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Timing of Fetal Exposure to Stress Hormones: Effects on Newborn Physical and Neuromuscular Maturation

ABSTRACT: The purpose of the study was to determine the specific periods during pregnancy in which human fetal exposure to stress hormones affects newborn physical and neuromuscular maturation. Blood was collected from 158 women at 15, 19, 25, and 31 weeks' gestation. Levels of placental corticotropin-releasing hormone (CRH) and maternal cortisol were determined from plasma. Newborns were evaluated with the New Ballard Maturation Score. Results indicated that increases in maternal cortisol at 15, 19, and 25 weeks and increases in placental CRH at 31 weeks were significantly associated with decreases in infant maturation among males (even after controlling for length of gestation). Results also suggested that increases in maternal cortisol at 31 weeks were associated with increases in infant maturation among females, although these results were not significant after controlling for length of gestation. Findings suggest that stress hormones have effects on human fetal neurodevelopment that are independent of birth outcome. © 2008 Wiley Periodicals, Inc. *Dev Psychobiol* 50: 232–241, 2008.

Keywords: fetal; HPA-axis; cortisol; CRH; pregnancy; fetus; New Ballard Maturation Score; stress; infant; human

INTRODUCTION

Insults or disruptions of normal fetal maturation can lead to long-lasting neurobiological and health outcomes that persist across the lifespan. Barker's (1995) pivotal studies linking adverse birth outcomes, such as low birth weight, to adult diseases promoted the idea that vulnerabilities to mental and physical diseases could be programmed during the fetal period. Deleterious birth outcomes likely

reflect aberrant processes beginning in intrauterine development that may contribute to health and disease risk later in life (Gluckman & Hanson, 2004; Maccari et al., 2003). One emerging area of interest is the effects of maternal stress on the intrauterine environment and its consequences for fetal and infant development.

The fetus is involved in a dynamic communication with the mother over the course of gestation, including exchanges in markers of biological stress that originate from the maternal-placental-fetal unit. One of the major placental stress signals in pregnant primates is the peptide corticotropin-releasing hormone (CRH). This peptide plays a key role in the maturation of the fetal hypothalamic-pituitary-adrenal-axis (HPA-axis) and other systems, and coordinates events that underlie both fetal growth and maturation and the time of onset of parturition (Hobel, Dunkel-Schetter, Roesch, Castro, & Arora, 1999; Sandman et al., 2006; Sandman et al., 2003; Sandman, Wadhwa, Chicz-DeMet, Porto, & Garite, 1999; Sandman et al., 1999b; Wadhwa et al., 2004; Wadhwa, Porto, Garite, Chicz-DeMet, & Sandman, 1998).

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The HPA-axis during pregnancy in human and nonhuman primates is significantly different than that of nonpregnant adult primates. In contrast to the typical suppressive effects of glucocorticoids on CRH synthesis in the hypothalamus, there is a positive feedback loop between placental CRH and cortisol from the adrenal cortex of the mother (Jones, Brooks, & Challis, 1989; King, Smith, & Nicholson, 2001; Trainer, 2002). The difference in behavior of the CRH gene in the placenta and hypothalamus is due to the expression of different transcription factors, coactivators, and corepressors in these two tissues (King, Smith, & Nicholson, 2002). Subsequently, levels of both CRH and cortisol rise steadily throughout gestation (Gemzell, 1953; Goland, Wardlaw, Blum, Tropper, & Stark, 1988). Despite increases in cortisol in maternal serum during pregnancy, only 10–20% of maternal cortisol crosses the placenta to reach fetal circulation in humans between 13 and 35 weeks' gestation (Benediktsson, Calder, Edwards, & Seckl, 1997; Gitau, Cameron, Fisk, & Glover, 1998; Sandman et al., 2003). The enzyme 11 β -hydroxysteroid dehydrogenase-2 (11 β -HSD-2) has been isolated in the human placenta and likely accounts for the relatively low transfer of maternal cortisol to the fetus by converting cortisol to its inactive form cortisone (Benediktsson et al., 1997; Bernal, Flint, Anderson, & Turnbull, 1980). 11 β -HSD-2 shows steady increases throughout gestation until the last few weeks of gestation when decreases in 11 β -HSD-2 are observed (Lopez Bernal, Anderson, & Turnbull, 1982; Murphy & Clifton, 2003; Sandman et al., 2003; Schoof et al., 2001; Shams et al., 1998). Although, 11 β -HSD-2 provides protection for the fetus from maternal cortisol, it does not provide complete protection; thus, some maternal cortisol continues to reach the fetus.

Fetal exposure to glucocorticoids and CRH has both beneficial and detrimental consequences for fetal growth and maturation. Specifically, during the last trimester of pregnancy, exposure to cortisol is critical for the maturation of fetal physiological systems and organs, such as the cardiovascular system, the pulmonary system, renal systems, and overall fetal growth (Murphy, Smith, Giles, & Clifton, 2006; Trainer, 2002; Welberg, Seckl, & Holmes, 2001). Despite the necessity of cortisol for fetal maturation, it has been repeatedly demonstrated that women receiving synthetic corticosteroids (administration commencing typically around the 24th week gestation) were more likely to deliver infants with fetal growth restriction and low birth weight, even when controlling for length of gestation, suggesting that high levels of glucocorticoids may be detrimental to fetal growth (Bloom, Sheffield, McIntire, & Leveno, 2001; French, Hagan, Evans, Godfrey, & Newnham, 1999; Reinisch, Simon, Karow,

& Gandelman, 1978). Similarly, exposure to CRH at 33 weeks' gestation has been associated with fetal growth restriction (Wadhwa et al., 2004). Nevertheless, no study has examined whether the timing of prenatal exposure to stress hormones affects fetal growth and maturation.

Stress hormones also have been consistently associated with preterm delivery and shorter gestational periods (Hobel et al., 1999; Mancuso, Schetter, Rini, Roesch, & Hobel, 2004; Mullings et al., 2001; Sandman et al., 2006; Sandman et al., 1994; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993). In addition, the timing of exposure to stress hormones during pregnancy may be critical in determining the length of gestation. Specifically, recent evidence suggests that elevated maternal cortisol at 15 weeks' gestation led to a surge of placental CRH at 31 weeks' gestation, which, in turn, was associated with preterm delivery (Sandman et al., 2006). These data suggest that a stress-related signal early in pregnancy could prepare the placenta to activate labor-inducing signaling mechanisms later in gestation.

Cumulatively, the existing empirical findings suggest that fetal exposure to elevated levels of stress hormones leads to shortened length of gestation and disruptions in fetal growth. Further, the timing of exposure appears to be critical in determining the precise consequences to fetal well-being. Nevertheless, no study has determined the effects of timing of exposure to stress hormones on measurable characteristics of newborn physical and neuromuscular maturation, independent of the effects of gestational length. Fetal exposure to stress hormones could lead to at least two potential developmental trajectories for the fetus. First, fetal exposure to maternal stress signals could prime fetal systems to mature more rapidly to ensure survival in anticipation of a premature birth. This possibility is consistent with CRH-dependent accelerated metamorphosis observed in tadpoles exposed to environmental stress that threatens survival (Denver, 1997). The second possibility is that under stressful conditions, the fetus must compete with its host (the mother) for resources required for coping and survival (Stearns, 2005). This possibility is consistent with findings indicating that fetal exposure to stress hormones and synthetic corticosteroid administration are associated with fetal growth restriction (Bloom et al., 2001; French et al., 1999; Reinisch et al., 1978; Wadhwa et al., 2004). The purposes of the present study were to (1) test these competing hypotheses by evaluating the consequences of exposure to stress hormones during pregnancy for physical and neuromuscular development in infants and (2) determine the specific periods during pregnancy during which stress hormones influence newborn physical and neuromuscular maturation.

METHODS

Participants

Participants in this study were part of the Multi-Site Behavior in Pregnancy Study (MS-BIPS), which examined the effects of stress during pregnancy on birth outcomes. Four hundred ninety-nine women with singleton, intrauterine pregnancies were recruited from 1997 to 2003 at two major medical centers in Southern California. Ballard neonatal assessments were conducted as part of routine neonatal care at only one site (UC Irvine), therefore the sample was limited to this study site. The sample was comprised of 158 women and their infants. In addition, part of the sample (89 women) was recruited earlier than 18 weeks' gestation; therefore, an additional earlier assessment was obtained for these women.

As presented in Table 1, the majority of the women were high school graduates, married, and from middle-income households. The sample was ethnically and racially diverse, such that the demographics were representative of pregnant women seen at this university hospital. Women were not eligible for study participation if they had current or prior medical conditions related to cardiovascular, neuroendocrine, hepatic, or renal functioning. In addition, women were excluded if they had medical conditions that placed them at high-risk for adverse birth outcomes (e.g., systemic maternal disease, placental or cord abnormalities, uterine anomalies, congenital malformations, or chromosomal abnormalities) and if they smoked or used controlled substances during or up to 3 months prior to their pregnancies.

As Table 1 indicates, the majority of the infants was born at term, was delivered vaginally, was stable at delivery, and was approximately equally distributed in terms of sex.

Procedures

All procedures and methods were approved by the Institutional Review Boards of the participating institutions and all women

Table 1. Subject Characteristics

Age (years)	30 ± 5.74
Marital status (% married)	74%
Education (%)	97% high school graduates 34.7% college graduates
Race/ethnicity (%)	45% non-Hispanic White 31% Latina 14% Asian 4% Multi-ethnic 3% African American 3% Other
Income	\$57,662.34 ± \$29,351.20
Primiparous (%)	46%
Vaginal delivery (%)	67%
Infant male sex (%)	49%
Gestational age at birth	39 ± 1.84
1 min APGAR scores	8.16 ± 1.21

Data are presented as means ± standard deviations.

provided informed consent to participate in the study procedures. Blood samples were collected at four time points during gestation (15.10 ± 1.22; 19.20 ± 0.94; 24.85 ± 0.97; and 31.00 ± 0.88 weeks' gestation). Samples were collected in the afternoon (mean times (nautical hours): 15 weeks = 13:24; 19 weeks = 13:36, 25 weeks = 12:54, and 31 weeks = 13:14). A clinical ultrasound performed at the first study session confirmed gestational age estimates based on last menstrual period. Infants were evaluated with the New Ballard Neonatal Examination by attending physicians within 48 hr after birth (mean 8.9 hr, SD = 7.94) to assess newborn physical and neuromuscular maturity.

Measures

Endocrine Assessments. Twenty-five milliliters of blood were collected through antecubital venipuncture (within 20 s of venipuncture). Blood was deposited into siliconized EDTA (purple top) vacutainers, placed on ice, and then centrifuged at 2000g (15 min). Plasma was decanted into polypropylene tubes containing a protease inhibitor (500 KIU/mL aprotinin; Sigma Chemical, St. Louis, MO) to arrest enzymatic degradation and then stored at -70°C until assayed.

Unbound, bioactive placental CRH concentrations (pg/mL) were determined by radioimmunoassay (RIA; Bachem Peninsula Laboratories, San Carlos, CA). Hypothalamic CRH is not detectable in human peripheral sera; therefore, multiple studies have demonstrated that CRH measured from maternal sera during pregnancy is from placental origins (King et al., 2001; Lowry, 1993; Petraglia, Sawchenko, Rivier, & Vale, 1987; Petraglia, Sutton, & Vale, 1989). Plasma samples were extracted (1–2 mL) with 3 volumes of ice-cold methanol, mixed, allowed to stand for 10 min at 4°C by the modified method of Linton et al. (1995). The pellets were washed with 0.5 mL methanol and the combined supernatants were dried down on a Savant SpeedVac Concentrator (Savant Instruments, Holbrook, NY). Reconstituted samples in assay buffer were incubated in human anti-CRH serum for 48 hr at 4°C followed by a 24-hr incubation period with ¹²⁵I-CRH. Labeled and unlabeled CRH were collected by immunoprecipitation with goat anti-rabbit IgG serum and normal rabbit serum after 90 min incubation at room temperature. Samples were centrifuged at 1700g for 20 min at 4°C and the aspirated pellets were quantified with a gamma scintillation counter. The CRH assay had less than 0.01% cross-reactivity with ovine CRH, 36% cross-reactivity with bovine CRH, and nondetectable reactivity with human adrenocorticotrophic hormone (ACTH). The intra-assay and inter-assay coefficients of variance ranged from 5% to 15%, respectively. Data reduction for the RIA assay was performed with a computer assisted four-parameter logistics program (Rodbard, Munson, & De Lean, 1978).

Plasma cortisol levels were determined by immunofluorescence using an automated procedure on an Abbott TDx Analyzer (Abbott Laboratories, Abbot Park, IL). The assay was less than 5% cross-reactive with 11-deoxycortisol, corticosterone, and less than 1% cross-reactive with 10 other naturally occurring steroids. The inter- and intra-assay coefficients of variance were less than 9% with a minimum detectable level of 0.45 µg/dL (95% confidence).

Newborn Physical and Neuromuscular Maturity. The New Ballard Maturation Score was used to assess physical and neuromuscular maturation of the newborns used in this study (Ballard et al., 1991). Although the New Ballard Score correlates with gestational age ($r = 0.6807$ in present study), about 54% of the variance in the Ballard Scores was not accounted for by gestational age, indicating that the Ballard assesses infant maturation in a manner not fully explained by length of gestation. Moreover, this is the first study to explore the relationship between fetal exposure to stress hormones and infant maturation, using a measure composed of multiple assessments of newborn characteristics that likely correspond to a better metric of developmental age than gestational length. Specifically, the scoring uses neuromuscular and physical characteristics of the newborn that develop over the course of gestation. The neuromuscular portion of the score consists of measures of muscle tone, distinct posture, and angles of resistance in key muscle groups. For example, in extremely premature infants flexibility is mostly absent at the wrist and passive flexor tone is mostly absent at the knee, shoulder, and hip (Ballard et al., 1991). Physical characteristics examined in the New Ballard Score include skin, lanugo, plantar surface of the foot, breast, eye/ear, and genitals. These characteristics develop at distinct periods throughout gestation, such that extremely premature infants typically have fused eyelids, sticky, transparent skin, no lanugo, absent breast markings, and undifferentiated genitalia (Ballard et al., 1991). The New Ballard Scores range from -10 to 50 . In the present study, scores ranged from $+12$ to $+49$ (mean = 37.68 , SD = 5.37).

Statistical Analyses. Statistical analyses were conducted using SAS version 8.2 software (SAS, Inc., Cary, NC) and STATA version 8.1 (STATA Corporation, College Station, TX). Initially, general multiple linear regression models were estimated for hormones at each study time point to determine whether placental CRH and/or maternal cortisol were associated with infant maturation, as assessed by the New Ballard Score. Models controlled for infant sex, infant's age at examination, parity, caesarian-section delivery, maternal age at delivery, and ethnic/racial categories. Primiparity, or giving birth for the first time, and fetal sex have been associated with differences in birth weight in previous studies; therefore parity and infant's sex were entered as covariates in all of the models (Ballard et al., 1991; Cogswell & Yip, 1995; Feldman, Dunkel-Schetter, Sandman, & Wadhwa, 2000; Lu & Halfon, 2003). Moreover, ethnicity and race have been associated with changes in maternal cortisol and placental CRH during pregnancy, as well as disparities in birth outcomes, such as low birth weight and premature delivery (even after controlling for other risk factors, such as income, education, parity, maternal age, and prenatal care; Glynn, Schetter, Chicz-DeMet, Hobel, & Sandman, 2007; Lu & Halfon, 2003). Given the aforementioned findings, analyses controlled for race/ethnic categories, which consisted of non-Hispanic White, Latina, Asian, African American, and other (multi-ethnic was combined with other due to small sample sizes). In addition, infant's age at exam, maternal age, and mode of delivery were significantly associated with Ballard scores and other independent variables used in the models; therefore, these variables were entered into the models to be conservative.

Additional models were constructed that controlled for gestational age at birth to assess whether there were effects of stress hormones during pregnancy on fetal maturation, independent of the length of gestation. Supplementary analyses were conducted separately for males and females to determine whether fetal exposure to stress hormones differed by sex.

RESULTS

Results are displayed in Table 2. The findings indicated that increases in maternal cortisol at 15 and 19 weeks were associated significantly with decreases in infant physical and neuromuscular maturation, with and without controlling for length of gestation. Without controlling for length of gestation, every unit increase in maternal cortisol ($\mu\text{g/dL}$) at 15 and 19 weeks' gestation was associated with a $.411$ decrease ($p < .0001$) and $.148$ ($p = 0.026$) decrease in Ballard total scores, respectively. These results were consistent after controlling for length of gestation, such that every unit increase in maternal cortisol ($\mu\text{g/dL}$) at 15 and 19 weeks' gestation was associated with a $.363$ decrease ($p < .0001$) and $.107$ ($p = 0.047$) decrease in Ballard total scores, respectively. In addition, maternal cortisol at 31 weeks was associated with a significant increase in total Ballard scores, such that for every unit increase in maternal cortisol ($\mu\text{g/dL}$) at 31 weeks, there was a $.071$ ($p = .040$) increase in total Ballard scores, without controlling for gestational age at birth; however this result was nonsignificant after controlling for length of gestation. In addition, placental CRH at 31 weeks was significantly associated with decreases in infant physical and neuromuscular maturation. Specifically, each unit increase in maternal placental CRH (pg/mL) was associated with a $.006$ decrease ($p < .0001$) in total neuromuscular/physical maturity. Moreover, this finding was statistically significant after controlling for length of gestation ($.005$ decrease in total Ballard scores for every unit increase in placental CRH; $p < .0001$).

In supplementary analyses examining male and female infants separately, results indicated that the findings linking increases in maternal cortisol at 15 and 19 weeks and increases in placental CRH at 31 weeks with decreases in infant physical and neuromuscular maturation only remained significant for male infants (see Tab. 3). In addition, after separating analyses by infant sex, every unit increase in maternal cortisol ($\mu\text{g/dL}$) at 25 weeks' gestation was associated with a $.203$ decrease ($p = .036$) in Ballard scores for males, but this finding was only significant when controlling for length of gestation. Lastly, additional analyses suggested that the relationship between increases in maternal cortisol at 31 weeks and increases in total Ballard scores was restricted to female infants, which was only significant without controlling for length of gestation (see Tab. 3).

Table 2. Associations of Cortisol and CRH with New Ballard Scores with and without Controlling for Length of Gestation

Hormones	Coefficient (95% CI)/Standardized Coefficient ^d		<i>t</i> (SE)	<i>p</i> > <i>t</i>	Coefficient (95% CI)/Standardized Coefficient ^b Controlling for Gestational Length		<i>t</i> (SE)	<i>p</i> > <i>t</i>
	Coefficient	Standardized			Coefficient	Standardized		
Cortisol								
15 weeks	-.411 (-.606, -.217)	$\beta = -.440$	-4.21 (.098)	0.000	-.363 (-.515, -.211)	$\beta = -.409$	-4.77 (.076)	0.000
19 weeks	-.148 (-.278, -.018)	$\beta = -.185$	-2.25 (.066)	0.026	-.107 (-.213, -.002)	$\beta = -.139$	-2.01 (.053)	0.047
25 weeks	-.011 (-.141, .119)	$\beta = -.015$	-0.17 (.066)	0.869	-.036 (-.142, .069)	$\beta = -.051$	-0.68 (.053)	0.497
31 weeks	.071 (.003, .138)	$\beta = .179$	2.08 (.034)	0.040	.043 (-.011, .098)	$\beta = .114$	1.57 (.028)	0.119
CRH								
15 weeks	-.257 (-.569, .055)	$\beta = -.193$	-1.64 (.157)	0.105	-.208 (-.453, .037)	$\beta = -.165$	-1.70 (.123)	0.094
19 weeks	.018 (-.042, .078)	$\beta = .051$	0.59 (.030)	0.559	.011 (-.038, .060)	$\beta = .033$	0.45 (.025)	0.653
25 weeks	.002 (-.018, .022)	$\beta = .015$	0.18 (.010)	0.859	-.004 (-.020, .012)	$\beta = -.036$	-0.51 (.008)	0.613
31 weeks	-.006 (-.008, -.004)	$\beta = -.381$	-4.91 (.001)	0.000	-.005 (-.006, -.003)	$\beta = -.302$	-4.53 (.001)	0.000

^dMultiple regression analyses controlled for maternal age, caesarian section delivery, parity, infant's age at exam, infant's sex, and mother's ethnicity/race.

^bMultiple Regression analyses controlled for maternal age, caesarian section delivery, parity, infant's sex, mother's ethnicity/race, and gestational length.

DISCUSSION

The results of this study provide the first evidence that fetal exposure to increases in levels of maternal cortisol at 15 and at 19 weeks and increases in levels of placental CRH at 31 weeks' gestation are associated with significant decreases in newborn physical and neuromuscular maturation. These effects were observed after controlling for length of gestation, indicating that exposure to stress hormones portends poorer fetal maturation independent of maturation effects associated with gestational age. These findings also suggest that intrauterine exposure to certain key stress hormones influences the development of fetal systems that may have long-term sequelae. For instance, decreased scores on newborn neuromuscular measures similar to those used in the New Ballard Score have been associated with abnormalities detected with MRI in newborns, including basal ganglia and white matter lesions, as well as motor abnormalities that persist until age 4 in childhood (Haataja et al., 2001; Mercuri, Ricci, Pane, & Baranello, 2005). Moreover, in newborns regarded as healthy by obstetric and pediatric staff, deviant patterns on neurological examinations (including measures of posture, movement, tone, reflexes, and some behavior) have been associated with newborn cranial ultrasound abnormalities, including thalamic and periventricular densities and intraventricular hemorrhaging (Mercuri, Dubowitz, Brown, & Cowan, 1998). Hence, the findings of the present study could have implications for neurobehavioral consequences in later development.

Further support for this possibility comes from animal studies (rats and rhesus monkeys) that found alterations in multiple neuronal systems often persisting into adulthood following exposure to stress hormones such as CRH and cortisol. Specifically, exposure to stress hormones during the prenatal period has been associated with 50% increases in tyrosine hydroxylase positive cells in the ventral tegmental area and the substantia nigra, as well as increased dopamine D2-like receptors in the dorsal frontal cortex, medial prefrontal cortex, hippocampus and nucleus accumbens in adult rat brains (Berger, Barros, Sarchi, Tarazi, & Antonelli, 2002; McArthur, McHale, Dalley, Buckingham, & Gillies, 2005). Moreover, animal findings suggest that fetal exposure to stress hormones leads to hippocampal abnormalities (including cell death and reductions in cell proliferation) and corollary cognitive and behavioral consequences, including deficits in learning and memory, long-lasting delays in neuromotor development, distractibility, and increased ultrasonic vocalizations in response to maternal separation (Brunson, Eghbal-Ahmadi, Bender, Chen, & Baram, 2001; Hennessy, Davis, McCrea, Harvey, & Williams, 1999; Lemaire, Koehl, Le Moal, & Abrous, 2000; Roberts et al., 2004). In humans, salivary cortisol in late pregnancy

Table 3. Associations of Cortisol and CRH with New Ballard Scores by Infant's Sex (with and without Controlling for Length of Gestation)

Hormones		Coefficient (95% CI)/Standardized		<i>t</i> (SE)	<i>p</i> > <i>t</i>	Coefficient ^b (95% CI)/Standardized		<i>t</i> (SE)	<i>p</i> > <i>t</i>		
		Coefficient ^c	β			Coefficient ^b	β				
Cortisol	15 weeks	Males	-.412 (-.699, -.124)	β = -.419	-2.91 (.142)	0.006	-.402 (-.625, -.179)	β = -.442	-3.67 (.109)	0.001	
		Females	-.271 (-.551, .010)	β = -.319	-1.97 (.137)	0.058	-.198 (-.436, .039)	β = -.233	-1.70 (.116)	0.099	
	19 weeks	Males	-.265 (-.436, -.093)	β = -.368	-3.09 (.086)	0.003	-.211 (-.363, -.059)	β = -.311	-2.77 (.076)	0.007	
		Females	.192 (-.019, .404)	β = .203	1.81 (.106)	0.074	.123 (-.040, .286)	β = .130	1.50 (.082)	0.138	
	25 weeks	Males	-.177 (-.397, .043)	β = -.214	-1.61 (.110)	0.113	-.203 (-.392, -.014)	β = -.261	-2.15 (.094)	0.036	
		Females	.092 (-.060, .245)	β = .143	1.21 (.076)	0.232	.072 (-.047, .192)	β = .110	1.21 (.060)	0.230	
	31 weeks	Males	.050 (-.124, .224)	β = .073	0.57 (.087)	0.571	.082 (-.068, .231)	β = .128	1.09 (.075)	0.279	
		Females	.085 (.019, .150)	β = .282	2.57 (.033)	0.012	.033 (-.020, .087)	β = .111	1.24 (.027)	0.220	
	CRH	15 weeks	Males	-.329 (-.824, .167)	β = -.215	-1.35 (.244)	0.186	-.342 (-.731, .048)	β = -.243	-1.79 (.191)	0.083
			Females	-.110 (-.497, .277)	β = -.102	-0.58 (.190)	0.567	.021 (-.308, .350)	β = .020	0.13 (.161)	0.897
		19 weeks	Males	.048 (-.072, .168)	β = .117	0.80 (.060)	0.424	.037 (-.068, .142)	β = .094	0.71 (.052)	0.480
			Females	.017 (-.050, .085)	β = .057	0.51 (.034)	0.609	.008 (-.043, .059)	β = .026	0.31 (.026)	0.757
25 weeks		Males	.008 (-.035, .051)	β = .051	0.38 (.021)	0.704	.001 (-.037, .039)	β = .008	0.60 (.020)	0.949	
		Females	.001 (-.020, .022)	β = .010	0.10 (.011)	0.922	-.006 (-.022, .011)	β = -.056	-0.69 (.008)	0.496	
31 weeks		Males	-.007 (-.010, -.004)	β = -.516	-4.64 (.002)	0.000	-.006 (-.009, -.004)	β = -.511	-4.91 (.001)	0.000	
		Females	-.003 (-.007, .001)	β = -.150	-1.40 (.002)	0.166	-.001 (-.005, .002)	β = -.068	-0.82 (.002)	0.415	

^aMultiple regression analyses controlled for maternal age, caesarian section delivery, parity, infant's age at exam, and mother's ethnicity/race.

^bMultiple regression analyses controlled for maternal age, caesarian section delivery, parity, infant's age at exam, mother's ethnicity/race, and gestational length.

is associated with lower mental and motor scores at 3 months and lower motor scores at 8 months (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003). Moreover, lower placental CRH at 25 weeks of gestation predicted lower scores in infant fear and distress at 2 months of age (Davis et al., 2005).

Findings from this study also indicated that there were differential effects of fetal exposure to cortisol and CRH depending on the sex of the infant, such that fetal exposure to increases in maternal cortisol at 15 and 19 weeks and fetal exposure to increases in placental CRH at 31 weeks was associated with decreased infant maturation only among male infants. Further, increases in maternal cortisol at 25 weeks also led to decreased infant maturation among males, suggesting that the effects of fetal exposure to maternal cortisol at this time point were obscured by including female infants in initial analyses.

Multiple studies have found sex-specific effects of fetal exposure to maternal glucocorticoids in animal and human models, although many of these findings produce contrary results to the current study. Specifically, female rats appear to be more responsive to the effects of prenatal exposure to maternal psychosocial stress (e.g., restraint), exhibiting hypocorticism over the diurnal cycle, as well as increased ACTH and cortisol responses to restraint in adulthood (Koehl et al., 1999; McCormick, Smythe, Sharma, & Meaney, 1995). Moreover, data from mouse models suggest that there may be greater transport of corticosterone across the placenta for female versus male fetuses (Montano, Wang, & vom Saal, 1993); however, female guinea pigs exposed prenatally to dexamethasone showed increases in plasma cortisol levels only during the follicular and luteal phases of the cycles, suggesting a potential influence of sex steroids in the increased cortisol responses of females exposed to prenatal stress (Liu, Li, & Matthews, 2001). Nevertheless, male pigs from mothers treated with ACTH expressed higher corticosteroid-binding globulin (CBG), increased noradrenaline and increased brain monoamine turnover, suggesting that the sex-specific effects of exposure to prenatal stress hormones may vary depending on species (Kanitz, Otten, & Tuchscherer, 2006). Further, human fetal plasma testosterone has been significantly correlated with fetal plasma cortisol and male fetuses have significantly higher testosterone concentrations than female fetuses (Gitau, Adams, Fisk, & Glover, 2005). Higher concentrations of testosterone among male fetuses appear to be restricted to earlier periods of gestation, given that testosterone concentrations increase throughout gestation among female fetuses and are similar in the two sexes by term; therefore, the responsiveness of male fetuses to stress hormones in the current study could be due to baseline higher concentrations of fetal cortisol and testosterone

earlier in gestation (Beck-Peccoz et al., 1991; Gitau et al., 2005).

The present study also found that maternal cortisol at 31 weeks was associated with significant increases in newborn physical and neuromuscular maturation, which appeared to be restricted to female infants. Although these effects were not significant after controlling for length of gestation, these findings raise the possibility that exposure to maternal stress hormones may have multiple consequences for the fetus, in some cases possibly leading to acceleration in fetal maturation to ensure survival in anticipation of a premature birth (Denver, 1997). The pattern of findings raises the possibility that not only does the timing of exposure to stress hormones have consequences for fetal development, but the timing of the exposure may differentially affect males and females. In light of this possibility, it is important to critically examine the studies that have investigated sex-specific effects of fetal exposure to stress hormones, given that the preponderance of these studies have limited the stress exposures to mid to late gestation and/or throughout gestation in animal models, which may not map precisely on to gestational periods of humans (Kanitz et al., 2006; Koehl et al., 1999; Liu et al., 2001; McCormick et al., 1995; Montano et al., 1993).

In summary, the results from the present study indicate that fetal exposure to elevated levels of maternal stress hormones early in pregnancy and placental stress hormones late in pregnancy were associated with infant measures of maturation. These results are consistent with previous results from our research group in which elevated levels of maternal cortisol at 15 weeks' gestation was associated with a surge of placental CRH at 31 weeks' gestation, which, in turn, was associated with preterm delivery, suggesting that cortisol early in pregnancy primes the placenta later in pregnancy to initiate labor (Sandman et al., 2006). The present study found that similar mechanisms may be involved in fetal growth and maturation, even after controlling for the gestational length of the pregnancy, suggesting that an early maternal stress signal may alter the dynamic interchange between the mother, the placenta, and the fetus over the course of pregnancy, leading to fewer available resources for the fetus and poorer fetal maturation (Stearns, 2005). These findings, in humans, suggest that the timing of exposure to stress hormones is critical in determining the trajectory of fetal physical and neuromuscular maturation. Furthermore, this is the first study to find that the timing of fetal exposure to stress hormones has sex-specific effects on infant maturation, which calls for future studies to examine the interactions between fetal exposure to stress hormones and sex-specific influences, such as sex steroids. Lastly, future studies are necessary to elucidate whether stress hormones interact with other potentially

important systems, such as maternal nutritional intake and metabolism, oxygen transfer to the fetus, and immune factors.

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REFERENCES

- Ballard, J. L., Khoury, J. C., Wedig, K., Wang, L., Eilers-Walsman, B. L., & Lipp, R. (1991). New Ballard Score, expanded to include extremely premature infants. *Journal of Pediatrics*, 119(3), 417–423.
- Barker, D. J. (1995). Fetal origins of coronary heart disease. *British Medical Journal*, 311(6998), 171–174.
- Beck-Peccoz, P., Padmanabhan, V., Baggiani, A. M., Cortelazzi, D., Buscaglia, M., Medri, G., Marconi, A. M., Pardi, G., & Beitins, I. Z. (1991). Maturation of hypothalamic-pituitary-gonadal function in normal human fetuses: Circulating levels of gonadotropins, their common alpha-subunit and free testosterone, and discrepancy between immunological and biological activities of circulating follicle-stimulating hormone. *Journal of Clinical Endocrinology and Metabolism*, 73(3), 525–532.
- Benediktsson, R., Calder, A. A., Edwards, C. R., & Seckl, J. R. (1997). Placental 11 beta-hydroxysteroid dehydrogenase: A key regulator of fetal glucocorticoid exposure. *Clinical Endocrinology (Oxford)*, 46(2), 161–166.
- Berger, M. A., Barros, V. G., Sarchi, M. I., Tarazi, F. I., & Antonelli, M. C. (2002). Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. *Neurochemical Research*, 27(11), 1525–1533.
- Bernal, A. L., Flint, A. P., Anderson, A. B., & Turnbull, A. C. (1980). 11 beta-Hydroxysteroid dehydrogenase activity (E.C. 1.1.1.146) in human placenta and decidua. *Journal of Steroid Biochemistry*, 13(9), 1081–1087.
- Bloom, S. L., Sheffield, J. S., McIntire, D. D., & Leveno, K. J. (2001). Antenatal dexamethasone and decreased birth weight. *Obstetrics and Gynecology*, 97(4), 485–490.
- Brunson, K. L., Eghbal-Ahmadi, M., Bender, R., Chen, Y., & Baram, T. Z. (2001). Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), 8856–8861.
- Cogswell, M. E., & Yip, R. (1995). The influence of fetal and maternal factors on the distribution of birthweight. *Seminars in Perinatology*, 19(3), 222–240.
- Davis, E. P., Glynn, L. M., Dunkel Schetter, C., Hobel, C., Chicz-Demet, A., & Sandman, C. A. (2005). Corticotropin-releasing hormone during pregnancy is associated with infant temperament. *Developmental Neuroscience*, 27(5), 299–305.
- Denver, R. J. (1997). Environmental stress as a developmental cue: Corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Hormones and Behavior*, 31(2), 169–179.
- Feldman, P. J., Dunkel-Schetter, C., Sandman, C. A., & Wadhwa, P. D. (2000). Maternal social support predicts birth weight and fetal growth in human pregnancy. *Psychosomatic Medicine*, 62(5), 715–725.
- French, N. P., Hagan, R., Evans, S. F., Godfrey, M., & Newnham, J. P. (1999). Repeated antenatal corticosteroids: Size at birth and subsequent development. *American Journal of Obstetrics and Gynecology*, V 180 (1 Pt 1), 114–121.
- Gemzell, C. A. (1953). Blood levels of 17-hydroxycorticosteroids in normal pregnancy. *Journal of Clinical Endocrinology*, 13, 898–902.
- Gitau, R., Adams, D., Fisk, N. M., & Glover, V. (2005). Fetal plasma testosterone correlates positively with cortisol. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 90(2), F166–F169.
- Gitau, R., Cameron, A., Fisk, N. M., & Glover, V. (1998). Fetal exposure to maternal cortisol. *Lancet*, 352(9129), 707–708.
- Gluckman, P. D., & Hanson, M. A. (2004). Maternal constraint of fetal growth and its consequences. *Seminars in Fetal & Neonatal Medicine*, 9(5), 419–425.
- Glynn, L. M., Schetter, C. D., Chicz-DeMet, A., Hobel, C. J., & Sandman, C. A. (2007). Ethnic differences in adrenocorticotrophic hormone, cortisol and corticotropin-releasing hormone during pregnancy. *Peptides*, 28(6), 1155–1161.
- Goland, R. S., Wardlaw, S. L., Blum, M., Tropper, P. J., & Stark, R. I. (1988). Biologically active corticotropin-releasing hormone in maternal and fetal plasma during pregnancy. *American Journal of Obstetrics and Gynecology*, 159(4), 884–890.
- Haataja, L., Mercuri, E., Guzzetta, A., Rutherford, M., Counsell, S., Flavia Frisone, M., et al. (2001). Neurologic examination in infants with hypoxic-ischemic encephalopathy at age 9–14 months: Use of optimality scores and correlation with magnetic resonance imaging findings. *Journal of Pediatrics*, 138(3), 332–337.
- Hennessy, M. B., Davis, H. N., McCrea, A. E., Harvey, A. T., & Williams, M. T. (1999). Short- and long-term consequences of corticotropin-releasing factor in early development. *Annals of the New York Academy of Sciences*, 897, 76–91.
- Hobel, C. J., Dunkel-Schetter, C., Roesch, S. C., Castro, L. C., & Arora, C. P. (1999). Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *American Journal of Obstetrics and Gynecology*, 180(1 Pt 3), S257–S263.
- Huizink, A. C., Robles de Medina, P. G., Mulder, E. J., Visser, G. H., & Buitelaar, J. K. (2003). Stress during pregnancy is associated with developmental outcome in infancy. *Journal of Child Psychology and Psychiatry*, 44(6), 810–818.
- Jones, S. A., Brooks, A. N., & Challis, J. R. (1989). Steroids modulate corticotropin-releasing hormone production in

- human fetal membranes and placenta. *Journal of Clinical Endocrinology and Metabolism*, 68(4), 825–830.
- Kanitz, E., Otten, W., & Tuchscherer, M. (2006). Changes in endocrine and neurochemical profiles in neonatal pigs prenatally exposed to increased maternal cortisol. *Journal of Endocrinology*, 191(1), 207–220.
- King, B. R., Smith, R., & Nicholson, R. C. (2001). The regulation of human corticotrophin-releasing hormone gene expression in the placenta. *Peptides*, 22(11), 1941–1947.
- King, B. R., Smith, R., & Nicholson, R. C. (2002). Novel glucocorticoid and cAMP interactions on the CRH gene promoter. *Molecular and Cellular Endocrinology*, 194(1–2), 19–28.
- Koehl, M., Darnaudery, M., Dulluc, J., Van Reeth, O., Le Moal, M., & Maccari, S. (1999). Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. *Journal of Neurobiology*, 40(3), 302–315.
- Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, 97(20), 11032–11037.
- Linton, E. A., Perkins, A. V., Hagan, P., Poole, S., Bristow, A. F., Tidors, F., Corder, R., & Wolfe C. D. A. (1995). Corticotrophin-releasing hormone (CRH)-binding protein interference with CRH antibody binding: Implications for direct CRH immunoassay. *Journal of Endocrinology*, 146(1), 45–53.
- Liu, L., Li, A., & Matthews, S. G. (2001). Maternal glucocorticoid treatment programs HPA regulation in adult offspring: Sex-specific effects. *American Journal of Physiology, Endocrinology and Metabolism*, 280(5), E729–E739.
- Lopez Bernal, A., Anderson, A. B., & Turnbull, A. C. (1982). The lack of influence of labor on human placental 11 beta-hydroxysteroid dehydrogenase activity. *Journal of Clinical Endocrinology and Metabolism*, 54(6), 1251–1254.
- Lowry, P. J. (1993). Corticotropin-releasing factor and its binding protein in human plasma. *CIBA Foundation Symposium*, 172, 108–115; discussion 115–128.
- Lu, M. C., & Halfon, N. (2003). Racial and ethnic disparities in birth outcomes: A life-course perspective. *Maternal and Child Health Journal*, 7(1), 13–30.
- Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A. R., Cinque, C., & Van Reeth, O. (2003). Prenatal stress and long-term consequences: Implications of glucocorticoid hormones. *Neuroscience and Biobehavioral Reviews*, 27(1–2), 119–127.
- Mancuso, R. A., Schetter, C. D., Rini, C. M., Roesch, S. C., & Hobel, C. J. (2004). Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. *Psychosomatic Medicine*, 66(5), 762–769.
- McArthur, S., McHale, E., Dalley, J. W., Buckingham, J. C., & Gillies, G. E. (2005). Altered mesencephalic dopaminergic populations in adulthood as a consequence of brief perinatal glucocorticoid exposure. *Journal of Neuroendocrinology*, 17(8), 475–482.
- McCormick, C. M., Smythe, J. W., Sharma, S., & Meaney, M. J. (1995). Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Brain Research. Developmental Brain Research*, 84(1), 55–61.
- Mercuri, E., Dubowitz, L., Brown, S. P., & Cowan, F. (1998). Incidence of cranial ultrasound abnormalities in apparently well neonates on a postnatal ward: Correlation with antenatal and perinatal factors and neurological status. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 79(3), F185–F189.
- Mercuri, E., Ricci, D., Pane, M., & Baranello, G. (2005). The neurological examination of the newborn baby. *Early Human Development*, 81(12), 947–956.
- Montano, M. M., Wang, M. H., & vom Saal, F. S. (1993). Sex differences in plasma corticosterone in mouse fetuses are mediated by differential placental transport from the mother and eliminated by maternal adrenalectomy or stress. *Journal of Reproduction and Fertility*, 99(2), 283–290.
- Mullings, L., Wali, A., McLean, D., Mitchell, J., Prince, S., Thomas, D., et al. (2001). Qualitative methodologies and community participation in examining reproductive experiences: The Harlem Birth Right Project. *Maternal and Child Health Journal*, 5(2), 85–93.
- Murphy, V. E., & Clifton, V. L. (2003). Alterations in human placental 11beta-hydroxysteroid dehydrogenase type 1 and 2 with gestational age and labour. *Placenta*, 24(7), 739–744.
- Murphy, V. E., Smith, R., Giles, W. B., & Clifton, V. L. (2006). Endocrine regulation of human fetal growth: The role of the mother, placenta, and fetus. *Endocrine Reviews*, 27(2), 141–169.
- Petraglia, F., Sawchenko, P. E., Rivier, J., & Vale, W. (1987). Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature*, 328(6132), 717–719.
- Petraglia, F., Sutton, S., & Vale, W. (1989). Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. *American Journal of Obstetrics and Gynecology*, 160(1), 247–251.
- Reinisch, J. M., Simon, N. G., Karow, W. G., & Gandelman, R. (1978). Prenatal exposure to prednisone in humans and animals retards intrauterine growth. *Science*, 202(4366), 436–438.
- Roberts, A. D., Moore, C. F., DeJesus, O. T., Barnhart, T. E., Larson, J. A., Mukherjee, J., et al. (2004). Prenatal stress, moderate fetal alcohol, and dopamine system function in rhesus monkeys. *Neurotoxicology and Teratology*, 26(2), 169–178.
- Rodbard, D., Munson, P., & DeLean, A. (1978). Improved curve fitting, parallelism testing, characterization of sensitivity and specificity, validation, and optimization for radioligand assays. *Radioimmunoassay Related Proceedings in Medicine*, 1, 469–504.
- Sandman, C. A., Glynn, L., Schetter, C. D., Wadhwa, P., Garite, T., Chic-DeMet, A., et al. (2006). Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental

- corticotropin releasing hormone (CRH): Priming the placental clock. *Peptides*, 27(6), 1457–1463.
- Sandman, C. A., Glynn, L., Wadhwa, P. D., Chic-DeMet, A., Porto, M., & Garite, T. (2003). Maternal hypothalamic-pituitary-adrenal dysregulation during the third trimester influences human fetal responses. *Developmental Neuroscience*, 25(1), 41–49.
- Sandman, C. A., Wadhwa, P. D., Chic-DeMet, A., Porto, M., & Garite, T. J. (1999). Maternal corticotropin-releasing hormone and habituation in the human fetus. *Developmental Psychobiology*, 34(3), 163–173.
- Sandman, C. A., Wadhwa, P. D., Dunkel-Schetter, C., Chic-DeMet, A., Belman, J., Porto, M., et al. (1994). Psychological influences of stress and HPA regulation on the human fetus and infant birth outcomes. *Annals of the New York Academy of Sciences*, 739, 198–210.
- Sandman, C. A., Wadhwa, P., Glynn, L., Chic-DeMet, A., Porto, M., & Garite, T. J. (1999). Corticotrophin-releasing hormone and fetal responses in human pregnancy. *Annals of the New York Academy of Sciences*, 897, 66–75.
- Schoof, E., Girstl, M., Frobenius, W., Kirschbaum, M., Repp, R., Knerr, I., et al. (2001). Course of placental 11 β -hydroxysteroid dehydrogenase type 2 and 15-hydroxyprostaglandin dehydrogenase mRNA expression during human gestation. *European Journal of Endocrinology*, 145(2), 187–192.
- Shams, M., Kilby, M. D., Somerset, D. A., Howie, A. J., Gupta, A., Wood, P. J., et al. (1998). 11 β -hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction. *Human Reproduction*, 13(4), 799–804.
- Stearns, S. C. (2005). Issues in evolutionary medicine. *American Journal of Human Biology*, 17(2), 131–140.
- Trainer, P. J. (2002). Corticosteroids and pregnancy. *Seminars in Reproductive Medicine*, 20(4), 375–380.
- Wadhwa, P. D., Garite, T. J., Porto, M., Glynn, L., Chic-DeMet, A., Dunkel-Schetter, C., et al. (2004). Placental corticotropin-releasing hormone (CRH), spontaneous preterm birth, and fetal growth restriction: A prospective investigation. *American Journal of Obstetrics and Gynecology*, 191(4), 1063–1069.
- Wadhwa, P. D., Porto, M., Garite, T. J., Chic-DeMet, A., & Sandman, C. A. (1998). Maternal corticotropin-releasing hormone levels in the early third trimester predict length of gestation in human pregnancy. *American Journal of Obstetrics and Gynecology*, 179(4), 1079–1085.
- Wadhwa, P. D., Sandman, C. A., Porto, M., Dunkel-Schetter, C., & Garite, T. J. (1993). The association between prenatal stress and infant birth weight and gestational age at birth: A prospective investigation. *American Journal of Obstetrics and Gynecology*, 169, 858–865.
- Welberg, L. A., Seckl, J. R., & Holmes, M. C. (2001). Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: Possible implications for behaviour. *Neuroscience*, 104(1), 71–79.