFP, maternal serum alpha fetoprotein; uE3, unconjugated estriol.

* $P < 0.05$.

[a] Adjusted for maternal demographics/anthropometrics (race, parity, maternal education, Medicaid status, pre-pregnancy body mass index, maternal height), self-reported substance use (tobacco, alcohol), and all variables in this table.

[b] Sample sizes reflect placental samples evaluated to date.

Table 4. *Adjusted associations between pregnancy complication indices and birth weight z-scores among term POUCH subcohort deliveries (⩾ 37 weeks), before and following the removal of term SGA or LGA births*[a]

	Birth weight *z*-score					
	POUCH subcohort terms only, $n = 1036$		POUCH subcohort term SGAs removed, $n = 909$		POUCH subcohort term LGAs removed, $n = 934$	
	β	95% CI	β	95% CI	β	95% CI
Obstetric complications						
Hypertensive disorders						
None (ref)	–	–	–	–	–	–
Preeclampsia	−0.86*	−1.31, −0.41	−0.67*	−1.12, −0.23	−0.64*	−1.08, −0.20
Gestational hypertension	0.20	−0.20, 0.60	0.09	−0.31, 0.50	0.19	−0.09, 0.48
Chronic hypertension	−0.31	−0.73, 0.12	−0.49*	−0.89, −0.09	−0.10	−0.44, 0.23
Gestational diabetes (ref = none)	0.29	−0.14, 0.72	0.49*	0.10, 0.88	−0.04	−0.36, 0.27
Maternal serum screening						
High MSAFP (ref = none)	−0.31*	−0.47, −0.16	−0.27*	−0.43, −0.11	0.21*	−0.36, −0.06
uE3 (per 0.5 MoM decrease)	−0.31*	−0.42, −0.20	−0.21*	−0.31, −0.05	−0.27*	−0.37, −0.17
Placental pathology	$n = 829$[b]		$n = 727$[b]		$n = 750$[b]	
Maternal vascular pathology						
Obstructive (ref = not high)	0.45*	−0.68, −0.23	−0.33*	−0.56, −0.11	−0.29*	−0.51, −0.08

POUCH, Pregnancy Outcomes and Community Health; SGA, small-for-gestational age; LGA, large-for-gestational age; MSAFP, maternal serum alpha fetoprotein; uE3, unconjugated estriol; MOM, multiples of the median.

* $P < 0.05$.

[a] Adjusted for maternal demographics/anthropometrics (race, parity, maternal education, Medicaid status, pre-pregnancy body mass index, maternal height), self-reported substance use (tobacco, alcohol), and all variables in this table.

[b] Sample sizes reflect placental samples evaluated to date.

distributions for live births. Because growth-restricted fetuses preferentially deliver at earlier gestational ages, our operationalization of BW/GA likely underestimates the degree of growth restriction present among preterm infants and as such, may underestimate the contribution of preterm delivery and SGA birth to the findings reported here.[43,44] Although efforts to develop population-based intrauterine growth standards are underway, they require further validation. Second, as illustrated by models accounting for less than 27% of the variance in BW/GA*z*, the biological measures addressed here do not represent an exhaustive list of factors that might influence birth weight and/or long-term health outcomes. For example, original formulations of the DOHaD hypothesis and much of the associated animal literature have identified maternal undernutrition as one potential mechanism through which BW/GA might be associated with later disease.[8,45] Although data on maternal nutritional intake are unavailable in the POUCH Study, we used pre-pregnancy BMI as a very rough proxy for pre-pregnancy undernutrition. We did not use pregnancy weight gain because this measure incorporates the weight of the fetus. (However, analyses replacing pre-pregnancy BMI with categorical or continuous measures of pregnancy weight gain did not alter any study findings.) We recognize that pre-pregnancy BMI is not a proxy for nutritional information, but it might identify women at risk for extreme under- or over-nutrition during pregnancy. Third, we assumed that missing data (e.g. placental pathology) from a particular weighted stratum were representative of that stratum in our adjusted analyses.

It will be important to evaluate the associations among MSAFP, uE3, MV-O and health outcomes in childhood and beyond, determining whether these indices add risk information beyond BW/GA. This approach is consistent with recommendations by other investigators regarding the need to identify the biological mediators of long-term health outcomes.[2,46,47] That said, we recognize the limitations of the association models explored here, and although these associations are biologically plausible, they are not meant to imply direct causality. Additionally, given the breadth of work investigating the contribution of maternal malnutrition to size at birth, it will be helpful to contextualize the findings reported here using adjusted models that include more detailed information regarding maternal diet and/or nutritional status.

A number of interpretational caveats are also important to consider when thinking about the links between the indices of complications described here and the risk for postnatal health outcomes. First, although our investigation centered around factors that have been considered indicators of pathology during pregnancy, their relevance to understanding the development of later health problems depends on the biological plausibility of their contributions to the particular outcome of interest. Second, attempts to link *in-utero* biological measures with postnatal health problems may be undermined by the inability to differentiate *in-utero* 'programming' effects from genetic and postnatal environment effects that also mediate patterns of familial risk.[48,49] For example, do obstructive lesions in the placenta (i.e. high MV-O) decrease BW/GA and alter fetal physiological systems involved in the etiology of cardiovascular disease? Or, are these same placental lesions proxies for a maternal thrombophilic profile that places offspring at risk for reductions in BW/GA and cardiovascular disease later in life? The overarching goal of our analyses was intended to be hypothesis-generating and was not designed to provide causal or predictive models of BW/GA.

Our findings reinforce the idea that size at birth, including BW/GA, is a composite measure representing a myriad of influences, including those that are interpreted as innocuous (e.g. maternal height), helpful (e.g. adequate nutrition) or harmful (e.g. PE) to perinatal health. Therefore, we and others argue that it is important to move beyond measures of birth size in studies investigating the perinatal origins of adult disease and instead identify specific exposures relevant to the long-term outcome of interest.[25] Doing so will facilitate the development of effective prevention/intervention strategies designed to promote health during pregnancy and beyond. Nonetheless, we show that there are meaningful biological clues about where infants fall in the BW/GA distribution beyond the clinical diagnosis of pregnancy complications, and this may help elucidate why postnatal health outcomes vary across the entire BW/GA continuum.[5,6,50]

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