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# Prenatal cortisol, prematurity and low birthweight

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## **Abstract**

Three hundred depressed pregnant women were recruited at approximately 20 weeks gestation. They were then divided by a median split into high and low urinary cortisol level groups. The high cortisol group had higher CES-D depression scores and higher inhibition (BIS) scores prenatally. Their fetuses had smaller head circumference, abdominal circumference, biparietal diameter and fetal weight. The high cortisol group neonates were shorter gestational age and lower birthweight and they had lower Brazelton habituation and higher Brazelton reflex scores. Discriminant function analyses suggested that cortisol levels more accurately classified short gestation and low birthweight groups than CES-D depression scores.

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In a recent study, prenatal cortisol was a significant predictor of prematurity (Field et al., 2004). Prenatal mood and biochemistry levels were assessed in women with and without depressive symptoms during their second trimester of pregnancy. At the neonatal period, maternal and neonatal biochemistry, EEG and vagal tone levels were assessed, neonatal behavior states were observed, and the Brazelton Neonatal Behavior Assessment was conducted. The mothers with depressive symptoms exhibited a profile of higher prenatal cortisol levels and lower dopamine and serotonin levels. Those mothers were also more likely to deliver prematurely and have low birthweight babies. The newborns of mothers with depressive symptoms had higher cortisol levels and lower dopamine and serotonin levels, thus mimicking their mothers' prenatal levels. These newborns also exhibited greater relative right frontal EEG asymmetry and lower vagal tone than neonates of non-depressed mothers. On the Brazelton Scale, the newborns of depressed mothers had less optimal habituation, orientation, motor, range of state, and autonomic stability scores as well as higher depression scores. A path analysis conducted to assess the effects of prenatal depression and the mothers' prepartum biochemistry on gestational age and birthweight showed that prenatal depression was related to prepartum cortisol and norepinephrine levels. Cortisol levels were, in turn, related to prematurity, and norepinephrine levels were related to low birthweight (see Fig. 1).

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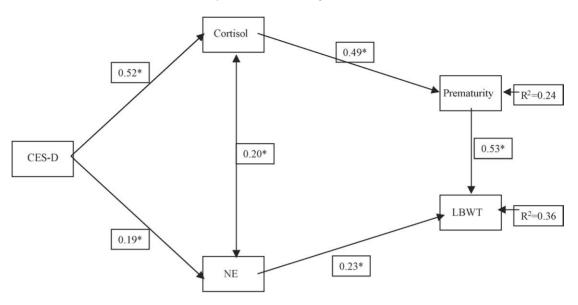


Fig. 1. Structural equations model in prenatal depression (CESD), prenatal cortisol and prematurity.

These findings replicated previous prenatal depression findings suggesting imbalanced maternal neuroendocrine function during pregnancy and less optimal neurobehavioral profiles in neonates of depressed mothers (Diego et al., 2004; Lundy et al., 1999). Furthermore, they expanded previous findings by our group, while replicating others' findings (Orr, James, & Prince, 2002; Orr & Miller, 1995; Steer, Scholl, Hediger, & Fischer, 1992) indicating that depressed mothers are more likely to deliver premature and low birthweight babies than non-depressed mothers. These findings suggest not only that prenatal depression affects fetal development, but also that these effects are mediated at least in part by elevated prenatal cortisol and norepinephrine.

The effects of cortisol on fetal weights were assessed in a subsequent study (Diego et al., submitted for publication). In this study on 98 pregnant women, maternal depression scores, cortisol and norepinephrine levels were significantly related to estimated fetal weight. Hierarchical stepwise multiple regression analyses were then conducted to evaluate the relations between maternal depression, biochemistry variables and estimated fetal weight. While controlling for gestational age and fetal gender, the analyses revealed that only cortisol was a significant predictor of estimated fetal weight, suggesting that the effects of maternal norepinephrine, depression and anxiety on fetal growth were mediated by cortisol. Maternal cortisol only accounted for an additional 1% of the variance in fetal weight after controlling for gestational age. Thus, we examined the percentage of fetuses of mothers with high and low cortisol values, who had an estimated fetal weight below or above average for their respective gestational week. These analyses revealed that 84% of mothers with high cortisol values had fetuses with below average estimated fetal weight.

The present study was designed to directly compare high and low cortisol pregnant women and their newborns. For this study, we recruited depressed pregnant women who had elevated depression scores (CESD > 16) and a SCID diagnosis of depression when they were approximately 20 weeks gestation. We then assigned them to high and low urine cortisol groups based on a median split of their 20week gestation cortisol levels. We followed them across pregnancy and assessed fetal development and newborn outcomes. We expected, based on the strong predictive value of cortisol in our previous study, that the high cortisol group would have less optimal fetal measures and less optimal neonatal outcomes including a greater incidence of prematurity and low birthweight and less optimal Brazelton Neonatal Behavior Assessment Scale scores.

## 1. Method

# 1.1. Participants

Three hundred depressed pregnant women were recruited at approximately 20 weeks gestation at a prenatal clinic. Eligibility criteria were as follows: (1) age greater than 18 years; (2) singleton pregnancy; (3) no HIV or major medical

illness; (4) no drug use as indicated by prenatal urine screens; and (5) elevated depression scores (CESD>16) and a SCID diagnosis of depression. The sample was lower-middle socioeconomic status women (M=3.5 on Hollingshead SES Index) who were distributed approximately 50% Hispanic, 25% African-American, 20% Non-Hispanic White, and 5% Asian. Any women with bipolar or other major psychiatric disorder, drug use or suicidal ideation were excluded from the study. In addition, mothers receiving psychotherapy, other forms of therapy and/or psychotropic medications were excluded because of potential confounding effects. Approximately only 10% of our previous samples had received medications or therapies for their depression during pregnancy.

## 1.2. Procedures and measures

## 1.2.1. Recruiting

Participants were recruited and tested over an approximately 24-month period. The participants were approached in the prenatal clinic where they were informed of the purpose of the study, given an opportunity to ask questions, and after consenting, were screened for eligibility. Mothers meeting study criteria were then asked to participate in the study. We attempted to recruit during the midmorning to control for diurnal variation in cortisol levels and extremely low levels mid-afternoon. Following consent, mothers then provided a urine sample, completed questionnaires and were observed for fetal movement and growth measures during their routine ultrasound examination.

#### 1.2.2. Prenatal assessments

The following prenatal assessments were made at the prenatal clinic.

*Maternal Questionnaires*: The mothers were given the following questionnaires at their first prenatal visit (at 20 weeks): (a) Sociodemograhic Questionnaire; (b) the Center for Epidemiological Studies – Depression Scale (CES-D); (c) the Structured Clinical Interview on Diagnosis (SCID); (d) the Trait Anxiety Inventory (STAI); (e) the Trait Anger Expression Inventory (STAXI); and (f) the Behavioral Inhibition and Behavioral Approach System questionnaire (BIS/BAS).

*Sociodemographic Questionnaire*. The Sociodemographic questionnaire is comprised of 11 items concerning age, education, occupation, income, length of time couples were in the relationship, marital status, number of children in the family, ethnicity and social support.

Center for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977). This 20-item scale was included to assess symptoms of depression. The participant is asked to report on his/her feelings during the preceding week. The scale has adequate test/retest reliability (.60 over several weeks), internal consistency (.80–.90) and concurrent validity (Wells, Klerman, & Deykin, 1987). Test-retest reliability over a one-month period on this sample was .79, suggesting some short-term stability of depressive symptoms. A score of 16 on the CES-D is considered the cutpoint for depression (Radloff, 1991).

Structured Clinical Interview of Diagnosis (SCID) (Multi-Health Systems Inc. North Tonawanda, NY). The SCID was used to determine whether subjects met criteria for DSM-IV depression (American Psychiatric Association, 1994). The SCID is a face-to-face interview for major DSM-IV disorders which takes approximately 45–90 min to complete. Questions address specific symptoms and their intensity, frequency, and duration to determine whether a person meets DSM-IV diagnosis. It also includes questions to determine whether the symptoms are due to medical conditions or drug/medication use, and whether the symptoms cause distress or impairment in social, occupational, or other important areas of functioning. After completing the questions, the interviewer determines whether a person meets criteria for depression. The reliability for assessing depression by relatively inexperienced clinicians is relatively high at .90 (Segal, Kabacoff, Hersen, Van Hasselt, & Ryan, 1995).

Trait Anxiety Inventory (STAI) (Spielberger, Gorusch, & Lushene, 1970). The Trait Anxiety Inventory is comprised of 20 items and is summarized by a score ranging from 20 to 90 and assesses how the subject usually feels in terms of severity ("not at all" to "very much so"). Characteristic items include "I feel nervous" and "I feel calm." Research has demonstrated that the Trait Anxiety Inventory has adequate concurrent validity and internal consistency (r=.83). Anxiety was assessed because of its noted co-morbidity with depression and its noted effects on pregnancy (Glover, Teixeira, Gitau, & Fisk, 1999).

Trait Anger Expression Inventory (STAXI) (Spielberger, 1988). The STAXI provides relatively brief, objectively scored measures of the experience, expression, and control of anger. The STAXI consists of 22 items on general angry reactions. Four point ratings range from "not at all" to "very much so". Examples of the items are "I am furious", "I

feel like screaming", "I am quick tempered"; "When I get frustrated, I feel like hitting someone", "When angry or furious I control my temper"; "I try to simmer down". The cut-off score for high anger is 52.

Behavioral Inhibition and Behavioral Approach System Questionnaire (BIS/BAS) (Carver & White, 1994). The BIS/BAS is a 24-item questionnaire consisting of personal statements followed by four severity options ranging from very true to very false. The BIS/BAS is designed to assess the tendency to have an approach response (BAS) or an avoidance or inhibited response (BIS) in situations. The BIS/BAS scales have adequate internal reliability (.66 to .74) and adequate convergent validity with other measures including Eysenck's Extraversion scale (Carver & White, 1994). BAS-BIS difference scores are obtained by subtracting *z*-transformed BIS scores from *z*-transformed BAS scores. The BAS-BIS scores can range from -2.53 to 2.79 with more positive scores denoting greater BAS activity. This method has previously been used to document the significant relation between the BIS/BAS and EEG activity (Diego, Field, & Hernandez-Reif, 2001; Sutton & Davidson, 1997).

Fetal Activity was measured by the same Doppler system we used in our pilot studies on depressed women (Diego et al., 2001; Dieter et al., 2001; Field et al., 2004). Fetal activity was assessed because significantly higher activity levels have been noted from 4 to 8 months gestation in fetuses of depressed mothers (Dieter et al., 2001). For this assignment the technician positions the scanner to obtain a lateral view of the fetus. The observer who is blind to the mother's group assignment then watches the fetus for five consecutive minutes. Every three seconds (a total of 100 samples) a tape-recorded cue (heard through an earphone) prompts the observer to record the fetal activity categories including: (a) single limb movements; (b) multiple limb movements; and (c) gross body movements. Interrater reliability, calculated on one-third of our pilot study sample for two observers, has yielded the following Kappa values: single limb movements = .82; multiple limb movements = .86; gross body movements = 1.00. For the data analyses, the percent time the fetus engaged in total movement, as well as each movement category, was calculated. No effort was made to discern fetal behavior states because they cannot be reliably identified prior to 36 weeks gestation without confirmation through fetal heart rate monitoring.

Fetal Growth Measures included femur length, head circumference, abdominal circumference and biparietal diameter measurements. These were conducted using standard clinical measurement protocols (Chitty, Altman, Henderson, & Campbell, 1994a, 1994b, 1994c). The Shepard, Richards, and Berkowitz (1982) fetal weight estimation algorithms were then used to estimate fetal weight from fetal ultrasound biometry measurements.

Maternal Urine Assays. The women were asked to provide first morning urine samples at their prenatal clinic visit. First morning urine samples were transferred to plastic vials which were stored at  $-20\,^{\circ}$ C without using acid or other preservatives. The samples were stored without information regarding group assignment and sent to our collaborators Drs. Saul Schanberg and Cynthia Kuhn at Duke University School for Medicine for analysis. Cortisol was measured in urine by radioimmunoassay using an extremely specific antiserum from Radioassay Systems Laboratories (Carson City, CA). The specificity of the assay is such that biological fluids can be assayed directly following heat inactivation of CBG eliminating the need for time-consuming extraction into organic solvents which is usually required for this assay. Specially purified 3H-cortisol from the same supplier is used as the labeled hormone. Bound and free hormones are separated by the dextran-coated charcoal technique. The sensitivity of the assay is .025 ng/tube. The inter-assay and intra-assay coefficient of variation is less than 10 and 5%, respectively. Standards are prepared from cortisol from the same supplier, and quality control samples representing low, medium and high values are run in every assay.

## 1.2.3. Neonatal assessments

Growth measures included gestational age, birthweight, birth length and head circumference taken from the birth records.

Obstetric and Postnatal complications were assessed on the Obstetric Complications Scale (OCS) (Littman & Parmelee, 1978) which is comprised of 41 items taken from the medical charts and rated as optimal or nonoptimal. Postnatal complications were quantified using the Postnatal Complications Scale (PNS) (Littman & Parmelee, 1978) which consists of 10 items rated as optimal or nonoptimal.

Brazelton Neonatal Behavior Assessment Scale. The Brazelton Neonatal Behavior Assessment Scale was given on the first afternoon after birth. The Brazelton assessments were performed by researchers who were trained to .90 reliability and were blind to the group classification of the mothers and infants. This neurobehavioral examination consists of 28 items, each scored on a 9-point scale, and 20 elicited reflexes, each scored on a 3-point scale. The infant's performance was summarized according to the traditional Lester, Als, and Brazelton (1982) clusters, and Lester and Tronick's (1992) depression, excitability and withdrawal factors.

## 2. Results

Multivariate ANOVAs were conducted comparing the low and high cortisol groups on the groups of measures including the prenatal questionnaire and cortisol measures, on the fetal activity and fetal growth measures and on the neonatal growth measures and Brazelton scores. Following significant MANOVAs on the grouped measures, ANOVAs were conducted on the individual measures.

## 2.1. Prenatal measures

As can be seen in Table 1, the high cortisol level group had higher depression (CES-D) scores and higher inhibition (BIS) scores. Not surprisingly, the high cortisol group had significantly higher cortisol levels.

# 2.2. Fetal activity and growth

As can be seen in Table 2, the high cortisol group fetuses were significantly more active and had significantly smaller head circumference, abdominal circumference, biparietal diameter and fetal weight.

# 2.3. Neonatal growth measures

The results given in Table 3 suggest that the high cortisol group neonates were shorter gestation and lower birthweight. No group differences were noted on the OCS (Obstetric Complications) or PNF (Postnatal Complications) scales.

## 2.4. Brazelton scores

Table 4 shows lower Brazelton habituation and higher Brazelton reflex scores for the neonates of the high cortisol group.

Table 1
Means for prenatal maternal questionnaires and cortisol (and standard deviations in parentheses)

Measures	Cortisol level				
	Low	High	F	p	
Depression (CESD)	16.76 (9.60)	19.86 (11.16)	6.72	.01	
Anxiety (STAI)	39.75 (9.71)	41.60 (12.48)	1.95	.16	
Anger (STAXI)	19.21 (5.95)	21.30 (6.24)	2.29	.13	
Inhibition (BIS)	17.74 (3.78)	19.13 (3.98)	5.71	.02	
Activation (BAS)	36.27 (9.01)	37.15 (9.14)	.39	.54	
Cortisol	128.12 (46.87)	333.24 (110.06)	444.77	.000	

Table 2
Means for fetal activity and fetal growth measures (and standard deviations in parentheses) taken at approximately 20 weeks gestation

	Cortisol level			
Measure	Low	High	F	p
Movement (%time)	33.81 (22.12)	41.40 (24.70)	3.81	.05
Femur length (mm)	3.59 (.06)	3.54 (.06)	1.02	.32
Head circumference (mm)	18.77 (.12)	18.13 (.14)	11.66	.001
Abdominal circumference (mm)	19.93 (.14)	16.21 (.16)	11.43	.001
Biparietal diameter (mm)	5.12 (.14)	4.96 (.04)	9.27	.003
Fetal weight (g)	535.29 (13.99)	485.89 (16.10)	5.36	.02

Table 3
Means for neonatal growth measures (and standard deviations in parentheses)

	Cortisol level			
Measure	Low	High	$\overline{F}$	<i>p</i>
Gestational age (weeks) (R = 35–41)	38.77 (1.36)	37.15 (3.02)	10.85	.001
Birthweight (g) $(R = 2807 - 3619)$	3392.87 (481.96)	3073.09 (587.56)	10.64	.001
Birth length (mm)	50.27 (2.72)	48.27 (6.53)	2.29	.14
Head circumference (mm)	34.00 (1.50)	33.45 (1.67)	.92	.34

Table 4
Means for Brazelton Scores (and standard deviations in parentheses)

Measure	Cortisol level				
	Low	High	F	p	
Habituation	5.99 (.59)	5.38 (1.19)	3.65	.05	
Orientation	5.79 (1.01)	5.71 (1.34)	.06	.82	
Motor	5.25 (.55)	5.31 (.72)	.11	.74	
Range of state	3.66 (.43)	3.65 (.98)	.00	.98	
Regulation of state	5.41 (1.15)	5.64 (1.37)	.43	.52	
Autonomic stability	6.01 (1.23)	6.09 (.95)	.07	.80	
Reflexes	1.52 (.99)	2.11 (1.41)	3.11	.05	
Withdrawal	2.78 (1.45)	2.80 (1.51)	.00	.97	
Excitability	2.12 (1.50)	1.56 (1.44)	1.8	.19	
Depression	1.87 (1.08)	2.13 (1.39)	.60	.44	

# 2.5. Incidence of prematurity and low birthweight

Chi-square analyses were conducted on the incidence of prematurity and low birthweight in the high and low cortisol groups. As can be seen in Table 5, the chi-square analysis on prematurity yielded a significantly higher incidence of prematurity in the high cortisol group. Similarly, the incidence of low birthweight was significantly higher in the high cortisol level group.

# 2.6. Discriminant function analyses

The discriminant function analyses in Table 6 show that the preterm/full-term group membership was not accurately predicted/classified by depression (CES-D) scores. However, a significant number of preterm infants were predicted by their cortisol levels (83%), suggesting that cortisol more accurately classified the preterm/full-term infants than CES-D depression scores.

Table 5
Chi-square analyses for incidence prematurity and low birthweight in high and low cortisol groups

	Low cortisol	High cortisol
Prematurity		
Full-term (%)	100	68
Preterm (%)	0	32
$\chi^2 = 6.10, p < .01$		
Low birthweight		
Avgerage birthweight (%)	100	68
Low birthweight (%)	0	32
$\chi^2 = 6.44, p < .01$		

Table 6
Discriminant function analyses for accuracy of prediction for preterm group membership using CES-D scores and cortisol levels as predictors

	Predicted		
Outcome	Full-term	Preterr	
Discriminant function for CES-D			
Full-term (%)	65	36	
Preterm (%)	43	57	
Wilk's Lambda = $.97$ , $p = .30$			
Discriminant function for cortisol			
Full-term (%)	59	41	
Preterm (%)	17	83	
Wilk's Lambda = $.89$ , $p = .05$			

Table 7
Discriminant function analyses for accuracy of prediction for low birthweight group membership using CES-D scores and cortisol levels as predictors

	Predicted		
Outcome	Average BWT	Low BWT	
Discriminant function for CES-D			
Average BWT (%)	71	29	
Low BWT (%)	29	71	
Wilk's Lambda = $.96, p = .19$			
Discriminant function for Cortisol			
Average BWT (%)	60	40	
Low BWT (%)	17	83	
Wilk's Lambda = $92$ , $p = .10$			

Similarly, as can be seen in Table 7, depression scores did not accurately predict low birthweight group membership. However, cortisol levels were at borderline significance in accurately classifying low birthweight infants.

## 3. Discussion

The high depression scores in the high cortisol group were not surprising given the commonly reported cortisol elevations in depressed individuals (Field et al., 2004; Lundy et al., 1999). The high withdrawal/inhibition (BIS) scores are consistent with other studies using the BIS/BAS and reporting withdrawal/inhibition EEG patterns (Diego et al., 2001; Sutton & Davidson, 1997). Surprisingly, anxiety was not co-morbid with depression in this study, as it frequently is, and anxiety scores were not higher in the high cortisol group even though elevated cortisol has been noted in high anxiety women during pregnancy (Field et al., 2003).

As predicted, the fetuses in the high cortisol group were more active and experienced growth delays including heard circumference, abdominal circumference, biparietal diameter and fetal weight. Inasmuch as 40% of the mothers' cortisol is noted to cross the placenta (Glover et al., 1999), these cortisol effects might be predicted.

Consistent with the Field et al. (2004) data, high prenatal cortisol and prematurity and low birthweight were related. The incidence of prematurity and the incidence of low birthweight were greater in the high cortisol group in this sample. And elevated cortisol was a better predictor of these outcomes than elevated depression scores in our discriminant function analyses. Potential underlying mechanisms are unknown for these consistent relationships between prenatal depression, elevated prenatal cortisol, prematurity and low birthweight. Also, future research is headed to determine the effects of prenatal cortisol on non-depressed women before recommending the monitoring of cortisol levels during pregnancy.

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