Running Title: Covariation of mood and cortisol during pregnancy

Psychological distress and salivary cortisol covary within persons during pregnancy

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1. Introduction

The observation that children born to mothers with elevated levels of psychological distress are at increased risk for poor birth and developmental outcomes (for reviews see Alder et al., 2007; Schlotz & Phillips, 2009) has given rise to the proposal that maternal experiences of stress and distress may 'program' fetal development. According to the fetal programming hypothesis, the morphological and functional organization of a variety of systems are sensitive to environmental input during critical windows of exposure and the nature of this exposure programs set points within those physiological systems (Barker et al., 1997).

1.1. Cortisol as a mediator of fetal programming effects

Results from both rodent and primate models of fetal programming indicate that exposing pregnant females to stressful environments (e.g., restraint) leads to increased basal glucocorticoid levels and prolonged glucocorticoid response to stress in offspring (Clarke et al., 1994) as well as enhanced vigilance and fearfulness (e.g., less time spent in open field; Vallee et al., 1997) and changes in brain structure and function (e.g., decreases in glucocorticoid and mineralocorticoid receptors; Matthews, 2002). These findings suggest that the effects of maternal psychological distress on fetal development may be indirect – that is, mediated, at least in part, by the maternal Hypothalamic Pituitary Adrenal (HPA) axis.

A substantial body of empirical evidence in pregnant and non-pregnant humans points to the HPA axis as a central feature of the stress response (see for example, McEwen, 1998; de Weerth & Buitelaar, 2005; Wadhwa, 2005). When individuals are

confronted with internal or external demands that exceed their self-perceived resources, the HPA axis initiates a complex set of neurophysiological changes, including the synthesis and/or release of 3 key hormones – corticotrophin-releasing hormone, adrenocorticotropin hormone, and cortisol – that facilitate adaptation (e.g., heightened vigilance, and defense-related learning and memory) and restoration of homeostasis through negative feedback to the pituitary (Miller & O'Callaghan, 2002). However, the typical functioning of this stress response may be fundamentally different during pregnancy because gestation is associated with profound alterations in HPA axis function.

Maternal cortisol increases 2- to 4-fold over the course of pregnancy, (Mastorakos & Ilias, 2003; Sandman et al., 2006). Despite this dramatic functional alteration, the circadian rhythm (with a peak shortly after waking and gradual decline over the day) remains intact (de Weerth & Buitelaar, 2005). Nevertheless, these changes appear to attenuate the HPA axis response to physical, pharmacological and psychosocial stress during pregnancy (de Weerth & Buitelaar, 2005; de Weerth et al., 2007; Entringer et al., 2010; Fink et al., 2010; Nierop et al., 2006). Given such powerful pregnancy-related alterations, it is not clear to what extent the maternal HPA axis remains responsive to psychological stress. Furthermore, such changes call into question the plausibility of cortisol as a biological link between maternal psychological distress and fetal development.

1.2. Associations between psychological distress and cortisol in pregnancy

The majority of what is known about the association between psychological stress and cortisol during human pregnancy comes from laboratory-based studies using acute

stressors. Few clinical studies have examined naturally occurring stressors and the majority of these have reported non-significant or low associations. This is especially true of studies using single measures of cortisol from amniotic fluid (Bergman et al., 2010), serum (Petraglia et al., 2001; Bergman et al., 2010; Davis, Glynn et al., 2010; Goedhart et al., 2010), or saliva (Davis et al., 2007; Davis & Sandman, 2010). Studies assessing diurnal variation in cortisol have provided stronger evidence of an association between psychological distress and cortisol during pregnancy, although the findings across studies are not consistent. Two studies reported flatter afternoon declines in cortisol for women with high levels of psychological distress in late gestation (Obel et al., 2005; Kivlighan et al., 2008) and another reported higher mid afternoon cortisol levels among women experiencing intimate partner violence (Valladares et al., 2009). In contrast, the most intensive assessment to date of stress and cortisol in pregnant women found no association between experiencing a recent stressor and daily levels of total cortisol (Harville et al., 2009).

Methodological differences between these studies can account for some of the differences in findings, including, for example, use of single versus multiple samples and single versus multiple days to estimate average cortisol as well as inconsistent inclusion of control variables that affect cortisol during pregnancy (e.g., previous pregnancy experience, maternal age, and gestational age; Kivlighan et al., 2008). The major limitation of these studies, however, is that they focus on individual differences between average levels of psychological distress and cortisol. Such approaches are concerned with determining whether individuals who experience more psychological distress also exhibit higher levels of cortisol. While this research has the potential to

inform our understanding of the relative ranking of individuals with regard to psychological distress and cortisol (or how predictable one is from the other), it is uninformative of the biobehavioural coordination of these processes *within* individuals. It is precisely such intra-individual approaches that are needed to evaluate the claim that maternal cortisol, consequent to psychological distress, is a plausible biological mechanism with the potential to affect fetal development.

Ecological Momentary Assessment (EMA) is a method that facilitates tracking of concurrent processes within individuals in the context in which they naturally occur. By assessing psychological distress and cortisol at various measurement moments over the course of several days, it is possible to accumulate a reliable record of variation in these processes and determine the extent to which they covary within persons (Hruschka et al., 2005). EMA approaches also eliminate recall bias and mental aggregation of moods over a prolonged period of time specified by retrospective methods (e.g., the usual paper-and-pencil self-report measures). This approach will help to clarify the nature and strength of the association between psychological and HPA axis changes during pregnancy because it directly assesses whether these process ‘track together’ within individuals.

1.3. Current Study

The current study was designed to clarify the intra-individual association between psychological distress and cortisol during pregnancy. Specifically, our goal was to determine whether: (1) moment-to-moment changes in psychological distress are associated with momentary changes in cortisol; (2) day-to-day changes in stress are related to daily changes in cortisol; and (3) any observed associations are moderated by

individual differences in gestational age (GA), maternal age, previous pregnancy experience, symptoms of anxiety or depression, and a history of stress exposure.

2. Materials and methods

2.1. Participants

A subsample of pregnant women between 6 and 37 week GA who were enrolled in an ongoing longitudinal study of nutrition during pregnancy (see www.apronstudy.ca for further details) were approached to participate. Participants were excluded if they reported any of the following: a) taking a steroid medication, b) smoking, c) consuming alcohol or drugs, d) recent dental work or tendency for oral bleeding (leading to falsely elevated cortisol values; Kivlighan et al., 2004), e) any known pregnancy or fetal complications (e.g., preeclempsia or fetal genetic anomalies), and illness during data collection (e.g., fever). Descriptive information for the study sample is shown in Table 1.

2.2. Materials and procedure

Participants attended an individualized training session where they received instruction about using the personal digital assistant (PDA) data collection device and saliva collection using Salivettes (Sarstedt, Germany). Saliva collection was done at home over 3 consecutive days (excluding weekends) on the following schedule: upon waking, 30-45 minutes after waking, and semi-randomly about the anchor times of 1100h, 1600h, and 2000h. The semi-random constraint was designed to reduce the possibility of changes in mood associated with anticipation of the signal received from the PDA. In order to facilitate adherence to the study protocol, the PDA was programmed to allow a 30 minute time window in which participants could respond after which data were considered missing.

Participants were instructed to turn on the PDA upon awakening, record their waking time, and collect a saliva sample. The PDA was programmed to ring 30-45 minutes later at which time participants collected their second saliva sample of the day. This procedure was used to assess the cortisol awakening response while allowing individualized wake times. Each time the PDA rang, it first provided a unique code corresponding to a prelabelled saliva tube and asked participants to click “OK” when they had placed the saliva roll under their tongue. This was designed to provide an exact time stamp for each saliva sample from the PDA record and to encourage adherence to the sampling protocol. The PDA administered the momentary mood questionnaire during the saliva collection. Questions about the most stressful experience of the day were included in the 2000h assessment only. Participants also completed a packet of paper-and-pencil standardized mood questionnaires that assess stable individual differences in psychological distress.

2.3. Measures

2.3.1 Cortisol

Participants were asked to refrain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing teeth in the 30 minutes prior to saliva collection and to report adherence to these guidelines. Whole saliva was obtained from under the tongue. Saliva samples were stored at -20 C until they were shipped frozen to Salimetrics, State College, PA. All samples were assayed for salivary cortisol in duplicate using a highly sensitive enzyme immunoassay. The test has a lower limit of sensitivity of 0.003 µg/dl, standard curve range from 0.012 to 3.0 µg/dl, and average intra-and inter-assay coefficients of variation 3.5 % and 5.1 % respectively. Method

accuracy, determined by spike and recovery, and linearity, determined by serial dilution are 100.8 % and 91.7 %.

2.3.2. Within-person psychological distress

Momentary negative mood was measured using the Profile of Mood States-15 (POMS-15), a 15-item multidimensional measure of mood, based on the larger 65 item Profile of Mood States (McNair & Heuchert, 2003), that was designed for momentary assessment with PDAs (Cranford et al., 2006). Participants rated each item on a 5-point Likert scale from *not at all* to *extremely,* based on how they were feeling in the previous 30 minutes. The scale assessed 5 mood dimensions: anger, sadness, anxiety, depression, fatigue, and vigour/positive affect). A total negative mood score was derived by subtracting the vigour subscale from the sum of the remaining subscales. Internal consistency for POMS is strong (coefficient alpha values range .79 - .93 (Bourgeois et al., 2010). In two separate samples, Cranford et al. (2006) demonstrated that the POMS-15 had appropriate reliability to detect within-person change processes. Construct validity of the POMS-15 was also supported by demonstrating sensitivity to changes in mood among participants experiencing a major life stressor.

2.3.3. Daily stress

Following Stone et al. (1998), peak daily stress was assessed via PDA by asking participants to reflect upon and rate their most stressful experience of the day. Ratings were on a 100-point scale with the following anchor points displayed: 0 – *not at all*, 50 – *moderately*, 100 – *extremely*. Using similar procedures, Smyth et al. (1998) demonstrated that stress was associated with increased salivary cortisol levels among a non-clinical sample of adults.

2.3.4. Individual differences in mood and stress

To assess relatively stable (as opposed to momentary) individual differences in mood and stress, a packet of standardized self-report instruments included the following measures.

2.3.4.1. Depression

The Edinburgh Postnatal Depression Scale (EPDS) is a 10-item instrument that is widely used to screen for perinatal depression. This instrument has demonstrated excellent reliability and validity among pregnant women (Cox et al., 1987; Jomeen & Martin, 2007). Sensitivity (79%) and specificity (85%) are also satisfactory when used with pregnant women (Jomeen & Martin, 2007).

2.3.4.2. Anxiety

The Symptom Checklist-90-R (SCL-90-R; Derogatis, 1994) is a multidimensional symptom inventory designed to reflect psychological symptom patterns. Only the 10-item anxiety scale was administered, which has good convergent and divergent validity (Morgan et al., 1998) and adequately discriminates between clinical and non-clinical samples (Bonicatto et al., 1997; Holi et al., 1998).

2.3.4.3. Stress history

The Stressful Life Events Questionnaire (SLEQ; Bergman et al., 2007) is a 26-item measure of exposure to stressful life events, adapted from Barnett et al. (1983) and designed for use with pregnant women. Construct validity of the SLEQ is supported by the finding that antenatal levels of stress, as measured by the SLEQ, predict cognitive development and fearfulness in offspring (Bergman et al., 2007). Respondents indicate which stressful events occurred and the perceived impact of the events. For present

purposes, the sum of pre-pregnancy and pregnancy events was multiplied by the perceived affect to obtain an overall estimate of stress history.

2.3.6. Statistical Procedures

Hierarchical linear modeling (HLM) was used to determine whether momentary within-person changes in daily cortisol were related to moment-to-moment changes in psychological distress, whether day-to-day changes in the diurnal cortisol pattern were related to day-to-day changes in stress, and whether stable or ongoing mood problems were related to the average or typical diurnal cortisol pattern over the days of testing. HLM does not require that all individuals complete assessments on the same schedule, which allows for individual differences in waking times without affecting the precision of parameter estimates. Furthermore, HLM is able to calculate slopes and intercepts even with missing data (which commonly occur with intensive measurement designs) and does not require even spacing or equal numbers of observations across individuals (Hruschka et al., 2005).

Multilevel equations were specified at three levels to account for the nested data structure (measurement moments nested within days and days nested within persons). In the models we tested, levels of cortisol for each person at each moment was the outcome, and was predicted by moment-level, day-level, and individual-level variables. Our primary goal was to determine whether cortisol and psychological distress ‘travel together’ over time. The secondary goal was to determine whether any of the day-level or individual-level variables moderate this association. Accordingly, the focus of the model was the time-varying covariation between momentary negative mood and cortisol. The following model served as the basis for these analyses:

Level 1: $\text{logCortisol}_{ijk} = \pi_{0jk} + \pi_{1jk}\text{CAR} + \pi_{2jk}\text{Time Since Waking}_{ijk} + \pi_{3jk}\text{Time Since Waking}_{ikj}^2 + \pi_{4jk}\text{Momentary Negative Mood}_{ijk} + \sigma_{ijk}$

Level 2: π_{0jk} through $\pi_{4jk} = \beta_{00k} + \beta_{01k}(\text{Day}_{jk}) + \beta_{02k}(\text{Stress}_{jk}) + \varepsilon_{0jk}$

Level 3: β_{00k} through $\beta_{40k} = \gamma_{000} + \gamma_{001}(\text{GA}_k) + \gamma_{002}(\text{Age}_k) + \gamma_{003}(\text{Gravida}_k) + \gamma_{004}(\text{Depression}_k) + \gamma_{005}(\text{Anxiety}_k) + \gamma_{006}(\text{Stress History}_k) + u_{00k}$

where $\underline{\text{logCortisol}}_{ijk}$ is the natural log of salivary cortisol for moment $\underline{i}$, day $\underline{j}$, and person $\underline{k}$. CAR represents a dummy variable (1 = sample taken 30-45 min after waking) included to model the initial increase in cortisol after waking.[1] Time was parameterized as both time since waking (in hours) as well as time since waking squared to better model the curvilinear shape of the diurnal cortisol curve over the course of the day. Momentary negative mood in the level 1 model refers to mood for measurement moment $\underline{i}$, day $\underline{j}$, and person $\underline{k}$. Negative mood and saliva were assessed concurrently allowing analysis of the extent to which changes in mood and cortisol were coordinated within persons. Stress in the level-2 model refers to peak daily stress for day $\underline{j}$ and person $\underline{k}$. The time parameter Day was included in the level-2 model to allow the effects of day to vary randomly from person-to-person. Individual-level variables in the level-3 model refer to GA, maternal age, previous pregnancy experience, EPDS, SCL-90-R anxiety, and stress history for person $\underline{j}$.

Data were analyzed with HLM 6.08 software (Raudenbush et al., 2004). Missing data were estimated using full information maximum likelihood. All HLM results reported here represent the final estimation of fixed effects with robust standard errors.

3. Results

[1] Only samples that were collected within 5 min of waking and 30 – 45 min after waking were used to model the CAR (Okun et al., 2010).

3.1. Missing data and adherence

Of the 96 participants, 13 did not complete the study. Noncompleters did not differ from participants on any demographic variables. The remaining 83 participants contributed a total of 1026 saliva samples (out of a possible 1245). Of the missing samples, 22 were missing because the quantity of saliva was insufficient for conducting the assay. Other reasons for missing samples included PDA failure (*n* = 11), participant was busy (*n* = 148), experimenter error (*n* = 15), and other reasons not specified (*n* = 23).

To estimate adherence to the protocol, self-reported wakeup time was compared to time of the 30-45 minute post-waking sample recorded on the PDA. If participants had responded immediately to the PDA signal, then all responses would have occurred within the 30 to 45 minute window and the mean would be 37.5. Mean and median response times were 48 (*SD* = 40) and 37 minutes respectively, suggesting good overall compliance. Because we were able to determine a precise time for each cortisol sample, “off time” samples were included in the multilevel model, however these samples were not included in estimating the cortisol awakening response.

To address concerns about validity of the psychological distress data, we assessed the reaction times for all responses and compared them to intentionally faked data. We generated validation data by asking 10 members of our research group to produce 2 kinds of data: 1) intentionally faked data in which responses were made as quickly as possible without reading the question or thinking about the response (n = 910), and 2) more realistic data from rapid but truthful responses (n = 875). The minimum and mean response times for intentionally faked and truthful responses recorded by our team were min = 1.1s, M = 1.6s and min = 1.8s, M = 4.0s, respectively.

In comparison, the minimum participant reaction time was 1.4s with a mean 5.4s. These reaction times are consistent with truthful responding. We also examined each set of responses to determine whether we could identify systematic patterns of response that may indicate faking. No such patterns could be detected by visual examination of each electronic diary.

The occurrence of tooth brushing and exercising were rare (3.6% and 1.2%, respectively), while consuming food within 30 minutes of sample collection was more common, 12.1%. There were no significant differences between samples affected and not-affected therefore these potential covariates were eliminated from further analyses.

3.2. Descriptive statistics

Descriptive statistics for study variables are presented in Table 2. As expected, there were diurnal changes in both cortisol and mood and increases in cortisol associated with gestational age.

Preliminary evaluation of the fully unconditional (empty) model revealed that 96.9% of the variability in cortisol levels was over measurements within moments (level-1 units); only 3.05% of the variability was at the individual level (level-3). This finding underscores the importance of specifying time-varying explanatory variables at level 1 of the model because there is more within person variability in cortisol over the course of a day than there is variability between persons in mean levels of cortisol.

3.3. Daily and diurnal variation in cortisol

The final multilevel model (see Table 3) revealed substantial day-to-day variability in waking cortisol levels, $\chi^2(109) = 261.43$, $p < .0001$, as well as significant variability in waking cortisol levels across individuals, $\chi^2(70) = 232.63$, $p < .0001$. This

variability in waking cortisol was associated with GA (β =.036, $p < .0001$) indicating significant increase in waking levels of cortisol with increasing GA. On average, each additional week of gestation resulted in a 3.6% increase in waking cortisol. Consequently, the average woman at 37 weeks gestation had 54.4% higher waking cortisol than the average women at the sample mean GA of 21.9 weeks.

In keeping with previous reports of a preserved diurnal pattern among pregnant women, cortisol in our model displayed a strong awakening response, β =.423, $p < .0001$, and then a gradual decrease over the course of the day, β =-.124, $p < .0001$. The CAR resulted in a 52.6% increase over waking levels while the remaining day slope resulted in an 11.6% decrease per hour at waking, when holding all other variables constant. This decrease was partially offset by a (statistically non-significant) .15% increase in cortisol per hour squared. The time squared parameter was retained in the final model because it was a significant predictor of cortisol in preliminary models.

3.4. Covariation between cortisol and mood

As seen in Table 3, the average effect of negative mood on cortisol, after accounting for the diurnal effects, was substantial, β =.121, $p < .01$, resulting in an average 12.8% increase in cortisol per 1 unit increase in momentary negative mood. Figure 1 illustrates how the average diurnal pattern is affected by changes in negative mood. It depicts a 'conceptual envelope' encompassing the possible trajectories implied by the model (see Singer & Willett, 2003). Figure 1 is a hypothetical case in which a woman begins the day on a low negative mood trajectory (mean of the lower quartile), shifts to a no negative mood trajectory, and finally shifts to a high negative mood trajectory (mean of the upper quartile). The dotted lines indicate shifts in trajectory associated with changes

in mood. It should be noted, however, that in the ordinary sequence of an actual day mood-related changes in cortisol occur in the context of the underlying diurnal cortisol decline. The mean diurnal decline is larger than the mean cortisol increase associated with negative mood. Accordingly, the effect of an average increase in negative mood is an attenuation of the cortisol slope, or a flattening of the diurnal curve.

3.4.1. Day-level moderators of momentary mood effects

The effect of mood on cortisol was stronger on the first day of data collection compared to subsequent days of collection, $\beta = -.013$, $p < .05$. To determine whether changes in mood or stress during the three days of assessment may have been responsible for this finding, we constructed separate multilevel models with peak daily stress and momentary negative mood as the outcome. The growth curve model for negative mood was not reliable, indicating no change in average mood over days. However, the model for peak daily stress indicated a significant decline in stress over days; $\beta = -3.28$, $p < .05$. These findings suggest that a decline in stress may be responsible for the decreasing effect of mood on cortisol over the 3 days. This interpretation, however, is inconsistent with the finding that daily levels of peak stress did not moderate the effects of mood on cortisol in the final multilevel model (see Table 3). It is possible that the novel experience of assessing mood and collecting cortisol may have initially enhanced their covariation (de Weerth & Buitelaar, 2005).

3.4.2. Individual-level moderators of momentary mood effects

At the individual level, there was evidence that momentary negative mood had less effect on cortisol for women with chronically elevated levels of depression, $\beta = -.006$, $p < .05$. There were also trends for maternal age such that negative mood had less effect

on cortisol among older women, β =-.003, $p < .07$; for gravida, such that mood had greater effect on cortisol with each additional pregnancy, β =.013, $p < .06$; and for anxiety, such that mood had a greater effect on cortisol in women with higher levels of chronic anxiety, β =.06, $p < .08$.

4. Discussion

The findings of this study help to clarify the intra-individual association between mood and cortisol during pregnancy. By examining momentary levels of cortisol concurrent with momentary levels of negative mood over 3 days, this study provides strong evidence for biobehavioural coherence between the HPA axis and psychological distress during pregnancy. That is, on occasions when negative mood was greater, cortisol levels were also higher within the same individual. We found no evidence for a progressive attenuation of coordination between the HPA-axis and psychological distress with advancing gestation. In keeping with previous reports, cortisol response to momentary negative mood among women with chronic levels of depression was blunted (Peeters et al., 2003).

To our knowledge, this is the first study to demonstrate the within-person association of momentary mood and cortisol during pregnancy. By examining variance at the momentary, daily, and between-person levels, this study helps to clarify the ambiguous pattern of previous findings. In particular, the fact that the majority of variance in diurnal cortisol was associated with processes occurring within individuals suggests that further advances in understanding biobehavioral coherence may be made by focusing on psychological processes that change within individuals. Although this study is the first to demonstrate a robust within-person association for momentary

negative mood and cortisol in pregnant women, similar findings in non-pregnant samples have demonstrated that within-person designs can detect associations between mood and cortisol where between person designs have yielded equivocal results (Hruschka et al., 2005; Steptoe et al., 2007).

Women in the current study displayed the expected patterns of cortisol increase in response to awakening and decline over the remainder of the day (de Weerth & Buitelaar, 2005). Furthermore, the daily declines in cortisol are nearly identical to those reported by Kivilghan et al. (2008) in late pregnancy, confirming that the diurnal patterns remain relatively unchanged across pregnancy. Interestingly, Goedhart et al. (2010) reported that cortisol levels in early pregnancy are lower in older women. One reason for this age related decrease may be that age moderates the association between mood and cortisol. Consistent with Goedhart's findings, mood had less effect on cortisol among older compared to younger women in our sample.

The current findings replicate and extend findings among non-pregnant women by researchers using similar operationalizations of psychological distress. Jacobs et al. (2007) assessed mood and cortisol 10 times per day over a period of 5 consecutive days in 556 non-pregnant women with demographic characteristics very similar to the current sample. Results of the multilevel model indicated that negative mood was significantly associated with cortisol. Comparable results were reported by Smyth et al. (1998) and van Eck et al. (1996). The effect sizes for momentary mood on cortisol in these two studies ranged from increases of 12% to 24.6%, respectively, for a shift in negative affect from the 25th to the 75th percentile. The estimated 29.9% increase over the same mood range in the current study is comparable to previous findings.

Furthermore, similar to Smyth et al., daily stress was not a significant predictor of cortisol level when momentary affect was included in the model.

Our finding of preserved biobehavioural coherence throughout pregnancy is in contrast to studies reporting decreasing neuroendocrine response to stress with advancing gestation (de Weerth & Buitelaar, 2005; Wadhwa, 2005; Kivlighan et al., 2008). We note, however, that covariation between psychological distress and cortisol in a naturalistic setting is not equivalent to the concept of physiological reactivity observed in laboratory settings. Furthermore, we note that longitudinal assessment of the association between mood and cortisol over the course of pregnancy are needed to clarify the effects of GA on biobehavioral coherence.

The findings may help explain non-typical diurnal curves (i.e. increasing or inconsistent) that are commonly observed, but poorly predicted by time-based parameters alone. For example, the shifts in trajectory depicted in Figure 1 imply that if mood stays constant, an individual stays on one cortisol trajectory. Those women who experience an increase in negative mood move to a higher trajectory. Conceptually, the model implies a shift in trajectory corresponding to each change in momentary mood. As such, the model implies that changes in mood can, in part, account for deviations from the typical diurnal pattern.

Ecological momentary assessment providing three days of diurnal cortisol with up to 15 measurements per woman, and the simultaneous assessment of psychological stress and distress at the momentary, daily, and individual level, are significant strengths of this study. Despite these strengths, several limitations require consideration. First, the time varying variables were measured concurrently, which

raises the problem of reciprocal causation. That is, increases in cortisol may be responsible for increases in negative mood. However, experimental studies suggesting that administration of exogenous glucocorticoids in healthy volunteers does not result in acute effects on mood (Wolkowitz et al., 1990; Schmidt et al., 1999) argue against this interpretation. Second, peak HPA-axis reactivity typically lags acute onset of a stress by approximately 20 min, although clear changes in cortisol are evident within 10 min (Kirschbaum & Hellhammer, 1989). The degree to which such a lag applies also to internal experience of mood is unknown, but it seems reasonable to assume that such delays exist (Smyth et al, 1998). We addressed this issue by asking participants to report on their mood *in the past 30 minutes*. To the degree that dramatic mood may have changed immediately prior to a mood sample, our assessment strategy will have missed the covariation of mood and cortisol. However, we note that such occurrences should result in underestimating the actual covariation of momentary mood and coritsol. Finally, the sample represents a relatively stable population of well-educated, employed, mature, and Caucasian women. Since social advantage is known to covary with psychological distress it is likely that the women in this sample experienced less psychological distress than more disadvantaged women. Given that our analysis revealed decreased biobehavioral coherence for women with higher levels of chronic depression, the intra-individual association between mood and cortisol may be decreased in more disadvantaged women.

In summary, we found that, within individual pregnant women, levels of psychological distress and cortisol covary over time. As such, these findings stand in contrast to previous research, based primarily on between-person analyses, suggesting

that average levels of cortisol and stress are weakly associated or unrelated during pregnancy. No changes in biobehavioral coherence as a result of advancing gestation were observed. The findings support the proposal that elevations in maternal cortisol, consequent to psychological distress, are a plausible biological mechanism for the observed relationship between maternal experience and fetal development. Further studies are needed to determine whether some forms of psychological distress (e.g., distress associated with intimate partner violence vs. job strain) have greater effect on the maternal HPA axis and therefore greater potential to affect fetal development.

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Table 1. Demographic information for the study sample (n = 83)

	Mean	S.D.	Range
Gestational Age (weeks)	21.87	8.67	6 – 37
Age (years)	32.42	4.36	25.33 – 43.04
Gravida	2.1	1.13	1 – 6
	Percentage		
Parity			
Nulliparous	34.9		
Primaparous	36.1		
Married or common law	97.6		
Education			
university degree	72.3		
less than high school diploma	1.2		
Annual household income			
more than $100,000/yr	54.2		
$70,001 to 100,000	13.0		
$40, 000 to $70,000	7.2		
less than $40,000/yr	9.6		
Employment			
Working full time	67.5		
Working part time	20.5		
Ethnicity			

Caucasian	89.0
Asian	4.8
Hispanic	3.8
African North American	1.2
First Nations	1.2

Note: Gravida refers to number of pregnancies, including the current pregnancy.

Table 2. Descriptive statistics for study variables by gestation

	1st Trimester			2nd Trimester			3rd Trimester		
Variables	**Mean**	**S.D.**	**Range**	**Mean**	**S.D.**	**Range**	**Mean**	**S.D.**	**Range**
Raw Cortisol (ug/dl)									
Waking	.36	.14	.06 – .72	.45	.19	.13-1.01	.50	.22	.12-1.18
Waking + 30 minutes	.38	.19	.12-1.00	.55	.23	.17-1.07	.65	.27	.15-1.39
Mid morning	.15	.07	.06-.38	.23	.12	.06-.81	.34	.13	.12-.81
Mid afternoon	.10	.04	.03-.26	.14	.09	.03-.53	.24	.12	.10-.69
Evening	.07	.03	.02-.26	.09	.06	.01-.46	.15	.07	.05-.35
Momentary Negative Mood									
Waking + 30 minutes	3.38	1.86	-.33 – 9.00	4.22	2.23	1.00-12.66	3.87	1.79	.99-8.00
Mid morning	3.07	1.99	.00-11.35	3.86	2.39	.00-13.67	3.24	2.29	-.67-12.33
Mid afternoon	3.74	1.94	1.00-11.01	3.36	1.88	.33-7.66	3.77	2.13	.33-10.66
Evening	3.94	1.84	.66-9.33	3.76	2.00	-.33-9.68	4.14	2.02	1.00-11.01
Peak Daily Stress									

Day 1	28.2	25.1	0-78	33.1	16.9	0-65	28.6	24.8	0-85
Day 2	27.4	23.8	0-80	33.0	17.1	0-80	28.3	26.1	0-97
Day 3	20.3	21.1	0-72	25.6	18.5	0-73	24.0	27.7	0-90
Chronic Mood									
EPDS	5.7	3.3	1-13	5.4	3.1	1-11	6.5	3.9	0-15
SCL-90-R (anxiety)	.29	.31	.00-1.20	.26	.29	.00-1.20	.22	.27	.00-.90
Stress history	11.1	23.4	0-108	4.5	14.2	0-78	13.2	21.6	0-84

Note: Raw cortisol values are presented for descriptive purposes but log transformed values are used in all analyses.

Table 3. Final model for covariation of momentary negative mood and cortisol with day-level and individual-level moderators

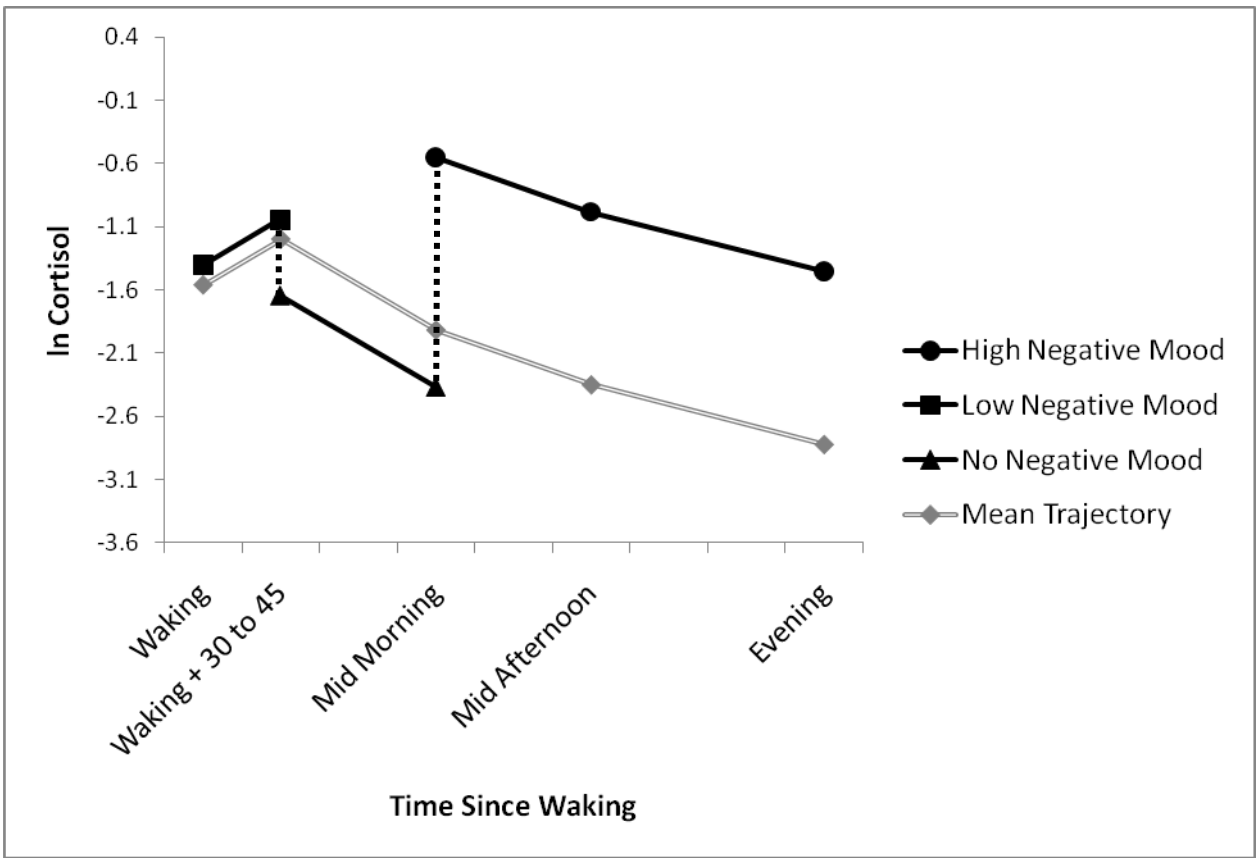
Fixed Effects	**Coefficient (SE)**	**t ratio**	**Interpretation**
Waking cortisol			
Average waking cortisol	-2.01(.28)	7.05***	Waking level = .13 ug/dl
Effect of daily stress	.001(.001)	1.12	n.s.
Effect of day	.026(.026)	1.00	n.s
Effect of GA	.036(.004)	9.41***	3.6% increase per additional week
Effect of maternal age	.002(.010)	.24	n.s
Effect of gravida	-.034(.038)	.89	n.s.
Effect of individual level depression	.014(.012)	1.21	n.s.
Effect of individual level anxiety	-.231(.220)	1.06	n.s.
Effect of stress history	-.027(.031)	.89	n.s.
CAR	.423(.073)	5.77***	52.5% increase 30 min after waking
Time since waking	-.124(.020)	6.07*	11.6% decrease per hour of waking
Time since waking^2	.002(.001)	1.37	n.s.
Momentary Negative Mood			
Average mood effect	.121(.045)	2.71**	12.8% increase per unit increase in mood disturbance
Daily stress	-.001(.001)	.26	n.s.
Sampling Day	-.013(.007)	1.93*	Effect of mood decreases 1.3% per day

GA	.001(.001)	.44	n.s.
Maternal age	-.003(.002)	1.82†	Effect of mood decreases by .03% per additional year
Gravida	.013(.007)	1.86†	Effect of mood increases 1.3% per additional pregnancy
Depression	-.006(.002)	2.59*	Effect of mood decreases 2.0% per 1 SD increase
Anxiety	.062(.035)	1.75†	Effect of mood increases 1.9% per 1 SD increase
Stress history	-.009(.006)	1.62	n.s.

Note: Effect sizes were derived using the following formula β% change = (e^{β} – 1)*100.

† = p < .10. * = p < .05. ** = p < .01. *** = p < .001

Figure 1. Example cortisol trajectory as a function of changes in negative mood over the day



Note: The black line segments illustrate a hypothetical case in which a woman begins the day on a low negative mood trajectory (mean of the lower quartile), shifts to a no negative mood trajectory, and finally shifts to a high negative mood trajectory (mean of the upper quartile). The vertical dotted lines indicate mood-related shifts in trajectory. The average trajectory, displayed in grey, is the trajectory for a person with average mood and *no mood change* over the day.