Placental 11β-HSD2 and Cardiometabolic Health Indicators in Infancy

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OBJECTIVE

Fetal excessive exposure to glucocorticoids may program cardiometabolic risk. Placental 11 β-hydroxysteroid dehydrogenase 2 (11β-HSD2) serves as a barrier to prevent fetal overexposure to maternal glucocorticoids. It has not been explored whether placental 11β-HSD2 levels are associated with cardiometabolic health in postnatal life.

RESEARCH DESIGN AND METHODS

In a prospective birth cohort study of 246 mother-infant pairs, we measured placental 11β-HSD2 expression and maternal (32–35 weeks of gestation) and cord plasma cortisol concentrations. The primary outcomes were HOMA of insulin resistance (IR) and blood pressure (BP) in infants at age 1 year. Other outcomes included fasting insulin, HOMA β-cell function, carotid intima-media thickness, weight *z* score, and skinfold thickness (triceps and subscapular) at age 1 year.

RESULTS

Placental 11β-HSD2 expression was negatively correlated with HOMA-IR ($r = -0.17$, $P = 0.021$) and fasting insulin ($r = -0.18$, $P = 0.017$) and marginally negatively correlated with systolic BP ($r = -0.16$, $P = 0.057$) but was not correlated with HOMA of β-cell function, diastolic BP, carotid intima-media thickness, and skinfold thickness (all $P > 0.1$) in infants at age 1 year. Cord plasma cortisol was negatively correlated to skinfold thickness ($r = -0.20$, $P = 0007$) but was not correlated with other outcomes at age 1 year. Maternal plasma cortisol was positively correlated with maximal carotid intima-media thickness ($r = 0.20$, $P = 0.03$) but was not correlated with other outcomes. Adjusting for maternal and infant characteristics, the associations were similar.

CONCLUSIONS

The study is the first to show that higher placental 11β-HSD2 expression is associated with lower IR in infancy. Independent cohort studies are required to confirm this novel finding.

Epidemiological and experimental studies (1–3) have shown that the vulnerability to cardiometabolic diseases such as type 2 diabetes in adulthood may originate from an adverse intrauterine environment. Exposures to excessive glucocorticoids (e.g., cortisol) may be involved in such fetal programming of cardiometabolic risk (4,5). Circulating cortisol levels are elevated in pregnancy, rise progressively over advancing gestation, and peak in the third trimester of pregnancy (6). The proper elevation of cortisol concentrations is critical for the development of fetal organs and

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maturation (7,8). However, excessive exposure to glucocorticoids may result in fetal growth restriction (9,10) and is associated with elevated cardiometabolic risk in adulthood (4,5,11).

Cortisol levels are substantially higher in maternal versus cord blood (12). Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme serves as a barrier to inactivate glucocorticoids from the maternal circulation to avoid fetal overexposure to high cortisol levels (13). Animal studies have shown that placental 11β-HSD2 insufficiency may enhance cardiometabolic risk in the offspring (14) and that overexposure to endogenous or exogenous glucocorticoids is associated with hypothalamus-pituitary-adrenal axis reprogramming and cardiometabolic dysfunction (14–16). However, the implications of placental 11β-HSD2 for postnatal metabolic health in humans are not yet known. In the current study, we sought to test the hypothesis that placental 11β-HSD2 expression is associated with cardiometabolic health indices in infancy. Validating the hypothesis would point to a new molecular target for developing effective interventions to safeguard against adverse programming of cardiometabolic health in early life.

RESEARCH DESIGN AND METHODS

Subjects and Specimens

This study was based on a prospective pregnancy cohort described previously (17,18). Briefly, a total of 339 women bearing a singleton fetus without major maternal illnesses (e.g., pregestational diabetes, essential hypertension) were recruited at 24–28 gestational weeks from three obstetric care centers in Montreal (Sainte-Justine, Jewish General, and Saint Mary's Hospitals) between August 2006 and December 2008. The women were followed up at 32–35 weeks of gestation and delivery, and the infants were followed up at 3 months and 1 year of age. A total of 246 singleton infants with placental specimens available for placental 11β-HSD2 assays were included in the current study. The study was approved by the Research Ethics Committee of Sainte-Justine Hospital Research Center, University of Montreal. Written informed consent was obtained from all participants.

Maternal venous blood samples were collected at 32–35 weeks of gestation, and cord venous blood samples were collected after the delivery of the baby but before the expulsion of the placenta. Fasting venous blood samples were collected from the infants at age 1 year. The study visits were scheduled in the morning. The parents/caregivers were instructed not to feed the infant (except for pure water) in the 12 h before the scheduled visit. A 5-mL blood sample was taken from a vein in the antecubital area of the infant's arm by an experienced pediatric research nurse with the application of a topic anesthesia. Milk, snacks, and toys were provided immediately after the blood sampling. Blood specimens were kept on ice and centrifuged within 30 min after collection. The separated plasma samples were stored in multiple aliquots at −80°C until biochemical assays. Fresh placental specimens (five pieces: three from the periphery and two from the center parts of the placenta, roughly evenly distributed over the entire surface of the placenta) were collected immediately after delivery by a research technician 24 h on call following a standardized protocol, flash frozen in a liquid nitrogen container, and stored at −80°C until assayed.

As previously described (18), weights at birth and at 3 and 12 months of age were measured by an electronic weighting scale to the nearest gram. Skinfold thickness at triceps and subscapular positions in an infant at 3 and 12 months of age was measured by a Harpenden skinfold caliper (Baty International, West Sussex, U.K.) to the nearest 0.1 mm. All anthropometric measurements were taken twice, and the average values were used in the final analysis data.

Weight *z* scores at birth and at 3 and 12 months of age were calculated based on the World Health Organization (WHO) child growth standards (19). Weight *z* score changes between 0–3 and 3–12 months of age were calculated and considered as covariables in the adjusted models in evaluating the associations of placental 11β-HSD2 with cardiometabolic health indicators at 1 year of age.

Blood Pressure

Blood pressure (BP) (mmHg) was measured in infants at 1 year of age. Diastolic BP values were obtained using a sphygmomanometer (GE Carescape Dinamap V100; GE Medical Systems, Milwaukee, WI). Before the measurement, the infant was on the mother's lap and kept in calm and quiet condition (if not possible, the measurement was cancelled). The upper right arm was placed at the heart level during the measurement. Resting BP (right arm artery) was measured two times at least 5 min apart; the average values were taken as the final values. If the difference in BP was >10 mmHg during the two measurements, a third measurement was taken, and the average of the two closer measurements was taken as the final value. Successful BP measurements were completed in 147 infants in the study cohort.

Carotid Intima-Media Thickness

Carotid intima-media thickness (cIMT) was measured (in mm) using an HDI 5000 apparatus (ATL; Philips, Bothell, WA) equipped with a 5.5–12.5 mHz linear array probe by an experienced senior ultrasound specialist (J.D., a coinvestigator). Measurements were assessed using the Math's system software (Math-sd; Metris, France). Ultrasound measures of the cIMT were taken with the infant lying supine, turning the head to a 45° angle away from the side to be scanned. Longitudinal acquisition of two-dimensional images of the right and left carotid arteries was carried out, and the anterior and posterior walls of each carotid were identified. The carotid bulb, internal carotid artery, and carotid bifurcation, and at least 2 cm below the bifurcation (i.e., into the common carotid artery) were imaged. The depth of scanning was 3.9 cm, with the ultrasound beam perpendicular to the far wall of the carotid artery. This caused two parallel lines representing the intima-media to be visualized. The image was frozen and cIMT was measured in two dimensions over a length of 1.5 cm. The software took a minimum of 100 measurements of the far wall of each carotid artery, over the length of 1.5 cm, and the minimum, maximum, and average IMTs for each carotid artery were then recorded. The mean cIMT was obtained by averaging values for the right and left arteries. Postprocessing of ultrasonic signals was performed off-line, at a single site (Sainte-Justine Hospital Research Center) by a study investigator (J.D.) blinded to participant identity,

using a computer-assisted image analysis software (Eurequa; Nutonian, Boston, MA) (20). Successful cIMT measurements were completed in 119 infants in the study cohort.

Biochemical Assays

Placental 11β-HSD2 protein expression was measured by Western blot in a single laboratory (A.M.N., the co-principal investigator of the study). Briefly, 2 g of placental tissue from the central pieces and 2 g from the peripheral pieces were used to extract proteins. The tissue was homogenized in a buffer containing 0.1 mol/L sodium phosphate, pH 7.4, and protease inhibitors. The Bradford technique was used to quantify the proteins. A classic electrophoresis and Western blot transfer was performed using 10 μg of proteins per sample; central and peripheral placental samples were loaded separately. We used the primary antibody anti–11β-HSD2 (1:500; catalog #PC545; The Binding Site Co., San Diego, CA) and the secondary antibody donkey anti-sheep (1:10,000; catalog #STAR88P Serotec; Bio-Rad, Hercules, CA). The antibodies have been validated by the manufacturers. The bands were revealed using BM chemiluminescence blotting substrate (catalog #11500694001; Roche Applied Science, Mississauga, Ontario, Canada). The bands were quantified using the ImageJ software. Placental 11β-HSD2 (45 kDa) content was expressed as the ratio of 11β-HSD2/β-actin. The 11β-HSD2 protein levels were similar between peripheral and central sections of placental samples (all $P > 0.2$), and the average values for each subject were taken as the final values.

Plasma cortisol concentrations (nmol/L) were measured by an automated competitive immunoassay with chemiluminescence detection on the DXI800 (Beckman Coulter, Brea, CA). The intra-assay and interassay coefficients of variation were in the range of 5.0–8.5%.

As reported previously, plasma glucose was measured by an automated glucose oxidase method, insulin by an automated ultrasensitive chemiluminescent immunometric assay, and proinsulin by a quantitative ELISA kit, respectively (17). The detection limits were 3.3 pmol/L for insulin and 2.0 pmol/L for proinsulin, respectively. The intra-assay and interassay coefficients of variation of these assays were in the range of 2.0–9.8%.

All assays were completed at 12–24 months after the specimen collection. There were no significant correlations between specimen storage (at −80°C) time and biomarker measurement values (all $P > 0.1$).

Outcomes

The primary outcomes were HOMA of insulin resistance (IR) and systolic BP in infants at 1 year of age, since elevated IR and BP have been reported in placental 11β-HSD2 insufficiency or fetal exposure to excessive glucocorticoids in animal models (14–16), and that higher cord blood cortisol/cortisone ratio, an indicator of reduced placental 11β-HSD2 activity, has been associated with increased systolic BP in infants (at age 3 years) (21). Other outcomes included diastolic BP, HOMA of β-cell function index (HOMA-β), fasting plasma insulin and proinsulin concentrations, glucose-to-insulin ratio (an indicator of insulin sensitivity), proinsulin-to-insulin ratio (an indicator of β-cell function), cIMT (mean, maximum), weigh *z* score and skinfold thickness (triceps + subscapular) (an adiposity indicator) in infants at age 1 year. HOMA-IR was calculated as (fasting insulin in mU/L) × (fasting glucose in mmol/L)/22.5, and HOMA-β as 20 × (fasting insulin in mU/L)/[(fasting glucose in mmol/L) − 3.5] (22).

Statistical Analysis

We presented the frequency (as percentage) for categorical variables, and median and mean ± SE for continuous variables. Log-transformation was applied for biomarkers with skewed data distribution in all comparisons, and correlation and regression analyses. Pearson partial correlation was used to evaluate the associations of placental 11β-HSD2, and maternal and fetal plasma cortisol with cardiometabolic health indicators at 1 year of age in infants adjusting for gestational age at delivery. Generalized linear model was used to assess the associations controlling for multiple covariables. The covariables included maternal gestational diabetes mellitus (GDM) (yes/no), gestational hypertension (yes/no), family history of diabetes (yes/no), prepregnancy BMI (kg/m^2), ethnicity (white, others), education (university: yes/no), age (≥35 years: yes/no), parity (primiparous: yes/no), smoking (yes/no) and alcohol use (yes/no), mode of delivery (cesarean or vaginal), infant sex, duration of labor (hours), gestational age (weeks), birth weight *z* score according to the Canadian sex-specific and gestational age-specific fetal growth standards (23), breast feeding (yes/no), and changes in weight *z* scores from birth to 3 months and 3 months to 1 year of age. Parsimonious regression models were fitted to obtain more stable adjusted effect estimates by excluding covariables that were not significant at $P > 0.2$ and did not affect the comparisons. We conducted mediation analyses to explore the potential mediation effects of cord plasma cortisol and skinfold thickness (an adiposity indicator) in any observed associations between placenta 11β-HSD2 and infant cardiometabolic health outcomes at 1 year of age using the product method (Baron and Kenny method) (24).

Two-tailed *P* values <0.025 were considered statistically significant, considering placental 11β-HSD2 in relation to two primary outcomes of interest (HOMA-IR and systolic BP). All data analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC).

To evaluate the robustness of the study findings to missing data, we conducted sensitivity analyses on the associations of interest with and without imputations for missing data. Multiple imputations for missing data were performed using the PROC MI in SAS to generate 25 data sets; the analysis results were obtained by pooling the statistics from the 25 data sets using the PROC MIANALYSIS.

RESULTS

Table 1 presents maternal, birth, and infant characteristics of the study cohort (n = 246). The majority of subjects were Caucasians (66.7%). There were 26 pregnancies (10.6%) complicated by GDM and 12 (4.9%) by gestational hypertensive disorders, 13 (5.3%) preterm deliveries (all mild preterm, 34–36 weeks), 16 infants (6.5%) being small for gestational age (SGA) (<10th percentile in birth weight for sex and gestational age) (24), and 23 infants (9.4%) being large for gestational age (LGA) (birth weight >90th percentile). The mean weight *z* scores (WHO child growth standards) were 0.35 at birth, 0.36 at 3 months,

and 0.50 at 1 year of age, respectively. Without imputations for missing data, the averages were 16.2 mm for the sum of triceps and subscapular skinfold thickness, and 88/55 mmHg for systolic/diastolic BP, and 0.56 and 0.65 mm for mean and maximal cIMT in infants at 1 year of age, respectively. Similar statistics were obtained with imputations for missing data.

Supplementary Figure 1 presents representative Western immunoblots of placental 11β-HSD2 expression. and Table 2 presents the descriptive statistics of placental 11β-HSD2 protein expression and maternal and cord plasma cortisol concentrations by mode of delivery (cesarean/vaginal), GDM (yes/no), preterm delivery (<37 weeks, yes/no), or SGA (yes/no) without imputations for missing data. Both maternal (P = 0.019) and cord plasma ($P < 0.001$) cortisol levels were lower in cesarean versus vaginal births, whereas placental 11β-HSD2 expression levels were similar (P = 0.583). Both maternal (P = 0.007) and cord plasma (P = 0.002) cortisol concentrations were lower in GDM versus nondiabetic pregnancies, but the differences would become narrower and nonsignificant if the analyses were restricted to cesarean deliveries (all $P > 0.2$) or to vaginal births (all $P > 0.05$) (data not shown). There were no significant differences in placental 11β-HSD2 expression between infants of GDM and euglycemic pregnancies (P = 0.94). Maternal ($P < 0.001$) and cord plasma ($P < 0.001$) cortisol concentrations were lower in preterm versus term infants, whereas placental 11β-HSD2 expression was not significantly different (P = 0.11). There were no significant differences in maternal and cord plasma cortisol concentrations and placental 11β-HSD2 expression between male and female infants (all $P > 0.1$; data not shown).

Maternal cortisol levels at 32–35 weeks of gestation and cord blood cortisol levels were positively correlated (r = 0.28, $P < 0.001$). Cord plasma cortisol concentrations were positively correlated with gestational age at delivery (r = 0.33, $P < 0.001$), whereas placental 11β-HSD2 expression was not (r = −0.06, $P > 0.2$). Adjusting for gestational age at delivery, higher cord blood cortisol levels were associated with lower placental 11β-HSD2 expression (r = −0.14, P = 0.03). Both cord plasma cortisol and placental 11β-HSD2 expression were not correlated to birth weight z scores ($P > 0.2$).

In the correlation analyses without imputations for missing data, lower placental 11β-HSD2 expression were correlated with higher fasting plasma insulin concentration (r = −0.18, P = 0.017) and HOMA-IR (r = −0.17, P = 0.021) and tended to be correlated with higher systolic BP (r = −0.16, P = 0.057) in 1-year-old infants (Table 3). HOMA-β, diastolic BP, cIMT, and skinfold thickness at 1 year of age showed no correlation with placental 11β-HSD2 expression (all $P > 0.1$). Cord blood cortisol was negatively correlated with skinfold thickness (r = −0.20, P = 0.007), but was not correlated with other outcomes (e.g., HOMA-IR, BP) in infants at 1 year of age. Higher maternal plasma cortisol values was associated with higher maximum cIMT (r = 0.20, P = 0.032) but not with other outcomes (all $P > 0.1$) in infants at 1 year of age. Similar correlations were observed in the analyses with imputations for missing data.

The adjusted associations of placental 11β-HSD2, and maternal and cord plasma cortisol concentrations with cardiometabolic health indicators are presented in Table 4. The associations were similar to those observed

Table 1—Maternal, newborn, and infant characteristics in the study birth cohort

	Cases with complete data	All (with imputations)
Mothers	n = 246	
Ethnicity (white)	164 (66.7)	
Parity (primiparous)	93 (37.8)	
Education (university)	131 (53.3)	
Age (years)	31.0 ± 0.29	
Age ≥35 years	55 (22.4)	
Prepregnancy BMI (kg/m^2)	23.9 ± 0.32	
Obesity (BMI ≥30)	31 (12.6)	
GDM	26 (10.6)	
Gestational hypertensive disorders	12 (4.9)	
Tobacco smoking	16 (6.5)	
Drinking alcohol	38 (15.4)	
Newborns	n = 246	
Cesarean section	72 (29.3)	
Sex (boy)	125 (50.8)	
Gestational age (weeks)	39.0 ± 0.1	
Birth weight (g)	3,419 ± 29	
z score (WHO standards)	0.35 ± 0.06	
z score (Canadian standards)	0.10 ± 0.06	
SGA	16 (6.5)	
LGA	23 (9.4)	
Preterm	13 (5.3)	
Infants at 3 months	n = 222	n = 246
Weight (g)	6,294 ± 45	6,293 ± 45
Weight z score (WHO standards)	0.36 ± 0.06	0.36 ± 0.06
ΔWeight z score (0–3 months)	0.02 ± 0.07	0.02 ± 0.07
Breastfeeding	199 (90.9)	222 (90.2)
Infants at 1 year	n = 197	n = 246
Weight (g)	9,871 ± 79	9,871 ± 79
Weight z score (WHO standards)	0.50 ± 0.07	0.50 ± 0.07
ΔWeight z score (3–12 months)	0.14 ± 0.05	0.14 ± 0.05
Skinfold thickness (mm)	n = 197	n = 246
Triceps	9.1 ± 0.15	9.1 ± 0.15
Subscapular	7.2 ± 0.12	7.2 ± 0.12
Sum	16.2 ± 0.23	16.2 ± 0.23
BP (mmHg)	n = 147	n = 246
Systolic	88 ± 1.0	88 ± 1.0
Diastolic	55 ± 0.7	55 ± 0.7
cIMT (mm)	n = 119	N = 246
Mean	0.56 ± 0.005	0.56 ± 0.004
Maximum	0.65 ± 0.007	0.65 ± 0.007

Data presented are n (%) for frequency variables and mean ± SE for continuous variables.

Table 2—Placental 11β-HSD2 and maternal and fetal/cord plasma cortisol concentrations by mode of delivery, GDM, SGA, or preterm status

	Maternal plasma cortisol, nmol/L, 32–35 weeks			Cord plasma cortisol, nmol/L			Placenta 11β-HSD2, 11β-HSD2/β-actin ratio		
	n	Median	Mean ± SE	*n*	Median	Mean ± SE	*n*	Median	Mean ± SE
All	245	562	573 ± 11	226	271	318 ± 13	246	0.83	0.88 ± 0.026
Mode of delivery									
Cesarean	72	518	**534 ± 19***	64	199	**250 ± 27‡**	72	0.81	0.90 ± 0.049
Vaginal	173	577	588 ± 13	162	306	345 ± 14	174	0.84	0.88 ± 0.030
GDM									
Yes	26	525	**518 ± 42†**	26	198	**273 ± 57†**	26	0.79	0.95 ± 0.11
No	219	567	579 ± 10	200	285	324 ± 13	220	0.83	0.88 ± 0.025
SGA									
Yes	16	569	593 ± 37	14	313	317 ± 28	16	0.75	0.78 ± 0.087
No	229	562	571 ± 11	212	271	318 ± 14	230	0.83	0.89 ± 0.027
Preterm									
Yes	13	416	**453 ± 58***	10	113	**276 ± 14†**	13	1.10	1.10 ± 0.10
No	232	568	579 ± 11	216	279	320 ± 12	233	0.82	0.87 ± 0.026

Data values in boldface type indicate $P < 0.05$ compared with the reference group. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ in *t* tests for differences in log-transformed biomarker data comparing gestational cesarean vs. vaginal, GDM vs. nondiabetic, SGA vs. non-SGA, or preterm vs. term births.

in correlation analyses. Adjusted for maternal and infant characteristics, each log unit increase in placental 11β-HSD2 expression was associated with a 20.0% (95% CI 4.3%, 33.1%) decrease in fasting insulin ($P < 0.01$), a 21.3% (4.5%, 35.1%) decrease in HOMA-IR ($P < 0.01$), a 23.9% (4.4%, 47.0%) increase in glucose-to-insulin ratio ($P < 0.01$), and 3.7 mmHg (−0.6, 7.9) decrease in SBP ($P = 0.07$) in infants at age 1 year, respectively. Maximal cIMT at 1 year of age remained positively associated ($P < 0.01$) with maternal plasma cortisol measured at 32–35 weeks of gestation.

There were no significant mediation effects of cord plasma cortisol or skinfold thickness (all $P > 0.6$) in the associations of placental 11β-HSD2 expression with fasting plasma insulin, HOMA-IR, or glucose-to-insulin ratio in infants at 1 year of age (Supplementary Table 1). Similar effect estimates for placental 11β-HSD2 were observed in the models with or without further adjustment for cord plasma cortisol or skinfold thickness.

HOMA-IR at 1 year of age was higher in SGA versus non-SGA infants (mean ± SE: 1.26 ± 0.21 vs. 0.85 ± 0.21; $P = 0.024$), whereas there was no difference in HOMA- β, BP, cIMT, or skinfold thickness (all $P > 0.3$). There were no significant differences in all outcomes (HOMA-IR and HOMA-β, BP, cIMT, and skinfold thickness) in infants at 1 year of age comparing GDM ($n = 26$) versus euglycemic, gestational hypertensive ($n = 12$) versus nonhypertensive pregnancies, or preterm ($n = 13$) versus term infants (all $P > 0.1$). There were no differences in all cardiometabolic health outcomes at 1 year of age comparing LGA versus non-LGA infants.

Excluding preterm and SGA infants from the analyses, the associations between placental 11β-HSD2 and cardiometabolic health indicators at 1 year of age were similar.

Table 3—Pearson correlation coefficients (without/with imputations for missing data) of placental 11β-HSD2 and maternal and cord plasma cortisol with cardiometabolic health parameters in infants at 1 year of age

Infant outcome (age 1 year)	Maternal plasma cortisol 32–35 weeks	Cord plasma cortisol	Placental 11β-HSD2
Weight and skinfold			
Weight (*z* score)	−0.07/−0.06	−0.05/−0.06	−0.06/−0.03
Skinfold thickness‡	−0.05/−0.04	**−0.20†/−0.17***	−0.03/−0.03
Fasting blood			
Insulin	−0.11/−0.07	−0.01/0.01	**−0.18*/−0.19***
HOMA-IR	−0.06/−0.06	−0.01/−0.01	**−0.17*/−0.18***
HOMA-β	−0.05/−0.10	0.03/0.09	−0.12/−0.13
Glucose-to-insulin ratio	0.07/0.08	−0.01/−0.01	**0.17*/0.18***
Proinsulin-to-insulin ratio	0.05/0.02	−0.01/−0.02	0.11/0.10
Proinsulin	−0.06/−0.06	−0.03/−0.02	−0.07/−0.09
BP			
Systolic	−0.12/−0.14	−0.01/−0.04	−0.16§/−0.16§
Diastolic	−0.13/−0.14	−0.01/−0.04	−0.03/−0.03
cIMT			
Mean	0.11/0.08	−0.03/−0.07	0.04/0.04
Maximum	**0.20*/0.18***	−0.04/−0.07	0.03/0.03

Data presented are Pearson correlation coefficients adjusting for gestational age at specimen sampling; biomarker data were log-transformed in the correlation analyses; the correlation coefficients are presented in subjects with complete data/in all subjects with imputations for missing data; the sample sizes in complete case data analyses were 197 for weight *z* score and skinfold thickness, 182 for fasting blood biomarkers, 147 for BP, and 119 for cIMT; the sample size was 246 in all analyses with imputations for missing data. *$P < 0.05$; †$P < 0.01$; §$P = 0.06$; correlation coefficients in boldface type were significant at $P < 0.025$. ‡Sum of triceps and subscapular skinfold thickness.

Table 4—Adjusted associations of maternal and cord plasma cortisol and placental 11β-HSD2 with metabolic health indices in infants at age 1 year

Infant outcome (age 1 year)	Maternal plasma cortisol (32–35 weeks)	Cord plasma cortisol	Placental 11β-HSD2
Complete data cases‡			
Skinfold thickness	0.1 (−1.1, 1.2)	**−0.7 (−1.4, −0.03)***	−0.2 (−1.0, 0.6)
Fasting insulin	−13.4 (−33.9, 13.5)	−3.7 (−18.6, 13.9)	**−17.3 (−30.5, −1.6)***
Glucose/insulin ratio	14.4 (−12.9, 50.2)	2.4 (−12.8, 20.3)	**21.4 (3.0, 43.1)†**
HOMA-IR	−10.4 (−34.57, 22.6)	−6.1 (−21.7, 12.7)	**−19.9 (−33.8, −3.7)†**
HOMA-β	−14.2 (−37.16, 17.1)	8.0 (−11.0, 31.0)	−14.0 (−29.0, 3.9)
Systolic BP	−3.7 (−10.1, 2.6)	−0.7 (−4.6, 3.1)	−3.8 (−7.7, 0.1)
Diastolic BP	−4.0 (−8.2, −0.2)	−0.9 (−3.4, 1.7)	−0.7 (−3.3, 1.9)
cIMT (maximum)	**0.05 (0.01, 0.08)†**	−0.01 (−0.04, 0.02)	0.01 (−0.03, 0.04)
All cases (n = 246, with imputations for missing data)			
Skinfold thickness	0.2 (−1.00, 1.3)	**−0.8 (−1.5, −0.1)†**	−0.1 (−0.9, 0.6)
Fasting insulin	−12.0 (−34.3, 18.0)	−0.4 (−16.5, 18.8)	**−20.0 (−33.1, −4.3)†**
Glucose/insulin ratio	12.0 (−14.6, 46.8)	−0.6 (−14.3, 15.4)	**23.9 (4.4, 47.0)†**
HOMA-IR	−5.7 (−30.3, 27.8)	−1.9 (−17.7, 16.9)	**−21.3 (−35.1, −4.5)†**
HOMA-β	−16.4 (−38.6, 13.9)	12.6 (−5.0, 33.4)	−16.4 (−31.5, 2.1)
Systolic BP	−5.2 (−11.2, 0.9)	−0.7 (−4.4, 2.9)	−3.7 (−7.9, 0.6)
Diastolic BP	−3.2 (−7.7, 1.22)	−0.80 (−3.1, 1.5)	−0.7 (−3.4, 2.0)
cIMT (maximum)	**0.05 (0.01, 0.09)†**	−0.02 (−0.05, 0.01)	0.01 (−0.03, 0.04)

For biomarker outcomes, the data presented are the percentage change (95% CI) in the dependent variable (e.g., HOMA-IR) per log unit increase in the predictor variable (e.g., placental 11β-HSD2); for skinfold thickness and cIMT, the data presented are the mean changes in mm; for BP, the data presented are the mean changes in mmHg. The effect estimates were from generalized linear models adjusting for GDM, gestational hypertension, family history of diabetes, prepregnancy BMI, ethnicity, age, education, parity, smoking, alcohol use, mode of delivery, infant sex, gestational age, birth weight z score, weight z score changes between 0–3 months and 3–12 months of age, and breast feeding status in the first 3 months of life. Parsimonious final models were fitted excluding covariables with $P > 0.2$ that did not affect the comparisons. Therefore, only up to five covariables (GDM, prepregnancy BMI, family history of diabetes, maternal smoking, and infant sex) were included in the final adjusted models. ‡The sample sizes without imputations for missing data were 197 for skinfold thickness, 182 for fasting blood biomarkers, 147 for BP, and 119 for cIMT. *$P < 0.05$; †$P < 0.025$; values in boldface type are significant at $P < 0.025$.

CONCLUSIONS

Main Findings

To our knowledge, this is the first study on placental 11β-HSD2 in relation to cardiometabolic health indicators in postnatal life. Our results show that lower placental 11β-HSD2 expression was associated with a higher fasting insulin level and HOMA-IR but was not associated with HOMA-β, weight z score, skinfold thickness, and cIMT in 1-year-old infants. The association between lower placental 11β-HSD2 expression and higher systolic BP did not reach statistical significance (adjusted $P = 0.07$).

Data Interpretation and Comparisons With Previous Studies

It is notoriously difficult to have comparable data on circulating glucocorticoid levels since various measured and unmeasured stressful events can cause fluctuations in circulating concentrations. Indeed, we observed that infants in cesarean deliveries had lower cord plasma cortisol levels than infants of vaginal deliveries, as previously reported (25). In contrast, placental 11β-HSD2 levels were not affected by mode of delivery. Plausibly, data on the associations of infant cardiometabolic health indicators with placental 11β-HSD2, as a reflection of chronic fetal exposure to maternal cortisol, may be more robust than the associations with cord blood cortisol.

We are unaware of any reports on the relationships of placental 11β-HSD2 or cord blood cortisol with IR and β-cell function later in life in humans. Supporting our findings, a smaller study of 38 term SGA infants reported a negative correlation between placental 11β-HSD2 mRNA abundance and cord blood insulin and HOMA-IR at birth (26). Animal studies (27,28) have shown that glucocorticoids may impair the development of fetal pancreatic β-cells, inhibit insulin release, and affect insulin sensitivity. Our study showed that placental 11β-HSD2 was negatively associated with IR but was not associated with β-cell function in infancy. The observation suggests that lower placental 11β-HSD2 levels may have a negative implication for postnatal metabolic health in humans.

Mediation analyses showed that cord blood cortisol or adiposity in infancy could not explain the associations of placental 11β-HSD2 with fasting blood insulin and HOMA-IR in infancy. Cortisol concentrations are much higher in maternal blood than in fetal/cord blood, and the maternal source of cortisol is the primary source of cortisol exposure for the fetus during pregnancy, although the fetal adrenal gland becomes mature in late gestation with the capacity to secrete glucocorticoids in response to stressful events (5,7,29). The observed lack of effect mediation may be due to the fact that cord blood cortisol levels at the time of delivery could not reflect fetal cortisol chronic exposure levels during pregnancy. Also, the mediation analyses suggest that the impact of placental 11β-HSD2 expression on IR in infancy may operate through mechanisms other than affecting adiposity.

Animal studies showed that the inhibition of 11β-HSD2 and excess exposure of the fetoplacental unit to maternal glucocorticoids could program hypertension in the offspring (14,16). A prospective

birth cohort study (n = 286) showed that higher cord blood cortisol/cortisone ratios (indicating lower placental 11β-HSD2 activity) were associated with higher systolic BP in infants at 3 years of age (21). This is consistent with our observed marginally negative association between placental 11β-HSD2 and systolic BP (adjusted P = 0.07) in infants at 1 year of age. Accurate BP measurement in young infants is a challenge. Although we made every effort to minimize measurement errors, they could still be present, which would increase random variations and bias the associations toward the null.

cIMT at 1 year of age was not associated with placental 11β-HSD2 expression, but our results reveal a positive correlation with maternal plasma cortisol at 32–35 weeks of gestation. Although it is known that chronic stress and elevated circulating cortisol levels are associated with increased cIMT even in children (30), the association between antenatal markers of glucocorticoids or stress exposure and cIMT in the child is novel and has not been reported. Despite technical challenges, the assessment of cIMT in nonsedated infants is feasible and reproducible (31). cIMT is increased in term SGA versus normal birth weight infants at birth and 4 years of age (32–34), although we could not detect a difference in cIMT at 1 year of age probably because of the small number of SGA infants in our cohort. Taken together, available studies indicate that perinatal factors, including fetal exposure to higher maternal cortisol, may affect cIMT early in life. The long-term impact of such exposure and how postnatal factors can modulate cIMT remains to be established.

Data have been limited on alterations in maternal and cord blood cortisol concentrations and placental 11β-HSD2 levels in GDM pregnancies. A small study (23 GDM, 22 control subjects) reported higher maternal blood but normal cord blood cortisol concentrations in GDM versus non-GDM pregnancies (35). In contrast, we observed lower maternal and cord blood cortisol levels in GDM versus non-GDM pregnancies, but the differences disappeared in the analyses stratified by mode of delivery. More frequent cesarean sections could largely explain the lower cord blood cortisol levels in GDM pregnancies in our study cohort.

Consistent with the findings in the literature (36), cord plasma cortisol concentrations were lower in preterm versus term infants. This is in agreement with the fact circulating maternal cortisol levels, which are elevated in pregnancy, are known to rise progressively over advancing gestation and peak in the third trimester of pregnancy (6). Interestingly, we also observed that women with preterm deliveries had lower cortisol concentrations at 32–35 weeks of gestation. This contrasts with the findings of a previous study (37) reporting elevated maternal cortisol levels at 18–20 and 28–30 weeks of gestation but not at 35–36 weeks of gestation in pregnancies ending in preterm versus term deliveries. Considering the small number of preterm births in our cohort, larger cohort studies are required to clarify these associations.

Previous studies (38,39) have reported lower placental 11β-HSD2 gene expression or protein levels comparing preterm versus term births and SGA versus non-SGA infants. Our study did not detect any differences in placental 11β-HSD2 protein levels between preterm and term infants or between SGA and non-SGA infants. Caution is warranted in data interpretation because of the relatively small numbers of preterm and SGA infants in our study cohort.

We observed a negative correlation between cord blood cortisol and skinfold thickness (a crude adiposity indicator) in infants at 1 year of age. This is a surprise since decreased placental 11β-HSD2 expression and elevated cord blood cortisol levels have been associated with fetal growth restriction, which has been linked to increased risk of obesity and IR in adulthood (1–3). Caution is warranted in data interpretation since cord blood cortisol levels may not be able to reflect fetal cortisol exposure levels during pregnancy and since the implication of adiposity in infancy for metabolic health in adulthood remains unclear.

Strengths and Limitations

The main strengths are the prospective birth cohort, high rates of follow-up and specimen collection, timely collection and processing of cord blood and placenta specimens, and high-quality biochemical assays. Further, this cohort represents the largest sample to date of cIMT at 1 year of age and could serve as reference values for future studies. The main limitation is that we did not have data on placental 11β-HSD enzyme activity, which is not amenable for reliable detection. Second, the measurements of β-function and IR in 1-year-old infants were based on a single fasting blood sample, which is what is feasible at this age. It is impractical (or inappropriate) to apply more accurate but invasive measurement procedures (e.g., oral glucose tolerance test or the euglycemic clamp) in small infants.

In conclusion, our study provides the first observation that lower placental 11β-HSD2 expression is associated with higher IR in infancy, indicating that placental 11β-HSD2 expression may have a positive implication for metabolic health in postnatal life.

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Author Contributions. L.C., J.G., and Z.-C.L. conducted the data analysis and drafted the manuscript. Z.-C.L., A.C., W.D.F., J.D., and A.-M.N. contributed to the acquisition of research data. L.C., J.G., W.-J.W., and M.-L.Y. conducted the literature review. Z.-C.L., W.D.F., and A.-M.N. conceived the study with input from all coauthors. All authors contributed in revising the article critically for important intellectual content and approved the final version for publication. Z.-C.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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