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Antenatal Glucocorticoid Treatment is Associated with Diurnal Cortisol Regulation in Term-Born Children

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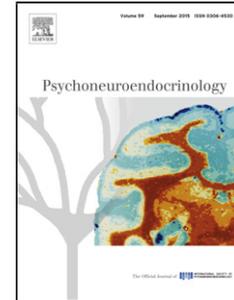
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Antenatal glucocorticoid treatment is associated with diurnal cortisol regulation in term-born children

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Research Highlights

- Antenatal glucocorticoids are given to mothers at risk for preterm delivery
- There are no known benefits of glucocorticoid treatment in term-born children
- Full term children with glucocorticoid treatment displayed altered HPA axis functioning
- Antenatal glucocorticoids were associated with lack of a CAR and a flattened diurnal slope

Abstract:

Due to the rapid developmental changes that occur during the fetal period, prenatal influences can affect the developing central nervous system with lifelong consequences for physical and mental health. Glucocorticoids are one of the proposed mechanisms by which fetal programming occurs. Glucocorticoids pass through the blood-brain barrier and target receptors throughout the central nervous system. Unlike endogenous glucocorticoids, synthetic glucocorticoids readily pass through the placental barrier to reach the developing fetus. The synthetic glucocorticoid, betamethasone, is routinely given prenatally to mothers at risk for preterm delivery. Over 25% of the fetuses exposed to betamethasone will be born at term. Few studies have examined the lasting consequences of antenatal treatment of betamethasone on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. The purpose of this study is to examine whether antenatal exposure to betamethasone alters circadian cortisol regulation in children who were born full term. School-aged children prenatally treated with betamethasone and born at term ($n=19$, mean (SD) = 8.1 (1.2) years old) were compared to children not treated with antenatal glucocorticoids ($n=61$, mean (SD) = 8.2 (1.4) years old). To measure the circadian release of cortisol, saliva samples were collected at awakening; 30, 45, and 60 minutes after awakening; and in the evening. Comparison children showed a typical diurnal cortisol pattern that peaked in the morning (the cortisol awakening response) and gradually decreased throughout the day. In contrast, children exposed to antenatal betamethasone lacked a cortisol awakening response and had a flatter diurnal slope (p 's <0.01). These data suggest that antenatal glucocorticoid treatment may disrupt the circadian regulation of the HPA axis among children born at term. Because disrupted circadian regulation of cortisol has been linked to mental and somatic health problems, future research is needed to determine whether children exposed to antenatal synthetic glucocorticoids are at risk for poor mental and physical health.

Keywords: betamethasone; glucocorticoid; cortisol; prenatal; fetal programming; HPA axis

1. Introduction:

The rapid developmental changes that occur during the fetal period are unmatched at any other stage of life. Because of the extraordinary pace of fetal development, the fetus is highly vulnerable to the consequences of stress exposure (Bourgeois, 1997). Prevailing models of early life stress propose that exposure to excess glucocorticoids is a key factor that shapes development of neural systems with lifelong consequences for physical and mental health (Moisiadis and Matthews, 2014a). Although there is extensive support for this model from experimental animal studies (Matthews, 2000), our understanding of the effects of antenatal glucocorticoid exposure on human neurodevelopment is limited.

For a number of reasons, glucocorticoids are a candidate mechanism underlying fetal programming. Glucocorticoids exert a wider array of metabolic, endocrine, and immune effects than any other biological ligand (Chrousos and Kino, 2009). Furthermore, glucocorticoids pass through the blood-brain barrier, target receptors throughout the central nervous system, and play a central role in normal brain development (Jacobson and Sapolsky, 1991). Although glucocorticoids are necessary for normative fetal brain development, exposure to excessive levels may disrupt basic neurodevelopmental processes including dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Buss et al., 2012). The risk may be even greater when glucocorticoids are administered exogenously, as they are to women in preterm labor, thus exposing the fetus to supraphysiologic levels.

Synthetic glucocorticoids, such as betamethasone, are routinely given as part of the standard of care to women who are at risk of delivery between 24 and 34 gestational weeks to reduce respiratory distress and increase survival among infants born preterm (McKinlay et al., 2012; American College of Obstetricians and Gynecologists, 2011). Unlike cortisol, betamethasone is not metabolized or inactivated by the placental enzyme 11-beta hydroxysteroid dehydrogenase and thereby crosses the placenta more readily to act directly on the developing fetus (Albiston et al., 1994; Kajantie et al., 2004). The benefits for lung maturation and survival among preterm infants are undisputed. However, the persisting consequences of antenatal glucocorticoids for neurodevelopment remain poorly understood. Preterm

birth is associated with increased risk for a wide range of neurodevelopmental consequences (Espel et al., 2014; Grunau et al., 2005). Thus, the effects of antenatal glucocorticoid treatment may best be observed among healthy children born at term independent of the neurodevelopmental effects of preterm delivery. Due to successful medical interventions and the difficulty of predicting who will deliver preterm, 25% to 30% of women who receive glucocorticoid treatment deliver at term (Davis et al., 2009). Recent studies have demonstrated that among children born full term, fetal exposure to glucocorticoid treatment is associated with restricted fetal growth (Davis et al., 2009), cortical thinning (Davis et al., 2013) and memory impairments (Grant et al., 2015).

One pathway by which antenatal glucocorticoid exposure is thought to influence later childhood outcomes is through altering the development of the fetal HPA axis. Term neonates treated with antenatal glucocorticoids have a larger cortisol response to the painful stress of the heel-stick blood draw when compared to term neonates without antenatal glucocorticoid treatment (Davis et al., 2011). The effects of antenatal glucocorticoids persist into childhood and are associated with increased cortisol reactivity to a social stress test (Alexander et al., 2012; Erni et al., 2012). These studies suggest that antenatal glucocorticoid treatment has long-lasting effects on the HPA axis response to stress. Less is known, however, about the effects of antenatal glucocorticoid treatment on circadian cortisol regulation. The HPA axis releases cortisol in a circadian pattern. Cortisol concentrations typically peak 30-45 minutes after awakening (the cortisol awakening response; CAR) and then gradually decline across the day. The CAR is a response to awaking (Clow et al., 2010; Wilhelm et al., 2007). Compelling evidence exists that the CAR is affected by exposure to early adversity including prenatal maternal depression and anxiety (Antonini et al., 2000; Lee et al., 2014; O'Donnell et al., 2013; Stalder et al., 2013). Further, among children who were born preterm, *postnatal* glucocorticoid treatment is associated with a blunted CAR during adolescence (Ter Wolbeek et al., 2015). During the prenatal period, evidence suggests that glucocorticoids shape the development of the fetal HPA axis. The offspring of mothers who received chronic prednisone treatment for rheumatoid arthritis throughout their pregnancy display altered circadian cortisol production and higher cortisol output over the day (de Steenwinkel et al., 2014). Studies

evaluating the association between early adversity and prematurity and development of the CAR indicate that prenatal glucocorticoid treatment may affect HPA axis development and be associated with a blunting of the CAR. The current study investigates whether antenatal betamethasone treatment is associated with altered diurnal cortisol patterns among school-aged children who were born full term.

2. Material and Methods:

2.1. Participants

Participants included 111 children, aged 6 to 10 who were born at term, and their mothers recruited using delivery records from a major medical center in Southern California. All children were full term at birth (gestational age more than 37 weeks based on American College of Obstetricians and Gynecologists, 2009, dating criteria; (American College of Obstetricians and Gynecologists, 2009)). Child inclusion criteria were appropriate weight for gestational age and singleton status. Exclusion criteria included child chromosomal or other congenital anomalies (e.g., trisomy 21), child exposure to postnatal systemic steroids, major neonatal illness (e.g., sepsis), maternal preeclampsia or HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, maternal drug use, and maternal disorders during pregnancy requiring corticosteroid treatment. Seven percent of glucocorticoid-exposed children and 5% from the comparison group failed to meet eligibility criteria and were not recruited.

Eligible participants were placed in two groups: those who received antenatal treatment with the glucocorticoid betamethasone due to risk of preterm delivery and those not treated with antenatal glucocorticoids (comparison group). Of those contacted, 86% of glucocorticoid exposed and 63% of nonexposed mother-child pairs consented to participate. Of the 26 glucocorticoid-exposed children, 4 declined participating in the home saliva sample collection and 3 participants provided data that was unusable because 3 or more time points were missing or of poor quality. Of the 85 comparison participants, 18 declined participating in home sample collection and 6 provided data that was unusable because 3 or more time points were missing or of poor quality. The glucocorticoid group consisted of 19

children born full term who received antenatal glucocorticoid treatment (betamethasone). Consistent with standard clinical practice, betamethasone was given via two intramuscular injections 24 hours apart between 24 and 34 gestational weeks ($M = 30.1$ weeks, $SD = 3.1$). The comparison group consisted of 61 children born full term and without antenatal exposure to synthetic glucocorticoids. We over recruited children in the comparison group to create a more stable characterization of child HPA axis regulation among unexposed children. The institutional review boards for protection of human subjects approved the study protocol. Written and informed consent from the mother and informed assent from the child were obtained before study enrollment.

2.2. Background Information

Sociodemographic characteristics were determined at the time of study entry by maternal interview. Children's *general intelligence* was assessed using the Perceptual Reasoning Index (PRI) of the Wechsler Intelligence Scale for Children (WISC-IV). The PRI is relatively language free and two of its subscales (Matrix Reasoning and Block Design) have been shown to be excellent indicators of general intelligence (Wechsler, 2002). Scores between 90 and 109 are considered average. Child global health was assessed with the 5-item global health scale from the MacArthur Health and Behavior Questionnaire (Armstrong and Goldstein, 2003). Possible scores ranged from 0 excellent health to 3 poor health. Child experience of life events was evaluated using the Coddington Life Events Checklist for Elementary School Aged Children (Coddington, 1972). This measure assesses both positive (e.g., "outstanding personal achievement") and negative (e.g., parental divorce) life events. Total score on this measure could range from 0 to 33. Maternal intellectual performance was determined by the Perceptual Organization Index (POI) of the Wechsler Adult Intelligence Scale (Wechsler, 1997). Scores between 90 and 109 are considered average. Maternal depression was evaluated with the Beck Depression Inventory II (BDI; Beck et al., 1996). Scores could range from 0 to 63 (14-19 mild depression; 20-28 moderate depression; 29-63 severe depression). Obstetric and neonatal medical characteristics, including birth outcome data, were determined by medical record abstraction.

2.3. Salivary Cortisol Assessment

Mothers collected saliva samples from their children five times over the course of one weekday at home. Samples were collected four times in the morning (at awakening and 30, 45, and 60 minutes after waking) and once in the evening (at 2000h). Salivary cortisol reflects the unbound or free cortisol and is highly correlated with plasma cortisol (Gunnar et al., 1989; Kirschbaum and Hellhammer, 1989). For each sample, a small, absorbent swab was placed in the child's mouth for approximately 30 seconds. The swab was then placed into the Swab Extraction Tube System (S.E.T.S., Roche Diagnostics) and returned to the lab by mail. Collection swabs were kept in Medication Event Monitoring System (MEMS)[®] 6 TrackCaps which recorded each date and time the cap was opened to remove a swab. Mothers were also asked to fill out a short survey including questions about sample collection time, awakening time, their child's sleep habits and health status. None of the samples were collected on days when the child was febrile. Mothers reported that medication use included analgesics (n=1), antibiotics (n=2) and an expectorant (n=1). Inclusion of these children did not alter study findings and thus they were included in analyses. Once returned to the lab, samples were spun in a centrifuge at 5,500 rpm for 15 minutes to extract saliva from the collection swab. Samples were stored at -20°C until assayed. Salivary cortisol levels were determined by a competitive luminescence immunoassay (LIA; IBL-America, Minneapolis, MN) with detection limits of 0.138 nmol/L. The intra- and inter-assay coefficients of variance are 5.5% and 7.6%, respectively. All samples were assayed in duplicate and averaged. For each time point, samples that were above 3 standard deviations above the mean were removed.

2.4. Data Analysis

Fisher exact and one-way analysis of variance tests were used to determine whether child (intelligence, health, experience of life events, race/ethnicity; sex; birth order; gestational age at birth; birth weight percentile; Apgar score; age at assessment; and time of awakening) or maternal (cohabitation status; cohabitation with the child's father; education level; household income; age at delivery;

intelligence; and depression level) characteristics differed between groups. Of these potential confounding factors, only gestational age at birth significantly differed between groups and thus was included as a covariate in all analyses. Because CAR was a primary outcome of interest, time of awakening was additionally included as a covariate. We used multiple imputations (n=10 datasets) as a strategy to manage the missing data, which consisted of 2 cortisol values, 5 collection times, and 3 awakening times (Little, 2002). No participants were missing more than 2 values. Cortisol values were positively skewed at all sampling periods and thus a square root transformation was applied. Data transformation did not alter the results, thus the untransformed data is reported in nanomoles per liter (nmol/L) to improve interpretability.

Multilevel modeling using hierarchical linear modeling (HLM) growth curve analysis was conducted to assess the association between antenatal betamethasone exposure and children's diurnal cortisol (Raudenbush, 2002). Initial testing indicated that a piecewise model provided the best fit for diurnal cortisol patterns. To create the piecewise model, data for each individual was split into two discrete phases, before and after their maximum cortisol value 30-45 minutes after awakening. In the full two-level model, the effects of betamethasone exposure on cortisol levels at awakening, the CAR, and the diurnal cortisol slope were examined. Specifically, the Level 1 variables (or the time-variant variables) included the cortisol values at each sample collection and the time of the sample collection in hours. The Level 2 variables (or time-invariant variables) included betamethasone exposure and relevant covariates (time of awakening and GAB).

3. Results:

As described in table 1, children were predominantly Hispanic or non-Hispanic white, of average intelligence, and of good physical health. Median income in this sample was 55,000 and mothers were predominantly cohabitating with the child's father (82.5%) (See Table 2). Although only term-born children were included in this study, those treated with betamethasone had a younger gestational age at

birth compared to those who did not receive glucocorticoids (38.5wks vs. 39.4wks; Table 1). The betamethasone group did not significantly differ from the comparison group on any other child (intelligence, child health, exposure to life events, race/ethnicity; sex; birth order; gestational age at birth; birth weight percentile; Apgar score; age at assessment; and time of awakening) or maternal (cohabitation status; cohabitation with the child's father; education level; household income; age at delivery; intelligence; and maternal depression level) characteristics (all p 's >0.05 , Tables 1 & 2).

Cortisol values for the betamethasone and comparison groups are illustrated in Figure 1. Children exposed to antenatal betamethasone failed to show a CAR and exhibited a flatter diurnal slope than the comparison participants ($\beta_{CAR}=-3.44$, $p<0.05$; $\beta_{diurnal}=0.19$, $p<0.05$; Table 3). These associations were strengthened when gestational age at birth and time of awakening included as covariates ($\beta_{CAR}=-4.77$, $p<0.01$, $\beta_{diurnal}=0.23$, $p<0.01$; Table 3). Figure 2 depicts the HLM modeled influence of betamethasone on cortisol levels after accounting for covariates. Gestational age at the time of betamethasone treatment was not associated with diurnal cortisol regulation ($p=0.80$).

4. Discussion:

The current study provides new evidence that antenatal glucocorticoid treatment is associated with persisting alterations in diurnal cortisol regulation among school-aged children. Children exposed to betamethasone during fetal development lacked a cortisol awakening response and had a flatter diurnal slope than children that were not treated with antenatal glucocorticoids. Interestingly, the diurnal cortisol profile, characterized by lack of a cortisol awakening response and a flattened diurnal slope, is similar to that seen among children experiencing prenatal (O'Donnell et al., 2013) or postnatal (Koss et al., 2014) adversity. The association between prenatal glucocorticoid treatment and diurnal cortisol regulation was observed among children who were born full term and persisted after considering potential covariates.

Our findings are consistent with a recent study by Ter Wolbeek and colleagues (2015) demonstrating that *postnatal* glucocorticoids are associated with a flattened CAR among children who

were born preterm. Premature delivery is associated with HPA axis dysregulation (Lee et al., 2014). Our observation that antenatal glucocorticoids are associated with a flattened CAR among children born full term provides important new evidence that glucocorticoid treatment is associated with HPA axis development independent of birth outcome. In conjunction with prior human research indicating that antenatal glucocorticoid treatment is associated with dysregulated cortisol responses to stress (Alexander et al., 2012; Davis et al., 2004; Davis et al., 2006; Davis et al., 2011; de Steenwinkel et al., 2014; Erni et al., 2012; Ter Wolbeek et al., 2015), these data suggest that antenatal glucocorticoid treatment exerts long term programming consequences on HPA axis development. The extant literature has relied on correlational designs. Future research should evaluate children who were part of randomized controlled trials for prenatal glucocorticoid treatment (Gyamfi-Bannerman et al., 2016). Long term follow up of children who received glucocorticoids as part of an experimental design is needed to fully determine the neurodevelopmental influence of prenatal glucocorticoid treatment.

It is plausible that the alterations to diurnal cortisol regulation we observed among children who received antenatal glucocorticoids indicate risk for poor physical and mental health outcomes. Neurological advances in middle childhood include synaptic pruning and myelination (Muftuler et al., 2011; Muftuler et al., 2012). These neurodevelopmental processes may be influenced by cortisol exposure. Although cortisol levels tend to be lower in middle childhood as compared to adolescence, there is evidence for individual stability in diurnal cortisol regulation from childhood to adolescence (Shirtcliff et al., 2012). Thus, children with antenatal betamethasone may continue to show dysregulated cortisol production into adolescence.

A flattened CAR and diurnal slope may indicate risk for metabolic disease or psychiatric dysfunction. Toddlers with a blunted CAR are more likely to have delays in cognitive functioning (Saridjan et al., 2014a). Children and adults with lower morning cortisol and a flatter diurnal slope have higher levels of internalizing problems (Bhattacharyya et al., 2008; Ruttle et al., 2011; Saridjan et al., 2014b) and in adulthood disrupted circadian cortisol regulation is associated with risk for metabolic, cardiac, and

autoimmune diseases (Chikanza et al., 1992; Matthews et al., 2006; Rosmond and Bjorntorp, 1998). Because a blunted CAR and a flat diurnal cortisol slope are associated with health risks, additional research is needed to determine the long-term consequences. The massive developmental changes occurring during gestation render the fetus vulnerable to exposures including elevated glucocorticoids. Glucocorticoids cross the blood-brain barrier and influence fetal neurogenesis, synaptogenesis, and myelination (McCabe et al., 2001; Moisiadis and Matthews, 2014b). These effects of glucocorticoids are greatest in regions that contain the highest levels of glucocorticoid receptors, such as the amygdala, hippocampus, and prefrontal cortex (Diorio et al., 1993; Jacobson and Sapolsky, 1991; Sanchez et al., 2000). During the gestational period when glucocorticoids are administered neurodevelopmental processes include neuronal differentiation, dendritic arborization, axonal elongation, synapse formation and collateralization, and myelination (Bourgeois, 1997; Volpe, 2008). Further, glucocorticoid receptors are present in the human hippocampus at by 24 gestational weeks rendering the fetal brain susceptible to glucocorticoids (Noorlander et al., 2006). By altering the development of hypothalamic, limbic, and cortical regions that play a central role in HPA axis regulation, antenatal glucocorticoids may exert persisting consequences on child HPA axis functioning. For example, glucocorticoids may downregulate the fetal HPA axis by activating epigenetic and feedback mechanisms that affect glucocorticoid receptors (Moisiadis and Matthews, 2014b). Fewer glucocorticoid receptors may result in desensitization of the HPA axis and a reduction in morning cortisol production (Jarcho et al., 2013). Fetal exposure to high levels of glucocorticoids may modify the development of the HPA axis and its regulatory brain regions resulting in alterations to diurnal cortisol regulation.

This study has several strengths. We examined healthy children 8-10 years of age who were full term at birth. Additionally, adherence to the cortisol sampling procedure was evaluated using both maternal-report and the Medication Event Monitoring System (MEMS), which records the time of sample collection. This technology allowed us to ensure good compliance, which is especially critical when measuring time-sensitive cortisol levels. A primary limitation of the present investigation is that

betamethasone treatment was not randomly assigned, and thus we cannot rule out the possibility of confounding factors. It is plausible that unmeasured prenatal experiences that related to the reason for betamethasone treatment contributed to group differences that affect HPA axis development. Further, because women and children were not prospectively evaluated during the prenatal period, we were not able to characterize other aspects of the prenatal environment (e.g. maternal stress, maternal cortisol) that may influence fetal HPA axis development. However, we examined several potential covariates. Groups differed on gestational age of birth, but not differ on other key clinical and demographic factors (Tables 1 and 2).

Our findings extend the existing literature by evaluating children born healthy and full term. Our results show that antenatal exposure to a single course of betamethasone is associated with absence of a cortisol awakening response and flat diurnal slope. Future research is needed to understand the clinical implications for health and development across the lifespan.

All authors have participated in the research and article preparation. EPD conceived and designed the study and acquired the data. MNE, EPD, CAS, and LMG analyzed the data. All authors contributed to the interpretation of the data, drafted or revised the manuscript, and have approved the final article.

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Figure Legends:

Figure 1: Salivary cortisol levels across the day for children treated (gray bars) or not treated (patterned bars) with antenatal betamethasone.

Figure 2: HLM model of the unique contribution of antenatal betamethasone exposure on diurnal cortisol trajectories. Even after accounting for covariates, children treated with betamethasone had an atypical decline in cortisol levels after awakening (gray line). In contrast, children without antenatal glucocorticoid treatment showed a typical peak in cortisol after awakening followed by a gradual decrease in cortisol across the day (black line).

Table 1: Descriptive information for children in the study sample

Child Characteristics	Betamethasone Group (n=19)	Comparison Group (n=61)	P
GA at first dose (weeks) ^b	30.1 (3.1)	N/A	N/A
Days between first dose and delivery ^b	59.5 (22.1)	N/A	N/A
Gestational age at birth (weeks) ^b	38.5 (0.9)	39.3 (1.4)	0.01
Birth weight (grams) ^b	3475 (465.5)	3414 (417.7)	0.59
Birth weight percentile ^b	60.1 (26.8)	51.1 (25.8)	0.19
Birth order (% firstborn) ^a	7 (36.8%)	19 (31.1%)	0.78
Race/Ethnicity ^a			0.83
Hispanic	9 (47.4%)	33 (51.6%)	
Non-Hispanic White	5 (26.3%)	15 (23.4%)	
Other	5 (26.3%)	13 (20.3%)	
Sex (% Male) ^a	8 (42.1%)	33 (54.1%)	0.44
Apgar score at 5-minutes ^b	8.95 (0.23)	8.90 (0.44)	0.65
Age at assessment (yrs) ^b	8.07 (1.19)	8.22 (1.42)	0.68
Physical health score	0.57 (0.42)	0.61 (0.56)	.74
WISC-IV: PRI	102.0 (11.5)	97.4 (13.2)	0.17
Life events score	6.9 (3.1)	7.4 (2.8)	0.56

Time of Awakening ^b	7:19am (1:08)	7:08am (0:57)	0.52
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^a: N(%). ^b: Mean (SD). Betamethasone group n=19, except gestational age at first dose and days between first dose and delivery where n=17. Comparison group n=61, except birth weight and Apgar score where n=60 and time of awakening where n=59.

Table 2: Descriptive information for mothers

Maternal Demographics	Betamethasone Group (n=19)	Comparison Group (n=61)	P
Maternal age at delivery (yrs) ^b	30.2 (5.5)	29.3 (6.9)	0.60
Living as married or cohabitating ^a	18 (94.7%)	53 (86.9%)	0.68
Cohabiting with baby's father ^a	18 (94.7%)	48 (78.7%)	0.17
Highest level of education ^a			0.49
High School or Less	4 (21.1%)	22 (34.4%)	
Some College or certificate	10 (52.6%)	26 (40.6%)	
Bachelor degree or higher	5 (26.3%)	13 (20.3%)	
Annual household income ^a			0.52
\$0-\$50,000	8 (42.1%)	26 (44.1%)	
\$50,001-\$100,000	7 (36.8%)	13 (22.0%)	
Over \$100,000	4 (21.1%)	17 (28.8%)	
WAIS: POI score ^b	95.3 (14.1)	93.4 (17.7)	0.67
BDI-II total score ^b	6.2 (6.3)	6.1 (6.4)	0.97

^a: N(%). ^b: Mean (SD). Comparison group n=61, except for household income where n=56.

Table 3: Betamethasone exposure and gestational age at birth predict cortisol rhythm in children.

<i>Effect</i>	<i>Estimate</i>	<i>SE</i>	<i>T</i>
Growth Curve Model			
Average Awakening levels	5.37	0.57	9.50***
Average CAR	-0.12	0.85	-0.14
Average Diurnal Slope	-0.34	0.04	-9.11***
Betamethasone Model			
Predicting Awakening levels			
Intercept	5.27	0.67	7.92***
Betamethasone	0.37	1.24	0.30
Predicting CAR			
Intercept	0.71	1.01	0.70
Betamethasone	-3.44	1.64	-2.10*
Predicting Diurnal Slope			
Intercept	-0.38	0.04	-8.71***
Betamethasone	0.19	0.07	2.63**
Multivariate Model			
Predicting Awakening levels			

Intercept	5.20	0.60	8.66***
Betamethasone	0.74	1.23	0.61
Gestational age at birth	0.70	0.44	1.57
Time of awakening	-0.94	0.16	-5.87***
Predicting CAR			
Intercept	1.01	0.97	1.04
Betamethasone	-4.77	1.72	-2.78**
Gestational age at birth	-1.48	0.60	-2.46*
Predicting Diurnal Slope			
Intercept	-0.40	0.04	-9.03***
Betamethasone	0.23	0.08	2.94**
Gestational age at birth	0.04	0.03	1.67^

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. CAR: Cortisol Awakening Response. All predictors for each model are represented in the table.

