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Diurnal cortisol patterns and psychiatric symptoms in pregnancy: Short-term longitudinal study

Thomas G. O'Connor[*], **Wan Tang**, **Michelle A. Gilchrist**, **Jan A. Moynihan**, **Eva K. Pressman**, and **Emma Robertson Blackmore**

University of Rochester Medical Center, United States

Abstract

Alteration in the HPA axis is a robust biomarker of anxiety and depression in adults, but questions remain about this association in pregnancy. We examined the longitudinal links between diurnal cortisol and mood symptoms from self-report questionnaire and diagnostic interview in an ethnically diverse, psychosocially at-risk sample of 101 women at mid-pregnancy and early third trimester. There were modest but significant associations between depression and elevated cortisol, indexed by a decreased morning level and diminished diurnal decline; the effects were strongest for diagnostic data from clinical interview. These effects were independent of socio-demographic factors and sleep disturbance. Associations with anxiety and trauma were generally non-significant. These findings extend prior work by showing that significant mood symptoms in pregnancy are associated with altered diurnal cortisol in pregnancy, which may have implications for maternal and child health.

Keywords

Depression; Anxiety; Pregnancy; Cortisol; HPA axis

1. Introduction

Research findings suggest that elevated cortisol is a robust biomarker of affective symptoms, stress reactivity and vulnerability (Hellhammer, Wust, & Kudielka, 2009). Whether or not mood symptoms are associated with elevated or otherwise altered cortisol production in pregnancy has attracted considerable recent research attention for several reasons. One is that pregnancy is a period of substantial neuroendocrine change that may offer a "natural experiment" to illuminate mechanistic links between neuroendocrine changes and psychiatric symptoms. Second, elevated cortisol in pregnancy may explain the widely-reported connection between affective symptoms and poor obstetric outcome (Federenko & Wadhwa, 2004; Goedhart et al., 2010; Grote et al., 2010; Kramer et al., 2009). Third, given that there is transmission of cortisol from mother to fetus in pregnancy (Sarkar, Bergman, Fisk, O'Connor, & Glover, 2007), elevated mood symptoms in pregnancy may signal

[*]Corresponding author at: Wynne Center for Family Research, Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642, United States. Tel.: +1 585 273 12212; fax: +1 585 273 1384. tom_oconnor@urmc.rochester.edu (T.G. O'Connor).

increased *in utero* exposure to cortisol in the fetus, a plausible mechanism underlying developmental programming of health outcomes (Connors et al., 2008; Glover, O'Connor, & O'Donnell, 2010; Wadhwa, Buss, Entringer, & Swanson, 2009). The current study contributes to this line of research by assessing the longitudinal connections between mood and diurnal cortisol from the 2nd to 3rd trimester in pregnancy in a consecutive series of women recruited from a clinic serving a disproportionately high-risk population.

An association between mood symptoms and altered HPA axis function in non-pregnant individuals is well-established; there is, however, considerable variation across studies in effect sizes reported and methodologies used. Knorr et al.'s (Knorr, Vinberg, Kessing, & Wetterslev, 2010) recent meta-analysis indicated small but significantly higher morning and evening cortisol values in depressed compared to non-depressed adults. Studies linking cortisol with anxiety and stress are similarly numerous and generally positive, e.g., (Michaud, Matheson, Kelly, & Anisman, 2008).

Research findings suggesting that mood symptoms may be associated with elevated diurnal cortisol in non-pregnant adults may not extend to pregnant women, however. That is, the association between mood symptoms and cortisol may be different in pregnancy because of the wide-ranging and substantial pregnancy-related changes in the HPA axis itself, and/or because pregnancy induces other changes that may alter the HPA axis and cortisol in particular, such as changes in the immune system and in levels of progesterone, e.g., (Robinson & Klein, 2012). Several studies have examined the hypothesis that mood symptoms and/or stress (a variety of constructs have been measured in studies reported so far) are associated with altered diurnal cortisol in pregnant women (see below), but substantial methodological variation among studies means that it is not yet possible to draw firm conclusions.

The current study builds on and extends the existing literature in several specific ways. First, in contrast to most studies that are based on a single assessment, we employ a short-term longitudinal design. That allows us to test the hypothesis that the association between mood symptoms and diurnal cortisol changes from the 2nd to the 3rd trimester of pregnancy. That possibility was raised by several cross-sectional studies showing a blunted cortisol reactivity to an acute stress in late but not mid- or early-pregnancy (DiPietro, Mendelson, Williams, & Costigan, 2012; Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001; Kammerer, Adams, Castelberg Bv, & Glover, 2002); see also Schulte, Weisner, and Allolio (1990). The mechanisms are not established, but are presumed to reflect the complex of pregnancy-related biological changes that directly or indirectly alter cortisol regulation. In the current study, we test an extension of this hypothesis for diurnal cortisol assessment: the association between diurnal cortisol and mood symptoms becomes weaker as pregnancy proceeds.

A second notable feature of the study is the sampling frame. Whereas most studies assessing cortisol and mood or stress in pregnancy are based on low-psychosocial risk samples, the current sample is ethnically diverse and includes women at elevated psychosocial (but not medical) risk. This is an important extension of prior work because individuals at high psychosocial risk and of minority race/ethnicity status may exhibit a different pattern of

diurnal cortisol than low-risk individuals, as has been shown in many studies of non-pregnant, e.g., (Cohen et al., 2006) and in a small number of pregnant (Bennett, Merritt, & Wolin, 2004; Glynn, Schetter, Chicz-DeMet, Hobel, & Sandman, 2007; Suglia et al., 2010) samples. The inclusion of a large proportion of pregnant women at elevated psychosocial risk also means that the findings obtained here may generalize to those for whom this research is most clinically relevant, i.e., those women who are at particularly high risk for mood disorder.

A third key feature of the study is that we assessed multiple overlapping phenotypes; depression and anxiety were measured using both self-report symptom measures and interview-based diagnostic assessments; trauma exposure was also ascertained. We adopted an assessment strategy of several putative risk phenotypes because, as noted, research on diurnal cortisol in pregnancy has included a wide range of constructs, and this may have led to inconsistencies among reports. For example, Shea et al. (2007) found decreased levels of awakening cortisol with retrospectively reported childhood trauma but no link between cortisol and anxiety or stress; Kivlighan, DiPietro, Costigan, and Laudenslager (2008) found that trait anxiety but not stress was associated with a flatter diurnal decline in cortisol at 36 weeks gestation; Giesbrecht, Campbell, Letourneau, Kooistra, and Kaplan (2012) reported momentary mood was associated with cortisol; and Obel et al. (2005) found that stressful life events were associated with elevated evening but not morning cortisol and in late but not early pregnancy. Furthermore, in their analyses of about 60 pregnant women in the third trimester, Pluess et al. (2012) reported that morning cortisol was significantly related to positive life events ($b = -.26$) but less so to negative life events ($b = -.22$); in a separate paper they (Pluess, Bolten, Pirke, & Hellhammer, 2010) reported that awakening cortisol was negatively related to trait anxiety, with stronger effects in the first compared with the third trimester. Finally, in a sample of 25 women, Entringer, Buss, Andersen, Chicz-Demet, and Wadhwa (2011) reported that an ecological momentary assessment of negative mood was associated with higher cortisol at awakening. Although the overall impression is that there may be a significant association between diurnal cortisol and mood symptoms or stress, no clear patterns have yet emerged. In the current study we focused on the dimensions of depression and anxiety because they have attracted considerable research attention in pregnant women, but made no *a priori* predictions about which dimension would be more strongly associated with altered diurnal cortisol. Particularly novel is the inclusion of diagnostic assessments, which complement self-report measures that have been exclusively used in previous studies. Diagnostic data offer a more direct index of the clinical significance of the variation in diurnal cortisol patterns in pregnancy and have methodological advantages over self-report questionnaires.

In summary, we enrolled a racially/ethnically diverse sample of women at high psychosocial risk and examined them in the 2nd and 3rd trimesters of pregnancy with self-report and diagnostic measures of depression and anxiety to test the hypothesis that mood symptoms would predict alteration in diurnal cortisol; we also examined the hypothesis that the association between mood symptoms and diurnal cortisol would diminish over time. Exploratory analyses considered the strength of the prediction from depression and anxiety, and if the association with diurnal cortisol was stronger for more severe disturbance (i.e., clinical diagnosis). Detailed assessments of socio-demographic risk and sleep were included

given their proposed influence on cortisol and possibly confounding effect on the association between mood symptoms and diurnal cortisol patterns.

2. Methods

2.1. Sample and procedures

Women were consecutively recruited from a university hospital-based obstetrics practice in a mid-sized city serving a generally low-income population. Exclusion criteria were (a) under 20 years of age or over 34 years, (b) history of psychotic illness, (c) medical high-risk pregnancy as determined from OB clinic assessment (including medical complications in the mother and prenatal drug use) based on detailed obstetric evaluation, (d) non-singleton pregnancy, (e) non-English-speaking. Because of the specific focus on cortisol in pregnancy, analyses for the current study are limited to the $n = 101$ women from the overall sample of $n = 149$ on whom cortisol data were available. Written consent was obtained from the mothers; the project was approved by the university institutional review board.

Women meeting criteria were asked by a nurse practitioner at the conclusion of the initial prenatal assessment if they were interested in participating in a research project on moods and feelings in pregnancy; this initial intake visit was typically conducted within 10–14 weeks gestation. Women agreeing to participate were then approached by a study team member who obtained written consent (it was not possible to determine precisely the percentage of women agreeing to participate). Participants were administered questionnaire measures and a clinical interview at approximately 20 weeks gestation and again at approximately 32 weeks gestation; each visit corresponded to a regularly scheduled obstetric visit. All questionnaires were read aloud to women to pre-empt comprehension problems. All interviewers were female with an advanced degree in a mental health field or considerable clinical research experience and formal training in clinical interviewing. At each assessment women were instructed in the procedures for collecting saliva (see below).

3. Measures

Affective symptoms from questionnaires and clinical interview. Two questionnaire measures of symptoms are the focus of this report. Depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (Cox, Holden, & Sagovsky, 1987), a 10-item self-report measure that is widely used in perinatal research with established reliability and validity (Murray & Carothers, 1990). The Penn State Worry Questionnaire (Meyer, Miller, Metzger, & Borkovec, 1990) is a widely-used index of worry that has been used in perinatal research (Swanson, Pickett, Flynn, & Armitage, 2011); it includes 16 items focusing on worry.[1] In addition, women were interviewed using the depression and anxiety segments of the structured clinical interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1995). For depression, diagnoses were made for the narrow phenotype of Major depressive disorder and the broader phenotype of any depression (e.g., including depression NOS) according to

[1]In addition, the 20-item state subscale of the State-Trait Anxiety Inventory was also administered (Spielberger, Gorusch, & Lushene, 1983); it is a widely-used index of anxiety in pregnancy (Sarkar, Bergman, O'Connor, & Glover, 2008). Findings from this measure parallel those of the PSWQ, with which it was highly correlated, and so are not discussed in detail.

DSM-IV criteria; we focus analyses on the broader phenotype because of the greater number of clinical cases and because we had no a priori reason to target the narrow MDD diagnosis. For anxiety, a diagnosis of anxiety disorder was made if the woman met DSM-IV criteria for any of the anxiety disorders with the exception of PTSD; we excluded PTSD from the anxiety disorder designation because of the suggestion that trauma may be associated with a different cortisol pattern from anxiety and worry. PTSD was considered as a separate diagnosis; we also include as an index of trauma exposure the endorsement of the initial screening item from the SCID that assessed history of trauma exposure.

Diurnal Cortisol

Diurnal Cortisol Women were instructed to provide diurnal cortisol according to a standard protocol (MacArthur research network on socioeconomic status and health, http://www.macses.ucsf.edu/research/allostatic/notebook/salivarycort.html). Five diurnal samples were collected: wake-up, 45 min after wake-up, and 2.5 h, 8 h, and 12 h after wake-up. Women were provided pre-labeled, color-coded tubes; collection was made using salivettes. A diary was provided on which women reported the time for each assessment; women were trained in the use of the salivettes and instructed to avoid brushing teeth and eating 30′ prior to sampling. Participants were instructed to provide saliva samples on an ordinary week-day immediately following the clinic visit and to place the samples in a refrigerator after collection. Samples were collected at the next regularly scheduled obstetrics visit or by other special arrangement. Reminder phone calls were used where needed to coordinate sample collection from a study team member. A new set of salivettes, tubes, time logs and envelopes were provided if the woman did not have her sample at the arranged pick-up or if requested. For visit 1, cortisol was collected at 21.26 [5.57] weeks, on average, with 82% occurring by the end of the 2nd trimester; cortisol was collected at 34.15 [2.69] weeks, on average, at visit 2, all in the 3rd trimester. Diurnal salivary samples were typically collected within 1–2 weeks of each prenatal assessment, but we aimed to collect samples wherever possible and examine if variation in gestational age at cortisol collection within each visit is associated with cortisol level (see below). Because of the interest in the cortisol awakening response (CAR), we excluded CAR samples that were not collected within an hour of wakening given the decline in cortisol >1 h after wake-up and a 1 h post wake-up period is a common window for assessing CAR (Federenko et al., 2004; Schmidt-Reinwald et al., 1999). Samples collected from the women were kept at −20 °C until assay. Saliva was assayed using commercially available high-sensitive enzyme immune-assay kits (Salimetrics, State College, PA), which have a range of sensitivity from .007 to 1.2 μg/dl. All samples were run in duplicate; average intra- and inter-assay coefficients of variation are 4.13% and 8.89%. Cortisol values were log transformed for analyses.

Socio-demographic, psychosocial, and sleep covariates. Women self-identified as Black/African-American, Black/other, Cau-casian/white, Hispanic, or Asian. The small number of women reporting biracial identity, all of whom included African-American as one race/ethnic group, were classified as Black/African-American. There were too few women reporting Asian ethnicity to constitute a separate group in analyses, and so we used the following ethnicity/racial groups in analyses: Black/African-American, Hispanic, and other. The findings were substantively unchanged when we excluded the few ($n = 3$) Asians from

analyses. Also considered in data analyses were maternal age, marital status (married/cohabiting, single), number of years of education, and household composition. Prenatal substance use was not included as a covariate because those who endorsed moderate use of any kind or who screened positive for substances from a standard urine screen would have been screened out in the initial obstetrics intake assessment as being at elevated medical risk. Seventeen percent of the women reported any level of current cigarette smoking; we included a dichotomous smoking variable as a potential covariate. Finally, women were given sleep diaries following the conclusion of each prenatal assessment and instructed in how to complete the diary for the subsequent 7-day period. Information derived from diaries included time to bed and time awake, number of hours slept, number of times awake after sleep onset, and how refreshed the women felt at wake-up; validity of self-report sleep diary data in pregnancy has been previously reported (Greenwood & Hazendonk, 2004). Sleep diary data were obtained on 89% of the sample.

Data analysis

After presenting descriptive data on the sample and key measures, we report analyses examining the associations between the main psychiatric constructs (depression, anxiety) and cortisol; socio-demographic, psychosocial, and sleep parameters are included as covariates. We report analyses on continuous ratings of symptoms and dichotomous diagnoses to examine the robustness of effect across method. Generalized estimating equations (GEE)/linear mixed modeling was used to analyze cortisol data. Four parameters index the diurnal cortisol pattern: main (overall mean) effect; initial value (i.e., at awakening); cortisol awakening response (CAR); diurnal decline (operationalized as the diurnal decline from wake-up to 12 h after wake-up excluding the CAR). We also included a quadratic effect for time, but it was not significant and so was dropped from analyses. That means that, for depression, we included the following predictors: a depression main effect, which indexes an overall (mean level) effect; a depression × wake-up effect, which assesses the effects of depression on wake-up cortisol value; depression × CAR, which assesses the effects of depression on the CAR; depression × time, which assesses the effects of depression on the diurnal decline across the day. Gestational age (a dichotomous variable indicating the 20 week or 32 week visit), gestation × wake-up time, gestation × CAR, and gestation × time since wake-up were used as covariates. GEE has several analytic advantages compared with parametric counterparts such as (generalized) linear mixed models. The semi-parametric approach of GEE makes no parametric assumptions such as multivariate normality and does not require a specification of correct correlation structure of the repeated measures of the same subjects. Thus, GEE provides estimates that are more robust to departures from parametric assumptions. All available samples were used in the GEE analysis. Analyses were first conducted for each diagnosis and dimension separately; subsequent models include both dimensions.

4. Results

Descriptive data (Table 1) indicate that the sample is at high psychosocial need, as shown by the low level of educational attainment, high percentage of single-parent status, and comparatively high level of disorder; the sample is also predominantly minority.

Comparisons of those with and without any cortisol data indicated no differences according to any symptom or socio-demographic measure with one exception: women providing samples were more likely to have a partner (85% versus 61%, chi-square (1) = 7.71, $p<.01$). The targeted prenatal assessments were 20 and 32 weeks gestation; in practice there was modest variation in these visits and cortisol collection because of canceled or re-scheduled OB visits, as noted. Preliminary analyses indicated that variation in gestational week within each visit was unrelated to cortisol values or the link between cortisol and symptoms, and did not improve the fit of the models (see below); therefore we include a dichotomous measure to index gestational age. Table 1 also displays the mean cortisol data for both visits (based on non-transformed data for illustration and comparison with other studies; prediction analyses are based on transformed data). The findings show the expected cortisol awakening response and diurnal decline at both visits; the slope is slightly flatter at the latter assessment.

Preliminary analyses indicated that sleep was a reliable predictor of cortisol, and so is retained in analyses below; the index of sleep used in analyses was number of total hours of night-time sleep. We also include, because it was of *a priori* interest, race/ethnicity. None of the other covariates was reliably associated with cortisol, and so were dropped from analyses.

The overlap between depression and anxiety was considerable. According to diagnostic data, for example, at the 20-week prenatal assessment, 12% were depressed only; 11% were anxious only; and 12% we co-morbid. Of those with a depression diagnosis, approximately one-half also received a diagnosis of anxiety, and vice versa. The overlap was similarly substantial at the 32-week prenatal assessment.

5. Prenatal diurnal cortisol and depressive and anxiety symptoms and disorder

Table 2 displays the base model for predicting cortisol. Results show the expected and substantial effect of time, that is, the diurnal effect: time of sample collection since wake-up was associated with lower cortisol value. There was also a significant effect of CAR, which indicated a significant rise in cortisol after awakening. There was not a significant effect of gestation overall, or on the specific cortisol parameters (wake-up, CAR, diurnal decline). Also, more total sleep was associated with lower overall cortisol levels.

Results from the second model in Table 2 indicate that a diagnosis of depression was significantly associated with cortisol. The strongest effect was that women with a diagnosis of depression had a lower initial waking level (depression × wakeup); other effects were more marginal. There was no evidence that depression altered the CAR (depression × CAR) or that the overall effect for depression varied across gestation (depression × Gestation).

Additional analyses (not shown; available from the first author) indicated that none of the three-way interactions (e.g., gestational age × depression × wake-up) was significant; these parameters are therefore not included in the final model. Given the interest in trauma and its overlap with depression and influence on cortisol, we also re-ran this model including

trauma exposure and PTSD diagnosis. There was no meaningful change to any of the depression diagnosis parameters or their significance (e.g., when trauma exposure was included in the model, the depression coefficients were depression × wakeup: estimate = −. 22, SE .08; depression × time since wake: estimate .05, SE .02; depression: estimate 1.54, SE .80).

Results from the third column in Table 2 show the results when depression was indexed by self-report ratings on the EPDS; the effects were weaker, with only a marginal effect of Depression on a flatter the diurnal decline. (Note that, given the differences in scales, the parameter estimates for the two depression measures are not directly comparable.) As was the case for depression diagnosis, there was no evidence that the effect of EPDS on diurnal decline was significantly different at the two prenatal visits or was altered by including Trauma or PTSD diagnosis in the model (not shown; details available from the first author).

Comparable analyses for anxiety based on diagnostic assessment from clinical interview (including all anxiety diagnoses other than PTSD) or self-ratings on the PSWQ indicated no reliable and robust predictions of cortisol parameters.

Given the high covariation between depression and anxiety, a final set of analyses was carried out that included both Depression and Anxiety diagnoses as predictors of cortisol in pregnancy. Results are reported in Table 3. Findings indicate that the effects for depression remain (and are trivially strengthened) when anxiety is included in the model. Specifically, women who were diagnosed as depressed in pregnancy show a lower initial (wake-up) cortisol and a less sharp decline over the day and a higher average level overall. Anxiety diagnosis did not predict cortisol measures. In follow-up analyses (not reported) we found no significance evidence of a statistical interaction between depression and anxiety diagnoses, i.e., co-morbidity – although this may be explained by too few cases of co-morbid diagnostic cases. Additional analyses using the continuous ratings for depression and anxiety symptoms yielded broadly similar but weaker pattern, consistent with what was found in the analyses of continuous ratings assessed separately (not shown; available from the first author).

6. Discussion

Findings from this short-term longitudinal study in pregnancy of a high psychosocial risk sample indicated a modest but significant association between depression and diurnal cortisol. Specifically, we found that depressed individuals demonstrated a lower initial (wake-up) cortisol level and a flattened, less sharply declining diurnal pattern; overall, they had a higher cortisol level across the day; these effects were obtained after accounting for a number of key covariates, notably sleep and co-occurring anxiety. Additionally, this effect was stronger for diagnostic data than self-reported symptoms on a depression questionnaire, and did not differ significantly between the 20 and 32 week assessment periods. Generally weak and non-significant effects were found for anxiety symptoms.

The findings support and extend prior studies including Suglia et al. (2010) who found that high-stress pregnant women at approximately 28 weeks gestation showed lower morning

levels and a flatter diurnal decline; that study also oversampled minority, high-stress subjects. The association between symptoms and cortisol decline was observed in both Kivlighan et al. (2008) and Obel et al. (2005) on more disparate samples; O'Keane et al. (2011) found evening cortisol (which empirically resembles a dampened diurnal decline) to be higher with depression. Mechanisms underlying these differential effects are not yet clear, and will require further study of both glucocorticoid and mineralocorticoid receptor activity. In any event, one implication is that a detailed characterization of the diurnal pattern will be needed to detect links with symptoms and stress, a point to which we return when considering clinical implications. It is interesting to note that a flatter diurnal decline is emerging as a marker of stress exposure in many studies, including in adolescents who were exposed to maternal prenatal anxiety (O'Donnell et al., 2013).

Findings from the current study offer much stronger support for a link between depression and diurnal cortisol than for anxiety (including trauma) and cortisol; moreover, the effects were stronger for a clinical diagnosis of depression based on clinical interview. The latter effect is novel in the literature and implies that altered diurnal cortisol effects may be more apparent at the clinical extremes. It is difficult to know how much the overall pattern of results contrasts with the existing literature. As described above, inconsistencies in findings across studies are somewhat difficult to reconcile given that measures of stress, depression, and anxiety overlap substantially, and several published studies assess one but not other of these constructs. There is not yet a compelling biological account for differences among putative risk phenotypes, where they exist. It may be that variation in findings across studies reflects a modest effect of a broad phenotype that is variously detected across study according to nuances in assessment timing, method or analysis or other factors. Alternatively, these results also imply that symptom or stress severity may also moderate the effects obtained. A related point is that the current study extends prior work by assessing a high proportion of women who were at high psychosocial risk and might be thought to be experiencing chronic stress; that compares with previous studies which typically assess low/normal risk samples. Furthermore, measurements of diurnal cortisol also differ across study and may drive differences in effects. In the current study we assessed diurnal cortisol using five measures; that likely provided a more reliable assessment of the dramatic and non-linear diurnal pattern in cortisol than studies using far fewer samples.

The finding of elevated cortisol in pregnant women with affective disturbance may suggest avenues for etiology or clinical treatment and monitoring, but arguably the most notable application of this effect is for studies of fetal programming. Whether or not elevated cortisol associated with mood symptoms in pregnancy translates into greater exposure to glucocorticoids for the fetus is a key mechanistic question for research linking prenatal mood with fetal and child outcomes. Data showing that mood disturbance is associated with elevated cortisol in pregnancy, along with data suggesting maternal-fetal transfer of cortisol (Gitau, Cameron, Fisk, & Glover, 1998; Sarkar et al., 2007), provide a basis for hypothesizing that prenatal cortisol exposure mediates the link between prenatal mood and child outcomes. However, we still lack clear evidence for this, and some studies indicate that the prediction from prenatal maternal stress or anxiety is independent of *in utero* cortisol exposure, e.g., (Bergman, Sarkar, Glover, & O'Connor, 2010). One possibility is that prenatal symptoms alter gene expression of 11BHSD2 (O'Donnell et al., 2011), the barrier

enzyme that metabolizes cortisol in the placenta. Further work is needed that considers this possibility alongside detailed measures of maternal symptoms and biomarkers in pregnancy. Additionally, there is a need to coordinate biological assessments with detailed longitudinal symptoms ratings from pregnancy into the postnatal period, given the sizable changes in mood symptoms that have been reported (Coelho, Murray, Royal-Lawson, & Cooper, 2011).

In addition to several positive findings, we also wish to note several potentially important non-significant findings. For example, we found that the cortisol awakening response, an index of HPA axis which has attracted considerable attention (Clow, Thorn, Evans, & Hucklebridge, 2004; Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010), was not significantly altered in pregnancy. Neither was there significant change in the diurnal pattern between assessments. We may not have found significant change in diurnal patterns across gestation because of the limited span of gestation covered. One study that did report a significant change in the CAR (Buss et al., 2009) included a wider gestational age span and a lower risk sample. Also, Jung and colleagues (Jung et al., 2011), who assessed plasma and urinary cortisol in 20 normal-risk pregnant women, found an increase in cortisol with gestation, although the size of the change varied according to sample type and collection procedures. Finally, Glynn et al. (2007) reported an increase in cortisol across gestational based on a single afternoon measure from 18–20 through 30–32 weeks; interestingly, however, cortisol in African-American women decreased from 24–26 to 30–32 weeks gestation, the approximate period of assessment in this study. The lack of an expected increase in cortisol from the 2nd to 3rd trimester may also be explained by the higher risk status of the sample, which may have elevated second trimester diurnal cortisol values and reduced the likelihood of finding a gestational age effect. Furthermore, it is known that placental CRH increases with gestation, although the detection of a downstream increase in maternal salivary cortisol will be regulated by desensitization of pituitary CRH receptors (Schulte et al., 1990; Smith, 1998). Plasma CRH also increases with gestation and is elevated particularly in those who will deliver early (McLean et al., 1995; Wadhwa et al., 2004). It may be that excluding high-risk pregnancies (i.e., those most at risk for pre-term birth) meant that we excluded those women for whom there would have been a detected increase in cortisol at the 32 week assessment. In any event, it is notable that the means obtained in this study are comparable to other reports, adjusting for gestational age (Giesbrecht et al., 2012; Kivlighan et al., 2008; Suglia et al., 2010) – although differences in risk exposure and prevalence of psychiatric disorder make comparisons across samples difficult. Finally, we did not find ethnicity/race differences in cortisol. It may be that ethnic/race differences were smaller in this sample because of the high concentration of psychosocial risk that was shared by all ethnic/race groups.

Several limitations of the study should be noted. One is that our conclusions are limited to diurnal assessment of cortisol. Second, the timing of the prenatal assessments may not have been optimal for tracking normative, pregnancy-related change in HPA axis function; assessments much earlier and later in gestation may have increased the normative changes observed and their association with mood symptoms. Third, findings linking cortisol and mood symptoms in pregnancy may vary across populations, e.g., pregnant adolescents, e.g., Dorn, Susman, & Petersen (1993). Fourth, considerable efforts were undertaken to insure the fidelity of the diurnal cortisol assessments, but were based on self-reports of time and day.

These limitations are offset by several strengths of the study, including socio-economic diversity, intensive short-term longitudinal assessment of cortisol data that included five diurnal assessments, detailed continuous and diagnostic measures of mood and trauma, and consideration of multiple confounds, including sleep, which is rarely included in studies of diurnal cortisol despite its significant influence.

Clinical implications of these findings are not yet clear. Given the demanding nature of cortisol assessment–in the case of a diurnal cortisol, multiple assessments across the day – it is not clear how practical it may be to incorporate this into routine clinical practice. Single cortisol assessments would be far easier, and practical, although a single assessment cannot approximate the dramatic diurnal pattern, and recommendations for assessing diurnal cortisol emphasize the need for multiple assessments within the day. It may be that cortisol assessments might be incorporated into clinical practice for only certain patient subgroups. Ultimately, the usefulness of cortisol in clinical assessment may rely on how effective prenatal interventions may be for maternal symptoms, and whether or not there is an attendant change in cortisol alongside improvements in mood. Psychological interventions for anxiety, stress, and depression in pregnancy, e.g., Duncan & Bardacke (2010) and Milgrom, Schembri, Ericksen, Ross, and Gemmill (2011) on prenatal cortisol profiles would seem a particularly suitable avenue for further clinical study.

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Table 1

Demographic characteristics of the sample.

	Mean (SD)/*n* (%)	
Maternal age (years)	24.91 (3.80)	
Race/ethnicity		
African American/black	50 (50%)	
Hispanic	18 (18%)	
Asian	3 (3%)	
Caucasian/white	29 (29%)	
Education		
Less than high school	26 (26%)	
High school	42 (42%)	
Less than 4-year college degree	19 (19%)	
4-year college degree or greater	13 (13%)	
Married/living with partner	29 (29%)	
Trauma exposure	53 (53%)	
	20 weeks	32 weeks
Depression diagnosis	23(23%)	13(13%)
Anxiety diagnosis	24 (24%)	23 (23%)
Cortisol (μg/dl)		
Wake-up	.40 (.33)	.37 (.49)
Wake-up + 45min	.45 (.35)	.49 (.77)
Wake-up + 2.5 h	.28 (.31)	.29 (.37)
Wake-up + 8 h	.20 (.35)	.25 (.51)
Wake-up + 12 h	.15 (.21)	.23 (.43)

Note: Depression diagnosis includes the broader phenotype of MDD plus depression NOS; anxiety diagnosis includes the broader phenotype of all anxiety disorders except PTSD. Cortisol values are in μg/dl after excluding outliers but are not adjusted for time. n =101(n = 81 for cortisol at 32 weeks).

Table 2

Association between prenatal depression and diurnal cortisol.

	Base model		Base model + diagnosis		Base model + EPDS	
Time/sampling factors	**Estimate (SE)**		**Estimate (SE)**		**Estimate (SE)**	
Intercept	−.95	(.52)	−1.31	(.49)	−.85	(.57)
Wake-up time	.05	(.05)	.09	(.05)	−.04	(.06)
Time since awake	−.07	(.01)***	−.08	(.01)***	−.10	(.02)***
CAR	.25	(.10)***	.24	(.11)***	.27	(.14)**
Gestation	.52	(.58)	.55	(.57)	.48	(.57)
× Wake-up	−.05	(.06)	−.06	(.06)	−.05	(.06)
× Time since awake	−.01	(.02)	−.02	(.02)	−.02	(.02)
× CAR	.17	(.13)	.13	(.12)	.16	(.13)
Covariates						
Race/ethnicity						
African-American	.10	(.13)	.07	(.13)	.09	(.13)
Hispanic	−.25	(.15)	−.24	(.14)	−.25	(.14)
Sleep (total hours)	−.13	(.05)*	−.11	(.04)*	−.11	(.05)*
Depression[a]			1.57	(.78)	−.03	(.06)
Depression × wake-up			−.22	(.07)*	.00	(.01)
Depression × time since wake			.05	(.02)	.004	(.002)
Depression × CAR			.07	(.16)	−.003	(.02)
Depression × gestation			.29	(.32)	.01	(.02)

Note: CAR = cortisol awakening response; EPDS = Edinburgh postnatal depression scale.

[a]In column 2, the effects for "depression" are given for a diagnosis of depression from clinical interview; in column 3, the effects for "depression" are given for self-report ratings from the EPDS. For gestation, the control condition is the 32 week visit; for race/ethnicity, Caucasian/other is the control condition. Significance is based on type 3 effects in GEE analysis. Estimates for depression diagnosis (dichotomous) and EPDS (continuous) are not directly comparable because of the scaling differences.

*$p<.05$.

**$p<.01$.

***$p<.001$.

Table 3

Prenatal depression and anxiety diagnoses as predictors of and diurnal cortisol.

	Estimate (SE)
Time/sampling factors	
Wake-up time	.02 (.03)
Time	−.09 (.01)***
CAR	.29 (.08)**
Gestation	−.05 (.11)
Covariates	
Race/ethnicity	
emsp;African-American/Black	.10 (.13)
emsp;Hispanic	−.18 (.15)
Sleep (total hours)	−.12 (.04)*
Symptoms	
Depression	1.86 (.70)*
Depression × wake-up	−.23 (.07)*
Depression × time since wake	.05 (.02)*
Depression × CAR	.04 (.16)
Depression × gestation	.08 (.35)
Anxiety	−.96 (.48)
Anxiety × wake-up	.07 (.05)
Anxiety × time since wake	−.003 (.02)
Anxiety × CAR	.15 (.14)
Anxiety × gestation	.48 (.27)

Note: CAR = cortisol awakening response. For gestation, the control condition is the 32 week visit; for race/ethnicity, Caucasian/other is the control condition. Significance is based on type 3 effects in GEE analysis

* $p<.05$.

** $p<.01$.

*** $p<.001$.