Diminished 11β-Hydroxysteroid Dehydrogenase Type 2 Activity Is Associated With Decreased Weight and Weight Gain Across the First Year of Life

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Context:

Low birth weight is associated with adverse metabolic outcome in adulthood. Exposure to glucocorticoid (GC) excess in utero is associated with decreased birth weight, but the prospective longitudinal relationship between GC metabolism and growth has not been examined.

Objective:

We have hypothesized that changes in GC metabolism leading to increased availability may impair growth.

Design:

This was a prospective, longitudinal study with clinical measurements and 24-hour urinary steroid metabolite analysis at 1, 4, 12, 26, and 52 weeks after delivery in mothers and their babies.

Setting:

The study was conducted with observations and samples collected in the volunteers' own homes.

Participants:

Healthy mothers and newborn babies/infants participated in the study.

Interventions:

There were no interventions.

Main outcome measures:

Urinary steroid metabolite excretion quantified by gas chromatography/mass spectroscopy across the first year of life in relation to change in weight was measured.

Results:

The total production of the GC metabolites quantified increased across the first year of life. Markers of 11β-hydroxysteroid dehydrogenase type 1 activity increased from the age of 3 months as did those of 5α-reductase activity. After correcting for confounding variables, low markers of 11β-hydroxysteroid dehydrogenase type 2 activity was associated with reduced absolute weight and decreased weight

gain over the first year of life. In the mothers, 5α-reductase activity was low at birth and progressively increased to normal over the first 6 months postpartum.

Conclusions:

Increased GC exposure as a consequence of reduced 11β-hydroxysteroid dehydrogenase type 2 activity is likely to be a critical determinant of growth in early life. This not only highlights the central role of GCs and their metabolism, but also emphasizes the need for detailed longitudinal analyses.

There is a wealth of epidemiological evidence to support the hypothesis that low-birth-weight infants are predisposed to metabolic disease in adult life (1). Despite this, and faced with the global epidemic of obesity, diabetes mellitus, and metabolic disease, identification of the mechanisms underpinning this observation have remained elusive. The adrenal gland is responsible for the coordinated and highly regulated secretion of glucocorticoids (GCs), mineralocorticoids, and androgens, including dehydroepiandrosterone. Changing patterns of corticosteroid secretion and metabolism have been described in many common conditions including obesity and insulin resistance in children and adults (2–4). In utero, exposure to exogenous GCs at high doses, although often clinically indicated, can also have detrimental effects, including transient hyperinsulinemia in neonates (5), impaired vascular function, and reduced pancreatic β-cell function later in life (6). In rodent models, GC exposure decreases birth weight as well as inducing dysglycemia and insulin resistance in the offspring (7, 8), potentially delaying pubertal development (8).

The regulation of GC action is complex and not simply determined by circulating concentrations. At a prereceptor level, the isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) are able to interconvert inactive cortisone and active cortisol. 11β-HSD type 1 (11β-HSD1) is expressed in liver, adipose tissue, muscle, and bone and regenerates cortisol from cortisone. In contrast, 11β-HSD type 2 (11β-HSD2) is expressed in mineralocorticoid target tissues, including kidney and large bowel, in which it inactivates cortisol to cortisone and thereby ameliorates the action of cortisol to bind and activate the mineralocorticoid receptor, for which it shares a similar affinity to aldosterone. Genetic defects in 11β-HSD2 cause the syndrome of apparent mineralocorticoid excess (AME), which can present with uncontrolled hypertension and life-threatening hypokalemia in childhood and in addition is associated with low birth weight (9). Furthermore, decreased placental 11β-HSD2 gene expression has been implicated in the pathogenesis of intrauterine growth restriction (10).

In parallel, the A-ring reductases, 5α-reductase types 1 and 2, (5αR1 and 5αR2) and 5β-reductase convert cortisol and cortisone to their dihydrometabolites, and these are subsequently converted to tetrahydrometabolites through the action of 3α-hydroxysteroid dehydrogenase. In addition, 5α-reductase (largely 5αR2) is crucial for converting T to the more potent androgen, dihydrotestosterone, and genetic defects in 5αR2 lead to 46XY disorder of sexual differentiation; genetic defects in 5αR1 have not been described. The role of the A-ring reductases in neonatal and infant growth and development has not been explored.

In this study, we have tested the hypothesis that increased cortisol exposure through increased 11β-HSD1, decreased 11β-HSD2 or decreased A-ring reductase activity, individually or in combination, contributes to changes in growth and development across the first year of life. To answer this question, we designed a prospective longitudinal study to characterize corticosteroid metabolite profiles in a cohort of healthy babies and their mothers across the first year of life.

Materials and Methods

Clinical Protocol

The clinical protocol received full ethical approval from Birmingham East, North, and Solihull Research Ethics Committee, United Kingdom (reference number 10/H1206/67). Research and development approval was granted by Birmingham Women's National Health Service Foundation Trust (reference number 10/BWH/NO95).

Recruitment took place on low-risk maternity units in Birmingham, West Midlands, United Kingdom, and women without complications during pregnancy and labor were identified and approached; fully informed written consent was obtained. Mothers and infants were visited at 1 week, 1 month, 3 months, 6 months, and 12 months postpartum. At all time points apart from 3 months, maternal and infant weight and infant length were measured. Infants were weighed without clothes. Weight was converted to a SD score (SDS), which adjusts measurements for age and gender (Child Growth Foundation, 1996). Infant length was measured to the nearest 0.5 cm using a Seca 210 mobile measuring mat. Mothers were weighed wearing light indoor clothing without shoes. Maternal height was measured at 1 week postpartum using a stadiometer.

During the 1-week home visit, mothers reported their pregnancy medical history and completed questionnaires ascertaining demographic information. At each time point, mothers provided a single maternal urine sample and a 24-hour collection of wet diapers to determine androgen and cortisol metabolites (see below).

Extraction of urine from diapers

Diapers in the 24-hour collections were counted and weighed. The wet material was removed and placed in a beaker and a measured volume of 4% saline solution was added (~1 mL/g of gel). The samples were squeezed by hand for 5 minutes and then left at 4°C overnight to equilibrate. The diaper extract was then put through a potato ricer containing a square of nylon gauze and the liquid collected and filtered through glass wool to remove residual particles of gel. The samples were then analyzed as described below. The total volume of the collection was calculated from the equation: total volume (milliliters) = [weight of wet diapers (grams) – expected total weight of dry diapers (grams)] + total amount of saline added. Prior development established that disposable diapers consist of cellulose fluff containing supersorb granules (polyacrylate resin) and that there were no material differences between the major brands, eg, Pampers (Proctor and Gamble) and Huggies (Kimberly-Clark). Pure samples of the resin were shown not to selectively retain steroids. The saline concentration was established at 4% to minimize gel swelling. Experiments involving dripping urine onto new diapers established that steroid recovery was close to 100%. The dry weight of a given diaper type was established by weighing 20 examples and obtaining the mean. Dry diaper weights were small in relation to the urine weight and showed low variation. Therefore, possible errors in the calculated urine volume (due to an inaccurate estimation of dry diaper weight) were considered to be negligible.

Urinary steroid analysis

Urinary androgen and corticosteroid metabolite analysis was performed by gas chromatography/mass spectroscopy as described previously (4, 11). Briefly, free and conjugated steroids were extracted from urine by solid-phase extraction. Steroids were enzymatically released from conjugation and reextracted. An internal standard (stigmasterol) was added prior to derivitization to form methyloxime-trimethylsilyethers. The final derivative was dissolved in cyclohexane, which was transferred to a gas chromatography/mass spectroscopy autosampler vial. The samples were run on an Agilent 5973 instrument in selected ion monitoring mode. It should be stated that only a few classical cortisol (F) metabolites were measured, dependent on their availability as authentic compounds necessary for instrument calibration. Newborns in particular have important metabolites that were not included, especially 1β- and 6α-hydroxylated metabolites of tetrahydrocortisone and the cortolones (12). In this document, use of the term, total F metabolites (see definition below), means the total of measured metabolites, not the complete excretion of all metabolites. We have estimated that the percentage of measured metabolites as compared with the complete excretion of all metabolites is approximately

49% at 1 week of age, 60% at 1 month, 74% at 3 months, 85% at 6 months, and 91% at 12 months of age.

Interpretation of totals and indices of urinary steroid excretion

The sum of total cortisol metabolites [tetrahydrocortisol (THF), tetrahydrocortisone (THE), 5α-THF, α-cortolone, cortisone (E), F, β-cortolone, β-cortol, α-cortol] provides a reflection of cortisol secretion rate (total F metabolites). The ratio of tetrahydrometabolites of cortisol (THF + 5αTHF) to those of cortisone (THE) provides a reflection of 11β-HSD1 activity when considered with the ratio of urinary F to E, which more accurately reflects renal 11β-HSD2 activity (higher F to E ratios indicative of decreased 11β-HSD2 activity). Urinary free F and E, which provide the most accurate assessment of 11β-HSD2 activity were not measured (11,13), although our own in-house analysis has shown a good correlation between hydrolyzed (free measurements) and nonhydrolyzed samples (cortisol r = 0.80, cortisone r = 0.71). In addition, the ratio of cortols to cortolones reflects 11β-HSD1 activity. Evidence to support the interpretation of these ratios is provided from studies using selective 11β-HSD1 inhibitors and patients with mutations causing functional defects in 11β-HSD1 (14, 15). The activities of 5α- and 5β-reductases can be inferred from measuring the ratio of 5αTHF to THF and androsterone (An) to etiocholanolone (Et).

Creatinine assay

Creatinine was measured using a QuantiChrom creatinine assay kit (BioAssay Systems), which is designed to measure creatinine directly in biological samples without any pretreatment. The assay is based on a kinetic Jaffe reaction, which uses picrate that forms a red-colored complex with creatinine. The intensity of the color, measured at 510 nm, is directly proportional to creatinine concentration in the sample.

Statistical approach

Data are presented as median and interquartile range (IQR) unless otherwise stated. Statistical analysis was undertaken using the R software language and the mixed-effects models were calculated using the LME4 package (http://cran.r-project.org/package=lme4); the Markov Chain Monte Carlo estimates of the random effects parameters and confidence intervals were calculated using the languageR package. Data were modeled using a mixed fixed and a random factor linear effects model, which used random effects to model those sources of variation attributable to the design of the study. In this model, time of measurement and type of steroid were treated as random factors within a random intercept model. Because each of the steroid levels was measured on a different metric, these data were standardized to z-scores prior to the subsequent analysis. For infant samples, the additional effect of gender was incorporated in to the statistical models to determine the two-way interactions (eg, gendersteroid and gendertime) and three-way interactions effect (eg, gendertimesteroid). Detailed statistical methods and model descriptions are presented in Supplemental Tables 1–4.

Results

Fifty-nine mothers [mean age 29 ± 5 y, mean body mass index (BMI) 26.9 ± 3.9 kg/m^2] and their infants [36 males, 23 females; mean birth weight 3.51 kg (SD 0.36)] were recruited from low-risk maternity units after delivery (all provided informed written consent). Thirty-four percent (20 of 59) were in their first pregnancy, 19% (11 of 59) continued to smoke during pregnancy, and 24% (14 of 59) consumed alcohol, although all within recommended limits. None had any significant past medical history and none were taking any regular medication. Any mother with gestational diabetes, preeclampsia, or pregnancy-induced hypertension was excluded from the study. Infants born prematurely (prior to 36 wk gestation) or small for gestational age were not eligible for this study. All births were spontaneous normal vaginal deliveries without compilations. Birth weight (absolute and SDS) and growth across the first year of life of the neonatal/infant cohort are presented in Table 1. Steroid ratios from one infant were excluded from the analysis because the ratios reflecting the 5α-reductase activity were more than 2 SD above the mean.

Table 1. Changes in Weight and Length Over the First Year of Life in a Cohort of Healthy Babies

Sex	Birth		1 Week		1 Month		6 Months		12 Months	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
n	37	22	29	11	29	14	24	13	29	20
Age at visit, wks			1.3 ± 0.3	1.3 ± 0.4	4.8 ± 0.6	4.7 ± 0.5	26.7 ± 1.3	26.9 ± 0.7	53.1 ± 1.9	53.3 ± 1.8
Weight, kg	3.54 ± 0.39	3.46 ± 0.30	3.56 ± 0.38	3.41 ± 0.32	4.63 ± 0.47	4.28 ± 0.39	8.28 ± 1.11	7.63 ± 0.81	10.41 ± 1.36	9.37 ± 1.01
Weight SDS	0.13 ± 0.77	0.38 ± 0.71	−0.55 ± 0.75	−0.53 ± 0.64	0.05 ± 0.78	−0.05 ± 0.71	0.13 ± 1.25	0.03 ± 0.99	0.15 ± 1.27	−0.23 ± 1.07
SDS weight gain 1 to 12 mo									0.13 ± 1.27	−0.30 ± 1.13
Length, cm	52.2 ± 2.7	51.9 ± 2.3	53.6 ± 2.2	53.1 ± 1.7	58.4 ± 2.5	56.6 ± 1.6	73.1 ± 2.9	70.0 ± 2.4	79.4 ± 2.7	77.2 ± 3.2
Length SDS	0.74 ± 1.25	1.12 ± 1.47	0.70 ± 1.07	0.98 ± 0.85	1.59 ± 1.17	1.34 ± 0.77	2.36 ± 1.26	1.81 ± 1.08	1.42 ± 1.13	1.18 ± 1.33

Data presented are mean ± SD. Although urine samples were collected at 3 months, clinical parameters were not measured.

Changes in corticosteroid metabolism across the first year of life

There were no significant differences in steroid metabolites or their ratios between boys and girls, and therefore, the analysis against time of the complete cohort is presented. The absolute data divided according to gender are presented in Supplemental Table 5. Steroid excretion data are summarized in Figure 1 and Table 2.

Figure 1. Neonatal and infant cortisol metabolite production (micrograms per 24 h) (A) and urinary steroid metabolite ratios (B–F) across the first year of life. The F to E ratio (B) reflects 11β-HSD2 activity; 5αTHF+THF to THE (C) and cortol to cortolone ratios (D) ratios reflect 11β-HSD1 activity and 5αTHF to THF (E) and An to Et (F) ratios reflect 5α-reductase activity. DHEA, dehydroepiandrosterone. There was no impact of gender on the patterns of change in steroid hormone metabolites with time, and *P* values reflect the interaction of individual steroid metabolites or ratios (boys and girls combined) with time.

Table 2. Longitudinal 24-Hour Urinary Steroid Metabolite Analysis in a Cohort of Healthy Babies Across the First Year of Life

		1 Week (n = 50)	1 Month (n = 56)	3 Months (n = 55)	6 Months (n = 49)	12 Months (n = 47)
Total F metabolites						
Median		280.7	539.8	767.9	970.6	999.4
	IQR	273.1	405.0	602.2	639.3	852.9
THF						
Median		1.50	2.35	15.1	51.6	87.7
	IQR	1.93	2.47	12.5	48.3	94.9
5α-THF						
Median		0.97	5.76	95.1	260.6	255.6
	IQR	2.05	11.25	96.7	207.6	293.0
THE						
Median		188.8	309.1	404.1	382.2	382.7
	IQR	157.9	247.7	397.2	278.4	398.4
Cortisol						
Median		10.5	11.4	9.4	8.0	10.7
	IQR	10.3	10.0	5.6	6.4	15.3
Cortisone						
Median		17.7	26.4	20.6	18.2	16.6
	IQR	20.1	24.7	12.9	11.4	18.4
α-Cortol						
Median		7.9	18.1	14.1	19.1	26.3
	IQR	9.1	22.5	12.7	14.9	25.6
β-Cortol						
Median		1.8	4.1	12.5	27.1	50.0
	IQR	2.1	4.4	11.4	23.9	63.7

		1 Week (n = 50)	1 Month (n = 56)	3 Months (n = 55)	6 Months (n = 49)	12 Months (n = 47)
α-Cortolone						
Median		7.8	25.8	40.3	55.3	71.3
	IQR	13.4	26.1	27.7	30.7	69.6
β-Cortolone						
Median		49.9	118.5	126.5	93.5	82.2
	IQR	64.7	128.2	125.4	75.5	97.1
An						
Median		2.1	4.4	4.1	3.6	3.8
	IQR	2.0	5.3	4.3	3.6	7.3
Et						
Median		0.73	0.67	0.55	0.62	1.75
	IQR	0.69	0.71	0.53	0.49	2.50
DHEA						
Median		2.83	2.82	1.47	0.85	0.91
	IQR	3.04	3.79	1.69	1.02	1.60
16α-OH-DHEA						
Median		191.6	294.0	57.9	14.3	5.1
	IQR	437.7	546.1	65.2	20.4	11.2
F to E ratio						
Median		0.53	0.38	0.47	0.50	0.70
	IQR	0.31	0.16	0.13	0.19	0.34
(THF+5αTHF) to THE ratio						
Median		0.02	0.03	0.35	0.91	0.95
	IQR	0.01	0.03	0.29	0.48	0.45
Cortol to cortolone ratio						
		0.16	0.16	0.17	0.34	0.53

		1 Week (n = 50)	1 Month (n = 56)	3 Months (n = 55)	6 Months (n = 49)	12 Months (n = 47)
Median						
	IQR	0.10	0.17	0.16	0.13	0.20
5αTHF to THF ratio						
Median		0.86	3.33	6.71	4.92	2.71
	IQR	0.70	2.56	3.47	3.27	1.36
An to Et ratio						
Median		2.74	6.25	7.53	5.51	2.63
	IQR	2.70	5.38	6.32	2.98	1.58

Abbreviations: DHEA, dehydroepiandrosterone; OH-DHEA, hydroxydehydroepiandrosterone. Data presented are median with the IQR. Individual steroid metabolites are quoted in micrograms per 24 hours. Statistical analysis was performed using Wald type II χ^2tests of deviation for the time by steroid interaction. There was no impact of sex on the statistical modeling.

Total cortisol metabolites increased across the first year of life ($P < .00001$) (Table 2 andFigure 1). However, this must be interpreted with caution because the proportion of quantified metabolites increased with time (see *Materials and Methods*). 11β-HSD1 activity, reflected by the 5αTHF+THF to THE and cortol to cortolone ratios, was almost undetectable until 3 months of age and then increased dramatically between 3 and 6 months, with stable values at 12 months ($P < .00001$) (Table 2 and Figure 1). 5α-Reductase activity, as estimated by the 5αTHF to THF and An to Et ratios, was also low at birth, rising significantly to a peak at 3 months of age and then showing a decrease at 6 months with a further decrease at 12 months of age ($P < .00001$) (Table 2 and Figure 1). Both 5α- and 5β-reduced metabolites of cortisol (5α-THF and THF, respectively) increased progressively, but the excretion of the 5α-epimer was 5-fold higher (Table 2 and Figure 1). The F to E ratio also increased with time, indicative of decreasing 11β-HSD2 activity, although the change was less marked than that observed for 11β-HSD1 and 5α-reductase activity ($P < .0005$) (Table 2 and Figure 1).

Changes in maternal corticosteroid metabolite analysis postpartum

Spot urine analysis rather than 24-hour samples were analyzed and absolute corticosteroid concentrations were corrected per gram of urinary creatinine to allow for the assessment of absolute production rates. Total F metabolite production and urinary F to E ratio did not change significantly over the first 12 postpartum months. Although the cortol to cortolone ratio was weakly associated with time ($P = .047$, Table 3), there was no relationship with the 5αTHF+THF to THE, suggesting that 11β-HSD1 activity did not change significantly.

Table 3. Longitudinal Changes in Urinary Steroid Metabolite Analysis in a Cohort of Healthy Mothers in the First Postpartum Year

		1 Week (n = 45)	1 Month (n = 53)	3 Months (n = 45)	6 Months (n = 46)	12 Months (n = 41)
Total F metabolites						
Median		6711	7476	6661	7214	6838
	IQR	2786	2552	3325	3015	2855
THF						
Median		1580	1480	1095	1229	1003
	IQR	722	662	609	558	379
5α-THF						
Median		265	551	793	996	831
	IQR	197	451	621	781	666
THE						
Median		2326	2764	2443	2394	2363
	IQR	1217	1026	174	1425	1386
Cortisol						
Median		84.0	48.0	44.0	50.5	62.0
	IQR	64.5	32.5	35.0	32.0	48.5
Cortisone						
Median		108.0	77.0	69.0	87.0	120.0
	IQR	76.0	40.0	42.0	43.8	92.5
α-Cortol						
Median		326.0	247.0	211.0	277.0	237.0
	IQR	179.0	146.0	92.0	110.3	118.5
β-Cortol						
Median		440.0	397.0	330.00	385.5	339.0
	IQR	258.5	225.0	206.5	224.5	163.0

		1 Week (n = 45)	1 Month (n = 53)	3 Months (n = 45)	6 Months (n = 46)	12 Months (n = 41)
α-Cortolone						
Median		1205	1112	856	1036	1072
	IQR	569	663	523	454	552
β-Cortolone						
Median		385.0	469.0	436.0	500.0	482.0
	IQR	196.0	255.5	241.0	264.8	223.0
An						
Median		583.0	679.0	1148.0	1326.0	1565.0
	IQR	560.5	852.0	921.0	1092.8	1119.0
Et						
Median		1237	1261	1262	1079	1152
	IQR	939	969	933	698	900
DHEA						
Median		146.0	88.0	125.0	104.0	159.0
	IQR	270.0	124.0	210.5	227.3	503.0
16α-OH-DHEA						
Median		244.0	212.0	246.0	240.0	400.0
	IQR	480.5	279.5	378.0	298.3	645.0
F to E ratio						
Median		0.80	0.62	0.63	0.63	0.63
	IQR	0.33	0.30	0.24	0.28	0.33
(THF+5αTHF) to THE ratio						
Median		0.86	0.79	0.90	1.02	0.77
	IQR	0.32	0.26	0.33	0.36	0.22
Cortol to cortolone ratio						
		0.47	0.40	0.45	0.48	0.39

		1 Week (n = 45)	1 Month (n = 53)	3 Months (n = 45)	6 Months (n = 46)	12 Months (n = 41)
Median						
	IQR	0.22	0.15	0.17	0.13	0.11
5αTHF to THF ratio						
Median		0.18	0.39	0.75	0.87	0.89
	IQR	0.12	0.32	0.51	0.82	0.53
An to Et ratio						
Median		0.45	0.57	0.99	1.15	1.20
	IQR	0.24	0.47	0.95	0.88	0.59

Data presented are median with the IQR. Urinary corticosteroid production is expressed per grams of urinary creatinine. DHEA, dehydroepiandrosterone; OH-DHEA, hydroxydehydroepiandrosterone. Statistical analysis was performed using Wald type II χ^2tests of deviation for the time by steroid interaction.

However, 5α-, relative to 5β-reductase activity, reflected in the 5αTHF to THF and An to Et ratios was low in the immediate postpartum period and increased over the initial 3 months and then stabilized (both $P < .00001$, Table 3). By 12 months, values were similar to those seen in a reference cohort of women (data not shown). Changes in the 5αTHF to THF and An to Et ratios were driven by lower absolute concentrations of both 5αTHF ($P < .0001$) and An ($P < .05$), indicative of changes specifically in 5α- rather than 5β-reductase activity.

The relationship between maternal and neonatal/infant corticosteroid metabolism

Partial correlation analysis was performed, adjusting for confounding variables (including infant gender and weight SDS, maternal age, BMI, and smoking) to examine any potential relationships between maternal and neonatal/infant urinary steroid metabolites; no significant relationships at any time point were identified (data not shown).

Corticosteroid metabolism as a regulator of neonatal/infant growth

Partial correlation analysis was performed adjusting for confounding variables including infant gender, maternal age and BMI, smoking during pregnancy and the age of introduction of solids. Elevation of the urinary F to E ratio at week 1, consistent with impaired 11β-HSD2 activity, was inversely related to weight at week 1 ($R^2 = –0.46$, $P < .01$). Furthermore, at 12 months of age, there was a negative relationship between F to E ratio and 12-month weight SDS ($R^2 = –0.40$, $P < .05$) as well as weight gain SDS over the first 12 months of life ($R^2 = –0.51$, $P < .01$). In addition, length SDS at 12 months was also negatively associated with F to E ratio ($R^2 = –0.35$, $P < .05$), suggesting that impaired 11β-HSD2 activity with consequent increased cortisol availability may limit growth (Table 4).

Table 4. Partial Correlation (2-Tailed) Analysis Examining the Relationship Between Neonatal/Infant Corticosteroid Metabolite Ratios and Weight Across the First Year of Life With Adjustment for Infant Gender, Maternal Age and BMI, Smoking During Pregnancy and the Age of Introduction of Solids (N = 31)

Urinary Steroid Metabolite Ratio	1-Week Weight SDS	1-Month Weight SDS	6-Month Weight SDS	12-Month Weight SDS	1- to 12-Month SDS Weight Gain
Concurrent F/E	−0.46[a]	−0.21	−0.08	−0.40[b]	−0.51[a]
Concurrent THF+5αTHF/THE	0.09	0.03	−0.15	−0.09	−0.11
Concurrent 5αTHF/THF	−0.05	−0.00	−0.06	0.05	0.09
Concurrent Total F Metabolites	0.20	0.19	0.04	0.03	−0.09

a*P* < .01;
b*P* < .05.

In contrast, there was no relationship between urinary steroid metabolite ratios reflecting 11β-HSD1 activity, 5α-reductase activity, or total GC metabolite production rate with absolute weight at the time of analysis or weight gain across the duration of the study (Table 4). However, after correcting for confounding variables, increased duration of breastfeeding was associated with higher 5α-reductase activity at 6 and 12 months and increased 11β-HSD1 activity at 12 months (Supplemental Table 6). There were no relationships between urinary steroid metabolite ratios and smoking status (data not shown).

Discussion

In this study, we have performed the first longitudinal characterization of corticosteroid metabolism across the first year of life. We have highlighted dramatic changes in the pattern of corticosteroid metabolite production and identified a potential role for GC metabolism, notably 11β-HSD2, to regulate growth and weight gain.

Both GC receptor and mineralocorticoid receptors (MR) are expressed in most tissues throughout fetal development (16, 17) and are critical to development; GC receptor knockout mice die shortly after birth (18). Clinical treatment with exogenous GC is indicated to promote organs, notably lung maturation (19), but in both human and rodent models, GC excess is associated with decreased birth weight (20–22).

We have shown that decreased 11β-HSD2 activity was associated with decreased weight gain across the first year of life as well as decreased length at 12 months, and it is possible that this is a reflection of decreased GC clearance. 11β-HSD2 is highly expressed in the human placenta, in which it serves to protect the developing fetus from high levels of active GCs. Although expressed in the fetus (16), a recent study has suggested that renal neonatal 11β-HSD2 activity is low in comparison with placental activity (23). In our study, the F to E ratio increased across the first year of life (suggesting decreasing 11β-HSD2 activity), and at 12 months, the levels were similar to those seen in the maternal cohort and to those observed in an adult control population (4); it is possible that this reflects changing patterns of enzyme expression. In our cohort, F to E ratios were, if anything, lower than those measured in the maternal samples at week 1, and this would tend to suggest increased (rather than

decreased) 11β-HSD2 activity in the first weeks after birth. The discrepancies in data may reflect differences in methodology, but perhaps most importantly, the use of 24-hour samples rather than spot urines. Unfortunately, we did not have access to serum or placental samples as part of this study. In rodent models, the levels of 11β-HSD2 correlate with fetal size (24), and there is evidence to support this in humans in whom the mRNA levels and the activity of 11β-HSD2 are associated with lower birth weight (25), although this is not a consistent finding in all studies (26). However, pharmacological inhibition of 11β-HSD2 with carbenoxolone and the genetic manipulation of 11β-HSD2 activity decrease birth weight (27, 28).

More than 50 different mutations in *HSD11B2*, the gene encoding 11β-HSD2, that cause AME have now been described (29). Cortisol and aldosterone share a similar affinity for the MR, and therefore, in the absence of 11β-HSD2, cortisol (which circulates at levels > 1000-fold that of aldosterone) will become the predominant ligand for the MR, leading to hypertension and hypokalemia. Several cases of AME presenting in the first year of life have been described and are associated with low birth weight, and this is believed to be due, at least in part, to increased fetal exposure to GCs (30). In our study, no child had urinary steroid metabolite ratios that would suggest undiagnosed AME, and it is possible therefore that a functional defect in 11β-HSD2 is responsible for the observations in this study. Although the absence of 11β-HSD2 activity causing low weight gain is clearly detrimental, excessive weight gain across the first year of life is also associated with adverse outcomes in childhood (31). It is possible therefore that there is a balance of activity that is optimal for growth across the first year of life, and this would need to be tested in extended longitudinal studies across childhood.

11β-HSD1 generates active GCs within key metabolic target tissues. In our study, activity remained low until 3 months of age and then gradually increased. This is likely to have a functional importance because case reports have been described in which neonates born with congenital adrenal hyperplasia fail to respond to inactive cortisone acetate (which requires 11β-HSD1 for activation to cortisol) (32). Urinary steroid metabolite assessment of 11β-HSD1 activity is believed to largely reflect hepatic activity. Data from preclinical studies and clinical trials of selective 11β-HSD1 inhibitors have demonstrated a role for 11β-HSD1 in the regulation of glucose and lipid metabolism (33, 34). It is plausible that the predisposition of neonates to develop hypoglycemia could arise as a result of a decreased gluconeogenesis, due to lack of ability to locally regenerate active glucocorticoids, although this hypothesis remains entirely untested. The lack of a relationship between 11β-HSD1 activity and growth across the first year of life is perhaps to be expected. Lack of expression and activity in the first few months of life would suggest that the contribution that 11β-HSD1 makes to GC exposure over this time is limited. In addition, there is no evidence from rodent models that genetic deletion of 11β-HSD1 impairs growth and development in the early stages of life (35).

5αR1 is expressed in the neonatal liver, and this continues throughout life. In the nongenital skin, expression persists up until 2–3 years of age and then decreases but increases again around the time of puberty (36); it is thought to be contributory to pubertal virilization in patients with 5αR2 deficiency. 5αR2 is also expressed in the neonatal and adult liver. Expression remains in the nongenital skin for the first 2–3 years of life and then decreases, only persisting in the prostate, epididymis, seminal vesicles, and urogenital skin (37). Despite the evidence of a link between metabolic phenotype and 5αR in adults (4, 38–40), we did not observe any relationship with growth in the first year. However, activity was low at birth increasing dramatically at 3 months with levels decreasing by 6 and 12 months. Our longitudinal data are in agreement with published cross-sectional analyses that have shown a similar relationship with time (41).

In addition to these direct actions of GCs, there is evidence that exposure to GC excess has a detrimental impact later in life though programming. In a variety of animal models, GC exposure during gestation can impact adult phenotype (42, 43). Blood pressure is increased and this may be driven by alterations in the pattern of corticosteroid receptor expression, changes in sympathetic innervation, and vascular reactivity (44, 45). Similarly, adult insulin sensitivity can also be altered by prenatal GC exposure, leading to altered glucose homeostasis (46) and may contribute to the

development of conditions including nonalcoholic fatty liver disease (47) and impaired pancreatic β-cell development (48).

Prereceptor GC metabolism has also been implicated in the programming process. Dexamethasone treatment to pregnant rodents induces hypertension in adult offspring that is associated with decreased 11β-HSD2 expression in the distal nephron without any significant change in MR or 11β-HSD1 expression (49). Although in this study we failed to demonstrate any relationship between 11β-HSD1 activity and growth across the first 12 months of life, GC exposure during gestation in a primate model increases 11β-HSD1 expression in liver and adipose tissue later in life (50) and based on rodent models and clinical studies, this may contribute to the development of an adverse metabolic phenotype (51).

The data in humans are less clear, with discrepancies in the published data as to the impact of GC administration in the first few years of life (52, 53). The impact later into adult life also remains controversial, with some studies showing a detrimental impact on blood pressure and insulin action (54, 55), but this is not a consistent finding across all studies (56). We have examined outcomes only in the first year of life, but tracking changes in metabolic and steroid metabolite phenotype as this cohort ages will prove highly informative.

In conclusion, we have demonstrated a relationship between 11β-HSD2 activity and weight across the first year of life, suggesting that increased GC exposure through this mechanism may limit growth. Further studies are undoubtedly warranted, not only to see whether this longitudinal relationship is maintained later into childhood but also to understand its relevance in children who were small for gestational age or born after maternal preeclampsia and pregnancy-induced hypertension.
Abbreviations:

AME	apparent mineralocorticoid excess
An	androsterone
BMI	body mass index
E	cortisone
Et	etiocholanolone
F	cortisol
GC	glucocorticoid
11β-HSD	11β-hydroxysteroid dehydrogenase
11β-HSD1	11β-HSD type 1
11β-HSD2	11β-HSD type 2
IQR	interquartile range
MR	mineralocorticoid receptors
5αR1	5α-reductase type 1
5αR2	5α-reductase type 2
SDS	SD score
THE	tetrahydrocortisone
THF	tetrahydrocortisol.

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