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Comments

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Intra-individual consistency in endocrine profiles across successive pregnancies

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Context: It is yet unknown how similar women's hormone levels are during successive pregnancies, and very little is known about the degree to which siblings experience similar prenatal environments. Given the importance of understanding how women's reproductive life-histories exert cumulative effects on health *via* hormone exposure, and the importance of understanding how fetal programming *via* endocrine signaling affects sibling trait concordance, here we address this important lacuna in the literature.

Objective: To investigate how consistent are women's hormone profiles across two successive pregnancies.

Design and Main Outcome Measures: This longitudinal, prospective study followed a cohort of 28 women across two pregnancies (PREG 1; PREG 2). Women's circulating hormone levels were assessed from blood samples at 25, 31, and 37 weeks' gestation for adrenocorticotrophic hormone (ACTH), placental corticotropin-releasing hormone (pCRH), cortisol, estradiol, and progesterone. ACTH and cortisol levels were assessed 3-months postpartum. Research questions include: Are hormone levels in PREG 2 significantly different from levels in PREG 1? What proportion of variance in PREG 2 hormone levels is attributable to variance in PREG 1 levels? Are hormone levels more stable between PREG 1 and PREG 2 compared with postpartum phases following these pregnancies? Is pCRH, which is completely placentally derived, less similar than other hormones across successive pregnancies?

Setting: Psychobiology laboratory.

Participants: Pregnant women in California.

Results and Conclusions: Comparisons of hormone concentrations across women's successive pregnancies *via* paired t-test revealed substantial consistency from one pregnancy to another, with only significant differences between pregnancies for pCRH. Regressions revealed substantial predictability from one pregnancy to another, with between 17%–56% of PREG 2 variances accounted for by PREG 1 values. Women exhibited lower degrees of consistency and predictability in hormone levels across postpartum phases compared with gestational concentrations. This is the first study to describe maternal and placental hormone levels across successive pregnancies.

It is unknown how similar a woman's hormone levels during one pregnancy are to the same woman's hormone levels during a subsequent pregnancy. The extent to which hormone concentrations during pregnancy are variable within a woman's lifespan may be influenced by ma-

ternal and fetal physiology and genetics, as well as the mother's environment, psychology, and behavior. Circulating hormone concentrations during pregnancy are of major interest for understanding how gestational endocrinology affects lifespan health and development of both

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Abbreviations:

mother and child. Previous studies have estimated cumulative hormone exposures based upon reproductive life-history patterns for the purpose of understanding how the endocrinology of female reproductive physiology exerts long-term effects on women's health (1–4), but it remains unknown whether hormone exposures from different pregnancies across a woman's lifespan exert equivalent effects. Additionally, many studies compare monozygotic twins, dizygotic twins, and sibling pairs to determine the contributions of genetics, intrauterine environment, and postnatal environment to phenotype (5). These comparisons assume highly variable intrauterine conditions in different pregnancies of the same mother, and yet the degree of similarity between siblings' prenatal environments remains unknown.

The hormones of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-ovarian (HPO) axes are present at the highest concentrations of the female lifespan during the phase of pregnancy (6, 7). For this reason, in conjunction with the observations that HPA and HPO hormones have been broadly implicated in maternal health (1–4) and as effectors of fetal programming (8–10), this study focuses on HPA hormones adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), and cortisol, and HPO hormones estradiol and progesterone. We measure mean hormone concentrations across midto-late pregnancy in two successive pregnancies for each woman (hereafter PREG 1 and PREG 2), in addition to hormone concentrations at each timepoint. Our research questions can be summarized as the following. (1) *Are hormone levels in PREG 2 significantly different from hormone levels in PREG 1?* (2) *What proportion of variance in PREG 2 hormone levels is attributable to variance in PREG 1 hormone levels?* Additionally, we address the issue of whether pregnancy represents a phase of particularly consistent hormone levels across the lifespan, compared to the nonpregnant state. This brings us to another research question: (3) *Are hormone levels more stable between PREG 1 and PREG 2 compared with the postpartum phases following PREG 1 and PREG 2?* Furthermore, we predict that circulating CRH, which derives from the placenta, should exhibit less predictability in levels across successive pregnancies compared with the other hormones, which derive at least partly from maternal organs, because different fetuses are semiallogeneic to the mother. These differences in genetics could contribute to differences in phenotypes and adaptive strategies. This prompts our final research question: (4) *Is placental CRH (pCRH) less similar than the other hormones across PREG 1 and PREG 2?*

Materials and Methods

Cohort and procedures

Participants were women involved in a larger, prospective, longitudinal study of gestational and postnatal psychobiology at a large university medical center in Southern California. Women were recruited during their first trimester of pregnancy based on the following criteria: singleton pregnancy; over age 18; English-speaking; nonsmoking; absence of any medical condition that could dysregulate neuroendocrine function. The subset of the larger cohort analyzed in this study were selected because they enrolled in the study twice, and were included only if they attended all 8 study visits relevant to our analyses, which occurred at 24–26, 30–32, and 36–38 weeks' gestation and 12–14 weeks ("3-months") postpartum, and then at the same timepoints in a subsequent pregnancy (Table 1). A blood sample was obtained at each study visit. Protocols were approved by institutional review boards of participating institutions, and written informed consent was obtained from all women before participation.

Endocrine measures

Blood draw occurred in the afternoon. Two 10-mL samples were withdrawn by antecubital venipuncture into EDTA-treated (purple top) vacutainers for plasma analysis, which were chilled on ice immediately, and red top vacutainers for serum analysis, which sat at room temperature until clotted (vacutainers: Becton Dickinson and Company, Sumter, SC). Blood samples in purple top vacutainers were decanted into polypropylene tubes, and 500-kallikrein inhibitor units/mL of aprotinin (Sigma-Aldrich Corp, St. Louis, MO) were added. All samples were centrifuged at 2000g for 15-minutes, and then stored at -70°C until assaying.

Plasma ACTH levels were determined by solid-phase two-site immunoradiometric assay (IRMA) using human ACTH antibodies (Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma samples (200- μL) combined with ACTH-labeled antibody (100- μL) and a coated bead were incubated at room temperature, and the bound radiolabeled antibody complex was quantified using a gamma scintillation counter (Isoflex: ICN Biomedical, Costa Mesa, CA) following standard procedures (as described elsewhere (11)). The assay has nonsignificant cross-reactivity with α -endorphin and ACTH fragments. Intra-assay and interassay coefficients of variation (CV) were 4.4% and 10.8%, respectively, with a minimum detectable level of 1.0 pg/mL.

Plasma cortisol levels were ascertained by a competitive binding solid phase enzyme-linked immunosorbent assay (ELISA) (ELISA: IBL America). Plasma samples (25- μL) along with conjugated enzyme (200- μL) were added to the antibody-coated microtiter wells, and standard procedures were followed (as described elsewhere (11, 12)). The absorbance units were measured at 450 nm within 10 minutes of adding stop solution. The assay has < 9% cross-reactivity with progesterone and < 2% cross-reactivity with five other naturally occurring steroids (testosterone, estradiol, estrone, estriol, aldosterone). Intra-assay and interassay CV were each < 8%, with a minimum detectable level of 0.25 $\mu\text{g}/\text{dL}$.

Plasma CRH levels were determined by radioimmunoassay (RIA) (Bachem Peninsula Laboratories, San Carlos, CA) following standard procedures. Plasma samples (1–2 mL) were extracted with 3 volumes of ice-cold methanol, mixed, incubated, and centrifuged. The pellets were washed with methanol, and the

Table 1. Cohort descriptive statistics.

	Pregnancy 1	Pregnancy 2
Maternal age at delivery (mean (SD), years)	30.6 (4.5)	32.6 (4.5)
Parity (frequency (%))		
0	19 (67.9)	n/a
1	6 (21.4)	18 (64.3)
2	1 (3.6)	7 (25.0)
3	2 (7.1)	1 (3.6)
4	0 (0)	2 (7.1)
Number of obstetric risk factors (frequency (%))		
0	22 (78.6)	19 (67.9)
1	5 (17.9)	8 (28.6)
2	1 (3.6)	1 (3.6)
Baby sex		
Female	13 (46.4)	14 (50.0)
Male	15 (53.6)	14 (50.0)
Gestational age at birth (mean (SD), weeks)	39.5 (1.2)	39.2 (1.2)
Birth weight percentile by sex (mean (SD))	51.9 (27.4)	55.1 (29.6)
Study visit 24–26 weeks gestation (mean (SD))	25 (1.00)	26 (0.93)
Study visit 30–32 weeks gestation (mean (SD))	31 (0.69)	31 (0.80)
Study visit 36–38 weeks gestation (mean (SD))	37 (0.65)	37 (0.70)
Study visit 12–14 weeks postpartum (mean (SD))	13 (1.10)	13 (1.00)
Maternal ethnicity (frequency (%))		
White, European, North African, Middle Eastern	15 (53.6)	
Hispanic White	7 (25.0)	
Multi-ethnic	3 (10.7)	
Asian	2 (7.1)	
African American, Black	1 (3.6)	
Maternal education (frequency (%))		
Some college, vocational, or AA degree	10 (35.7)	
Bachelors degree	10 (35.7)	
Graduate degree	8 (28.6)	
Total household income before taxes at pregnancy 1, 15 weeks gestation (mean (SD), US\$)	57 679.0 (30 738.9)	
Time between pregnancy 1 delivery and pregnancy 2 conception (mean (SD), days)	475.2 (198.9)	
Are there any parous events between pregnancies 1 and 2? (frequency (%))		
No	27 (96.4)	
Yes	1 (3.6)	
Are there any gravid events between pregnancies 1 and 2? (frequency (%))		
No	22 (78.6)	
Yes	6 (21.4)	
Baby sex concordance (frequency (%))		
female, female	8 (28.6)	
female, male	7 (25.0)	
male, female	6 (21.4)	
male, male	7 (25.0)	
Breastfeeding 3-months postpartum (frequency (%))		
Pregnancy 1	22 (79.0)	
Pregnancy 2	22 (79.0)	
Both pregnancies	17 (61.0)	
Menstruation recommencement 3-months postpartum (frequency (%))		
Pregnancy 1	20 (71.0)	
Pregnancy 2	15 (54.0)	
Both pregnancies	13 (46)	
Hormone contraceptives 3-months postpartum (frequency (%))		
Pregnancy 1	7 (25.0)	
Pregnancy 2	3 (11.0)	
Both pregnancies	3 (11.0)	

Demographic and obstetric description of cohort ($n = 28$).

combined supernatants were dried down in a concentrator (SpeedVac: Savant Instruments, Holbrook, NY). Labeled and unlabeled CRH samples were collected by immunoprecipitation with goat antirabbit IgG serum and normal rabbit serum, and centrifuged again. The aspirated pellets were quantified with a gamma scintillation counter. For further methodologic details see (11). The assay has undetectable cross-reactivity for human ACTH. Intra-assay and interassay CV were 5% and 15%, respectively, with a minimum detectable level of 2.04 pg/mL.

Serum 17β -estradiol levels were ascertained by microtiter well competitive binding enzyme immunoassay (EIA) (ELISA: IBL America) following standard procedures (as described elsewhere (12)). Diluted samples (25- μ L) were incubated with conjugated enzyme (200- μ L) in each well, and substrate reagent (100- μ L) was added and incubated. Enzymatic reaction was halted with stop reagent (50- μ L), and within 10-minutes, absorbance readings were taken at 450 nm. The assay has less than 0.2% cross-reactivity with estriol and estrone, and nondetectable cross-reactivity with 17α -estradiol and 25 other naturally occurring steroids. Interassay and intra-assay CV are < 10% and 7%, respectively, with a minimum detectable level of 9.7 pg/mL.

Serum progesterone levels were determined by microtiter well competitive binding EIA (ELISA: IBL America) following standard procedures. Diluted samples (25- μ L) were incubated with conjugated enzyme (200- μ L) in each well, and substrate reagent (200- μ L) was added and incubated. Within 10-minutes of adding stop solution (50- μ L), absorbance readings were taken at 450 nm. The assay has 1.1% cross-reactivity with 11-desoxycorticosterone, <0.4% cross-reactivity with pregnenolone, 17α -OH progesterone, and < 0.1% cross-reactivity with corticosterone, estriol, 17β -estradiol, cortisol, and three other naturally occurring steroids. Interand intra-assay coefficients of variance are < 10% and < 7%, respectively, with a minimum detectable level of 0.045 ng/mL.

Data analysis plan

The timepoints of 25, 31, and 37 weeks' gestation were selected to capture the window when hormone levels are highest. In addition to each individual timepoint, the means of the three timepoints were also investigated, in order to minimize bias from the timing of any individual study visit and eliminate variability based on acute fluctuations. Thereby, this method optimized accuracy in reflecting hormone levels across the course of mid-to-late pregnancy.

(1) Are hormone levels in PREG 2 significantly different from hormone levels in PREG 1?

For mean values and for each timepoint individually, we evaluated the significance of the difference in PREG 1 and PREG 2 hormone levels by paired t test, and we assessed the magnitude of change in hormone concentrations by computing PREG 2 levels as a function of PREG 1 levels.

(2) What proportion of variance in PREG 2 hormone levels is attributable to variance in PREG 1 hormone levels?

First, we used regression models to investigate the following relationships in order to evaluate whether potential covariates

should be included in models: maternal age; gestational age; time of day at sample collection ("time of collection"). Time of collection was significantly related to cortisol concentrations at 25 and 31 weeks' gestation (Supplemental Table 1). Consequently, we residualized all cortisol values by time of collection, and used these residuals in all subsequent analyses. There were no other significant associations with covariates.

For regression models for each hormone, the independent (PREG 1) and dependent (PREG 2) variables used were hormone concentrations at each of three timepoints, as well as the mean across the three timepoints. Additionally, we investigate whether the changes in hormone levels from 25 to 37 weeks' gestation were consistent between PREG 1 and PREG 2. Each variable was transformed to improve distribution when necessary. Gaussian distribution was assessed by visual inspection of histograms and Shapiro-Wilk p -values > 0.10 (Supplemental Table 2). For each hormone, cases with missing data at any timepoint for either pregnancy were excluded from all models for that hormone, resulting in sample sizes of 19 to 21 women (Table 2).

Using visual inspection of curves fitted by locally weighted scatterplot smoothing (LOESS) and ANOVA comparisons, it was determined that linear models were the best fit for the data. Linear regression models measured the statistical reliance of PREG 2 hormone concentrations upon PREG 1 hormone concentrations. We investigated the following covariates as interaction terms in models for all hormones: parity, gravidity, time between pregnancies (days between PREG 1 delivery and PREG 2 conception), and child sex concordance for the two pregnancies. No interaction terms contributed significant effects for any model. To optimize accuracy of models, we omitted a small number of cases that had excessive leverage, influence, or outlier values, according to conventional criteria (13) (Supplemental Table 3). Plots of residuals vs fitted values revealed no indication of heteroscedasticity or nonlinearity.

(3) Are hormone levels more stable between PREG 1 and PREG 2 compared with the postpartum phases following PREG 1 and PREG 2?

We assessed how alike women's hormone concentrations were in the same cohort of women 3-months after PREG 1 and PREG 2 deliveries. We only investigated ACTH and cortisol because CRH is not detectable in circulation in nonpregnant women, and estradiol and progesterone would only have been relevant to measure in women who were not using hormonal contraception, leaving an insufficient sample size ($N=9$). Firstly, we determined by linear regression that time of collection was significantly related to postpartum cortisol for both PREG 1 and PREG 2, and unrelated to ACTH (Supplemental Table 1). Consequently, we used residualized postpartum cortisol values in all subsequent models. Next, we determined that neither postpartum ACTH nor cortisol concentrations were related to number of days since delivery. Final linear regression models adjusted for breastfeeding, hormonal contraception, and whether menstrual cycling had recommenced (Table 1). Lastly, we optimized the accuracy of our models by excluding women whose hormone concentrations had excessive leverage, influence, or outlier values (Supplemental Table 3). These exclusions did not change statistical significance of models.

Table 2. Descriptive statistics of hormone concentrations.

Hormone	Statistic	Pregnancy 1	Pregnancy 2	Postpartum pregnancy 1 (n = 24)	Postpartum pregnancy 2 (n = 24)
ACTH pg/mL n = 21	Mean	44.09	36.57	22.68	16.01
	Range	21.19 – 129.15	18.93 – 77.3	6.94 – 59.42	4.06 – 31.90
	SE. mean	5.36	3.89	2.50	1.61
	CI mean 95%	11.17	8.10	5.17	3.33
	SD	24.55	17.80	12.24	7.88
Cortisol μ g/mL n = 19	Mean	20.85	21.75	6.39	7.07
	Range	14.54 – 39.25	16.57 – 30.34	1.98 – 15.70	3.13 – 22.26
	SE. mean	1.23	0.84	0.66	0.91
	CI mean 95%	2.59	1.77	1.37	1.89
	SD	5.38	3.68	3.25	4.46
pCRH pg/mL n = 19	Mean	387.97	314.31		
	Range	115.37 – 657.83	84.07 – 587.97		
	SE. mean	37.76	35.52		
	CI mean 95%	79.33	74.63		
	SD	164.58	154.83		
Estradiol pg/ mL n = 19	Mean	4930.30	4927.02		
	Range	3230.37 – 6054.08	3403.98 – 7039.38		
	SE. mean	181.85	244.54		
	CI mean 95%	382.06	513.76		
	SD	792.67	1065.92		
Progesterone ng/mL n = 20	Mean	88.27	85.76		
	Range	43.18 – 189.77	42.18 – 131.96		
	SE. mean	7.34	5.24		
	CI mean 95%	15.36	10.98		
	SD	32.83	23.46		

Each hormone refers to the mean of hormone level measurements at 25, 31, 37 weeks' gestation for PREG 1 and PREG 2. Postpartum values refer to hormone levels measured at one time point for each pregnancy 3-months delivery. Total cohort size was $n = 28$. ACTH is adrenocorticotropic hormone; pCRH is placental corticotropin-releasing hormone; SE. is standard error; CI is confidence interval; SD is standard deviation.

Research question 4 is based on a comparison of regression and t test results of pCRH to the other hormones. This question does not require further statistical analyses, so it is addressed in the Discussion section. All analyses were conducted using the R programming language and RStudio (Version 0.98.1091) environment for statistical computing.

Results

Cohort descriptives

The cohort included 28 women, mean age 30.6 years at PREG 1 delivery and 32.6 years at PREG 2 delivery, with a mean of 1.3 years between PREG 1 delivery and PREG 2 conception (Table 1). Data include 27 participants for whom PREG 2 was the directly subsequent delivery after PREG 1, and one case in which there was an interim delivery not included in the study. Also, five participants experienced a miscarriage between PREG 1 and PREG 2. At PREG 1, 68% of women were primiparous. Demographic information is presented in Table 1.

Research question 1: Are hormone levels in PREG 2 significantly different from hormone levels in PREG 1?

Hormone levels in PREG 1 and PREG 2 are described in Table 2. Comparisons of group mean hormone con-

centrations in PREG 1 and PREG 2 via paired t test revealed that the only significant ($P < .10$) difference was for pCRH (Table 3). Comparisons of each hormone's concentration at each individual timepoint via paired t test revealed that the only significant ($P < .10$) differences were pCRH at 31 weeks' gestation (mean of differences = 120.23 pg/mL, $t(1, 18) = 2.38$, $P = .03$) and ACTH at 37 weeks' gestation (mean of differences = 17.71 pg/mL, $t(1, 20) = 2.95$, $P = .01$).

Also, we explored the stability of interpregnancy hormone levels by calculating PREG 2 levels as a percentage of PREG 1 levels for each hormone. Examining the means of these proportions, we found stable patterns for each hormone, reflecting the mostly-nonsignificance of interpregnancy differences. PREG 2 ACTH level was on average 4.0% lower than PREG 1 level. PREG 2 pCRH level was on average 8.1% lower than PREG 1 level. Cortisol was on average 7.2% higher in PREG 2 compared to PREG 1. Estradiol and progesterone were the most stable, <2% higher in PREG 2 than PREG 1 on average (Table 3).

Research question 2: What proportion of variance in PREG 2 hormone levels is attributable to variance in PREG 1 hormone levels?

Analyzing data as mean hormone concentrations across gestation as well as individual timepoints, we find

Table 3. Comparisons of hormone concentrations in PREG 1 and PREG 2

	Calculation	Comparison of means	Proportion
		Paired <i>t</i> test of PREG 1 and PREG 2 means	Mean of (PREG 2 ÷ PREG 1) × 100
Pregnancy	ACTH	<i>t</i> (20) = 1.6, <i>P</i> = 0.13	96.02%
	pCRH	<i>t</i> (18) = 1.8, <i>P</i> = 0.08	91.92%
	Cortisol	<i>t</i> (18) = -0.91, <i>P</i> = 0.37	107.22% ^a
	Estradiol	<i>t</i> (18) = 0.01, <i>P</i> = 0.98	101.89%
	Progesterone	<i>t</i> (19) = 0.45, <i>P</i> = 0.66	101.63%
Post-partum	ACTH	<i>t</i> (23) = 14, <i>P</i> = 7.8e-13	49.96%
	Cortisol	<i>t</i> (23) = -16, <i>P</i> = 5.2e-14	59.28% ^a

"Comparison of means" column lists results of paired *t*-tests in which mean hormone levels in PREG 1 and PREG 2 were compared. *P*-values reflect whether hormone levels in PREG 1 were significantly distinct from hormone levels in PREG 2. There are no statistically significant differences, besides pCRH (*t*(18) = 1.8, *P* = 0.08; mean (*M*) of differences = 73.67 pg/mL; *M* of the absolute value of differences = 142.77 pg/mL). The "Proportion" column lists the means of PREG 2 hormone levels as a function of PREG 1 levels. ACTH is adrenocorticotropic hormone, and pCRH is placental corticotropin-releasing hormone. Cortisol data were residualized by time of day at collection unless otherwise indicated.

^a Calculated using unresidualized cortisol values.

that ACTH and progesterone exhibit the most consistency across pregnancies, estradiol and cortisol are intermediate, and pCRH exhibits the least consistency. Figure 1 shows scatterplots with regression lines for PREG 1 × PREG 2 mean hormone levels. For mean hormone concentrations, ACTH exhibited the strongest effect magnitude, with 55.8% of variance in PREG 2 accounted for by values in PREG 1. For progesterone, 47.4% of variance in PREG 2 was accounted for by values in PREG 1. For cortisol it was 33.3% and for estradiol 26.0%. The weakest association was exhibited by pCRH with 16.8% of variance in PREG 2 accounted for by values in PREG 1 (Table 4). We also report the linear regression results for each individual timepoint across the two pregnancies (Table 5), which follow a similar pattern to the mean hormone level results. ACTH and progesterone show a significant cor-

relation between PREG 1 and PREG 2 levels for all three timepoints. Cortisol and estradiol show a significant correlation between PREG 1 and PREG 2 levels for two of the three timepoints. PCRH showed a significant correlation between PREG 1 and PREG 2 levels for only one timepoint, 37 weeks' gestation.

Additionally, we used linear regression to investigate whether the changes in hormone levels from 25 to 37 weeks' gestation were consistent between PREG 1 and PREG 2. Only pCRH exhibited significant consistency in change in concentration during gestation between PREG 1 and PREG 2 ($F(1, 15) = 4.6$, adjusted $R^2 = 0.18$, $P = .05$), while ACTH, cortisol (both unresidualized and residualized for time of collection), estradiol, and progesterone exhibited no significant consistency in change during gestation between PREG 1 and PREG 2 ($P > .10$).

Table 4. Stability of mean hormone concentrations across subsequent pregnancies

		N	F-statistic	DF	Adjusted R-squared	p-value
Pregnancy	ACTH	19	23.75	1,17	0.558	0.00 ***
	pCRH	18	4.43	1,16	0.168	0.05 .
	Cortisol	16	8.50	1,14	0.333	0.01 *
	Estradiol	18	6.97	1,16	0.260	0.02 *
	Progesterone	18	16.34	1,16	0.474	0.00 ***
Post-partum	ACTH	20	0.67	4,15	-0.075	0.41 (ns)
	Cortisol	22	0.68	4,14	-0.077	0.29 (ns)

Regression analyses measure the proportion of variance in PREG 2 hormone levels that is attributable to variance in PREG 1 hormone levels. Cortisol data were residualized by time of day at collection. Postpartum models control for breastfeeding, resumption of menses, and hormonal contraceptive use. For the null postpartum models, the negative adjusted R-squared values are interpretable as zero. ACTH is adrenocorticotropic hormone; pCRH is placental corticotropin-releasing hormone; ns is not significant; DF is degrees of freedom. *P*-values < 0.10 are in bold. See Supplemental Tables 2 and 3 for model details.

. $P < 0.10$

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$.

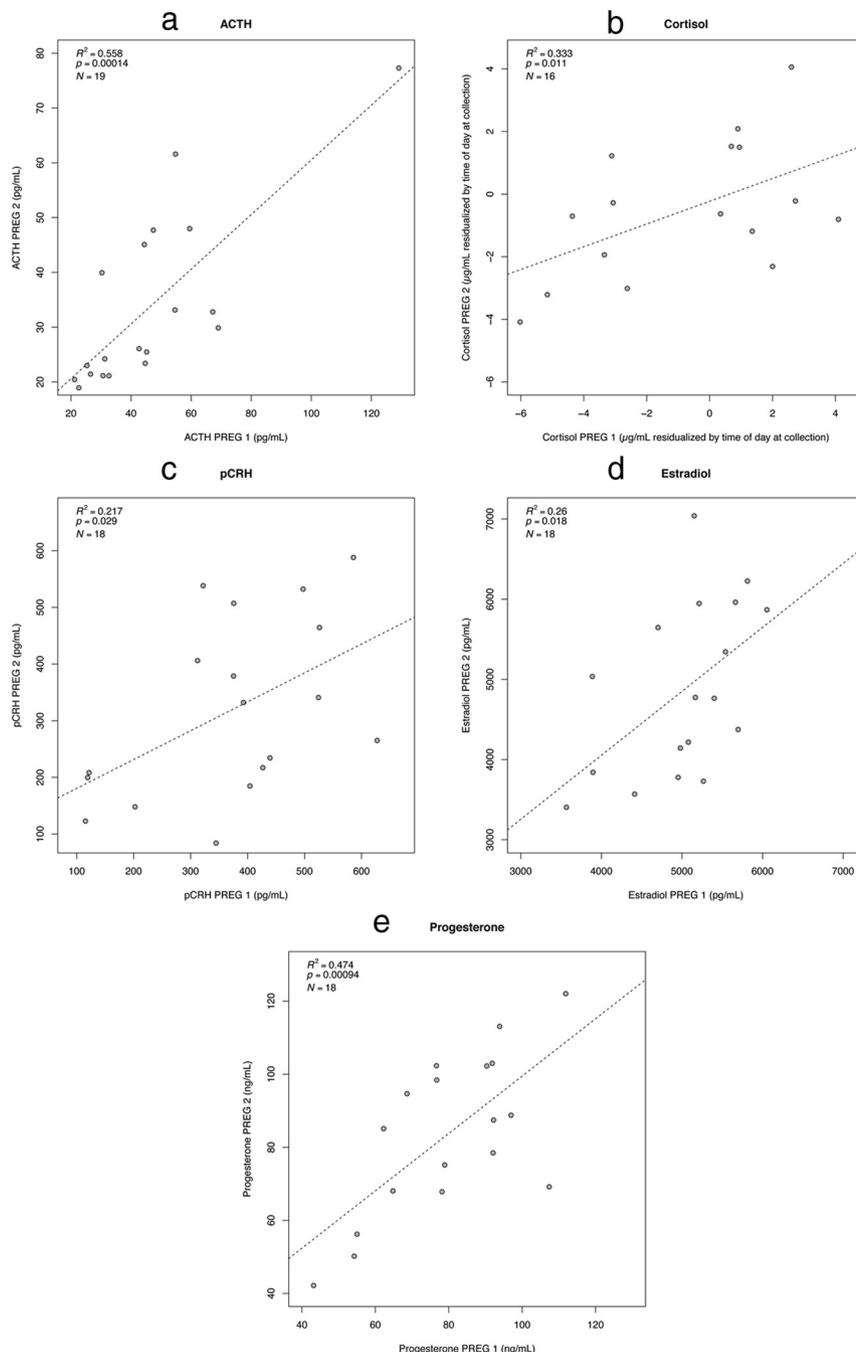


Figure 1. Correlations between PREG 1 and PREG 2 hormone levels. Scatterplots with regression lines use untransformed data to display the relationship between means of hormone concentrations at 25, 31, 37 weeks' gestation in PREG 1 and PREG 2. Regression results written in the corner of each plot use data transformations described in Supplemental Table 2. All cortisol values were residualized by time of day at collection (Supplemental Table 1). ACTH is adrenocorticotrophic hormone; pCRH is placental corticotropin-releasing hormone. Panels: (a) ACTH, (b) cortisol, (c) pCRH, (d) estradiol, (e) progesterone.

Research question 3: Are hormone levels more stable between PREG 1 and PREG 2 compared with the postpartum phases following PREG 1 and PREG 2?

Comparisons of group mean hormone concentrations at 3-months postpartum via paired *t* test revealed significant differences in both ACTH and cortisol between

Stability of pregnancy physiology across the lifespan

Concentrations of HPA and HPO hormones during pregnancy are substantially greater than during the nonpregnant state. Conceivably, factors that cause fluctuations in hormone concentrations during nonpregnant

PREG 1 and PREG 2 (Table 3). The changes in ACTH and cortisol levels from the postpartum phase of PREG 1 to the postpartum phase of PREG 2 were of substantially greater magnitude than the changes that occurred across the pregnancy phases. Hormone level during the PREG 2 postpartum phase as a percentage of level during the PREG 1 postpartum phase was a mean of 50.0% for ACTH and 59.3% for cortisol (Table 3). Altogether, these results reveal a low degree of stability in hormone levels across the postpartum phases of successive pregnancies.

Neither ACTH nor cortisol exhibited any significant correlation between PREG 1 postpartum and PREG 2 postpartum concentrations (Table 4). We repeated the postpartum ACTH and cortisol analyses restricting the cohort to those participants included in the pregnancy analyses, and the results remained null (ACTH: $F(4, 10)=2.87$, $P=.14$; cortisol: $F(4, 7)=0.39$, $P=.40$). These results reveal a low degree of predictability in hormone levels across the postpartum phases of successive pregnancies.

Discussion

Results suggest that hormones in maternal circulation during pregnancy are relatively stable from one pregnancy to another within a woman's life-history. Hormones during two nonpregnant states equally separated by time appear to be far less stable by comparison. These results have important implications for gestational biology, maternal health, fetal development, and child health.

Table 5. Stability of hormone concentrations at each timepoint across subsequent pregnancies

Hormone	Gestation timepoint	N	F-statistic	DF	Adjusted R ²	P
ACTH	25 wks	20	3.5	1,18	0.115	0.079 .
	31 wks	20	5.9	1,18	0.206	0.026 *
	37 wks	19	6.4	1,17	0.231	0.022 *
pCRH	25 wks	19	0.0	1,17	-0.058	0.921
	31 wks	19	1.3	1,17	0.015	0.273
	37 wks	19	6.4	1,17	0.232	0.0213 *
Cortisol	25 wks	17	5.8	1,15	0.230	0.030 *
	31 wks	18	9.9	1,16	0.342	0.006 **
	37 wks	18	1.6	1,16	0.032	0.228
Estradiol	25 wks	19	4.5	1,17	0.164	0.048 *
	31 wks	18	6.8	1,16	0.253	0.019 *
	37 wks	18	0.0	1,16	-0.061	0.877
Progesterone	25 wks	19	22.6	1,17	0.546	0.000 ***
	31 wks	20	5.0	1,18	0.176	0.038 *
	37 wks	18	12.4	1,16	0.401	0.003 **

Regression analyses measure the proportion of variance in PREG 2 hormone levels that is attributable to variance in PREG 1 hormone levels at each of three timepoints (25, 31, 37 weeks' gestation). Cortisol data were residualized by time of day at collection. For null results, negative adjusted R-squared values are interpretable as zero. ACTH is adrenocorticotropic hormone; pCRH is placental corticotropin-releasing hormone; ns is not significant; DF is degrees of freedom. P-values < 0.10 are in bold. See Supplemental Tables 2 and 3 for model details.

. $P < 0.10$

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$.

phases may exert less influence during pregnancy for two reasons. Firstly, endocrine systems may be less responsive to these factors because hormone levels are held close to their physiological maximum by gestational homeostatic systems (14, 15). The physiological demands of pregnancy can act as a challenge to the somatic system, enlisting all available, relevant, maternal resources towards meeting the demands of the developing fetus (16). Circulating concentrations of hormones may be relatively stable from one pregnancy to the next because pregnancy may reveal endocrine ceiling effects, and how ceiling effects may change across the life-history.

Secondly, stability of hormone levels across pregnancies may reflect desensitization of endocrine systems to external perturbation. This possibility is supported by previous evidence that imposing external stressors on pregnant women elicits a dampened physiological response compared to nonpregnant women, eg, blood pressure (BP) (17); HPA-axis activation (18); psychological responses to stress (19, 20). Whether endocrinological inflexibility or insensitivity plays a functional role in pregnancy remains unknown. Further research is necessary to explain the striking degree of stability we observe in hormone levels across successive pregnancies.

Previous studies of nonpregnant adults have shown low intra-individual stability of hormone levels in baseline conditions and higher stability in challenge conditions (ACTH, cortisol, lipotropic hormone (21); cortisol (22,

23); cortisol, luteinizing hormone (LH) (24)). Our observation that postpartum (ie, baseline) ACTH and cortisol levels were inconsistent compared to the consistency during pregnancy (ie, challenge) is congruous with these previous observations.

Research question 4: Is pCRH less similar than the other hormones across PREG 1 and PREG 2?

We predicted that pCRH should be less predictable from one pregnancy to the next compared with hormones that derive, entirely or in part, from maternal organs. Circulating levels of maternal plasma CRH during pregnancy is nearly exclusively derived from the placenta (25). In support of our hypothesis, we observed pCRH to be the least predictable from PREG 1 to PREG 2 of all the hormones investigated here. Notably, CRH was the only hormone that exhibited significant difference comparing group means for PREG 1 and PREG 2.

By comparison, maternal plasma cortisol during pregnancy is exclusively derived from the maternal adrenal glands, ACTH is nearly exclusively derived from the maternal pituitary, and estradiol and progesterone reflect both maternal (ovarian) and placental secretion (6). The relative stability we observed across two successive pregnancies for these hormones could reflect stability in the mother's reproductive strategy, compared with pCRH instability reflecting variation in strategies of semiallogeneic fetuses. Possibly, pCRH could be less stable across preg-

nancies than other hormones with partial placental contribution because it is more sensitive to environmental conditions, which vary stochastically between subsequent pregnancies.

Previous studies

Two previous studies analyzed gestational physiology across two successive pregnancies in cohorts of women. The first study found that women exhibited intra-individual correlations in weight, BMI, and offspring birth weight across the two pregnancies, and no association for maternal hemoglobin (26). The second study compared various aspects of gestational physiology in 106 women across two pregnancies (27). For indicators of maternal sympathetic activation, they found that maternal electrodermal activity was greater during the subsequent pregnancy, while respiratory sinus arrhythmia was greater during the earlier pregnancy, and no trend in directionality for maternal heart rate or respiratory period. They did not explore correlations across the two pregnancies. Similar to our results, they found no moderating effects of fetal sex concordance. Their study began the important exploration of the degree to which siblings share a prenatal environment. Our results expand this field of inquiry as the first investigation of intra-individual gestational endocrine concordance.

Implications for understanding maternal health

Knowing whether women experience similar concentrations of hormones in each of their pregnancies can improve our estimation of lifetime (cumulative) exposures to the endocrine conditions of pregnancy. Because pregnancy is characterized by the highest concentrations of glucocorticoids and gonadotropins in a woman's lifetime, and because these hormones have been implicated in disease etiology, this topic is of major interest for women's health. Glucocorticoids are involved in a wide range of immunological functions, including modulation of gene expression, suppression of certain pathways and promotion of others (28). Cumulative exposure to high concentrations of estrogens has been positively associated with risk of reproductive cancers (breast (1), ovarian (3), endometrial (4)), and negatively associated with risk of Alzheimer's Disease (2). Our calculations of the intra-individual stability in gestational hormone levels represent an important step in improving our estimation of the cumulative effect of reproductive life-history on later-life disease risk, via cumulative hormone exposure. Additionally, maternal endocrine profiles during gestation have been implicated in maternal cognitive performance (12), maternal sensitivity (29), and postpartum depression (30–32). Our results contribute to a better understanding of

how successive pregnancies (and postpartum phases) may influence a woman's health across her lifespan.

Reconceptualizing the early shared environment: Fetal programming and sibling effects

Appreciating the degree of consistency in a mother's hormone concentrations across pregnancies will improve our understanding of the underlying mechanisms involved in sibling trait concordance. Hormone exposures during the prenatal phase of life during sensitive periods moderate fetal developmental processes (8) in ways that have lifelong, often irreversible, consequences for offspring health and development (9, 10). This is part of the process of fetal programming. For certain traits, prenatal hormone exposures play a major role in shaping phenotype (9, 33–36). For such traits, two siblings exposed to similar endocrine environments in utero may exhibit trait concordance.

Until now, we did not know how similar siblings' fetal hormone exposures were, limiting our ability to draw accurate conclusions about prenatal and postnatal environmental influences on phenotypic development. Many "extended twin studies" have compared monozygotic twins, dizygotic twins, and siblings to discern the genetic, prenatal, and postnatal environment influences on a wide range of traits, eg, brain morphology (37), depression (38), drug abuse (39), cardiovascular disease risk (40), and diabetes risk (41). These study designs are based on the premise that nontwin siblings have the same genetic relatedness as dizygotic twins but experience different intrauterine environments. Thus, an underlying assumption of the study design is that within a mother, gestational physiologies during two of her pregnancies are different enough from one another to reveal the effects of prenatal programming. Maternal age, environmental circumstances that affect maternal somatic and placental function, and fetal identity differ across pregnancies. Yet, maternal identity remains consistent, maternal-placental genetics remain consistent, and fetal (and fetal-placental) genetic identity is still half of maternal origin, and, in some cases, has shared paternal genetic origin with the antecedent sibling. Therefore, some aspects of gestational biology are consistent across successive pregnancies, while other aspects vary, predicting some (but not total) consistency in endocrinology, as our results demonstrate. Because of the important role of hormones as effectors of fetal programming, the aspects of gestational biology that account for endocrinologic differences across successive pregnancies may promote divergent sibling phenotypic development, while the aspects of gestational biology that account for endocrinologic similarities across successive pregnancies may promote concordant sibling phenotypic develop-

ment. In conclusion, the way we interpret comparisons of dizygotic twin vs sibling trait concordance needs to be reconsidered based on a more informed understanding of a mother's interpregnancy physiological consistency.

Additionally, the correlation between fetal hormone exposure and maternal circulating hormone levels is not one-to-one and may vary (42, 43). Further studies are needed to investigate differences in hormones not only in maternal circulation but also in utero across successive pregnancies.

Conclusion

We find that in this cohort, up to 56% of the variance in hormone levels in a pregnancy can be predicted from hormone levels in a previous pregnancy. This interpregnancy consistency in hormone levels is absent during the non-pregnant state. Future studies should further investigate this topic in a larger cohort. Nonetheless, our results can inform future efforts to estimate women's cumulative hormone exposures based on reproductive life-history. Additionally, these results suggest that a substantial portion of siblings' shared environments may be prenatal, which should alter how we interpret observations of sibling trait concordance.

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