RUNNING HEAD: Biobehavioural Coherence During Pregnancy

Advancing Gestation Does Not Attenuate Biobehavioural Coherence Between Psychological Distress and Cortisol

Gerald F. Giesbrecht[a]*, Tavis Campbell[b], Nicole Letourneau[c], Bonnie Kaplan[a],

and the APrON Study Team

Author Note

[a] Department of Paediatrics, University of Calgary, Behavioural Research Unit, Alberta Children's Hospital, 2888 Shaganappi Trail N.W., Calgary, AB, T3B 6A8, Canada
[b] Department of Psychology, University of Calgary, 2500 University Dr. N.W. Calgary, AB, T2N 1N4, Canada
[c] Faculty of Nursing, University of Calgary, 2500 University Drive NW Calgary, AB, T2N 1N4, Canada

*Corresponding author
Behavioural Research Unit, Alberta Children's Hospital, 2888 Shaganappi Trail N.W., Calgary, AB, T3B 6A8, Canada Tel.: +1 403 955-2793; fax: +1 403 955-2773
Email address: ggiesbre@ucalgary.ca.

Abstract

Background

Despite little evidence to suggest that HPA axis responses to psychological provocation are attenuated during pregnancy, it is widely held that dampening of the HPA axis response to psychological distress serves a protective function for the mother and fetus. The current study was designed to assess changes in biobehavioral coherence between psychological distress and cortisol over the course of pregnancy.

Methods

Ambulatory assessment of ecologically relevant psychological distress and salivary cortisol were repeated in all three trimesters for 82 pregnant women. Samples were collected 5 times per day over the course of 2 days in each trimester.

Results

Psychological distress and cortisol were positively associated, $\beta = .024$, $p < .01$, indicating that increases in psychological distress were associated with increases in cortisol. Gestational age did not moderate this association, $\beta = .0009$, $p = .13$, suggesting that negative psychological experiences remain potent stimuli for the HPA axis during pregnancy.

Conclusion

Biobehavioral coherence between ecologically relevant experiences of psychological distress and cortisol is not attenuated with advancing gestation.

Keywords: Psychological Distress, Salivary Cortisol, Pregnancy, Stress Response, HPA axis, Biobehavioural coherence

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Psychological distress during pregnancy is associated with adverse obstetric (e.g., intra-uterine growth restriction, preterm birth) and developmental outcomes (e.g. exaggerated behavioral and hypothalamic-adrenal-pituitary [HPA] axis response to stress; Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006). Animal studies suggest that psychological distress generally has a larger effect on fetal development when the exposure occurs earlier rather than later in gestation (Mueller & Bale, 2007; Schneider, Roughton, Koehler, & Lubach, 1999). The effects of exposure timing on human development and the underlying mechanisms, however, are not as well understood. Moreover, in light of evidence for progressive adjustment within maternal physiological systems involved in stress responses (one of the pathways by which psychological distress may impact fetal development; de Weerth & Buitelaar, 2005) and data indicating that the prevalence of self-reported affective disorders may be as high as 51% in some groups (Halbreich, 2004) with approximately 14% of pregnant women meeting diagnostic criteria for an affective disorder (Alder, Fink, Bitzer, Hosli, & Holzgreve, 2007), understanding how and when psychological distress ‘gets under the skin’ of the fetus is an important conduit to improving infant health and development.

It is well-known that biological systems are more vulnerable to exposure during periods of rapid development (Davis & Sandman, 2010; Moore & Persaud, 1998), which may partially explain why the effects of psychological distress do not appear to be uniform across gestation or across developmental domains. For example, the effects of psychological distress during pregnancy on infant cognitive development may be greatest when the exposure occurs early (15 wks) in pregnancy (Davis & Sandman, 2010; Laplante et al., 2004) whereas adverse birth

outcomes have been associated with exposure during the 5th and 6th months of gestation (Class, Lichtenstein, Langstrom, & D'Onofrio, 2011). Findings from the Western Australian Pregnancy Cohort (Raine) Study, one of the largest prospective cohorts of pregnancy and childhood (Robinson et al., 2011), suggest that behavioral morbidity is higher in children who were exposed to maternal distress at 18 versus 32 weeks gestational age (GA). In contrast, reports from another large cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC; O'Connor, Heron, & Glover, 2002), suggest that maternal distress at 32 but not 18 weeks GA was associated with child behavioral problems. The inconsistent results may in part reflect differences in the measurement of maternal distress, infant behavior and parenting. The overall pattern of results, however, reinforces the notion that diverse developmental outcomes may have differing periods of vulnerability to psychological distress that are influenced by the type, intensity and duration of the exposure (Wadhwa, Sandman, & Garite, 2001).

In addition to the effects of developmental changes in biological vulnerability, the timing of exposure may affect development because the physiological "dose" associated with psychological distress may decrease as a function of gestational age. Changes in maternal physiological reactivity to negative psychological experiences during pregnancy are supported by research with animals suggesting that in most mammals, the major stress systems mount dampened responses (Slattery & Neumann, 2008), including marked attenuation of the HPA axis response to both physical and mixed physical and psychological stressors (Neumann et al., 1998; Russell, Douglas, & Brunton, 2008). The prevailing interpretation of these findings is that physiological changes during pregnancy may serve to protect the fetus and mother from the potentially negative effects of noxious psychological experiences (de Weerth & Buitelaar, 2005;

DiPietro, Mendelson, Williams, & Costigan, 2012; Petraglia et al., 2001; Slattery & Neumann, 2008).

As a consequence of significant species differences in the structure and function of the placenta and in the nature of stress responses, there is a need to exercise caution in extrapolating animal findings suggesting attenuation of reactivity to humans (Michael & Papageorghiou, 2008). Nevertheless, reports from human studies have typically supported the proposal that responses to negative psychological stimulation are progressively dampened throughout pregnancy (de Weerth & Buitelaar, 2005; DiPietro, Costigan, & Gurewitsch, 2005; Entringer et al., 2010; Klinkenberg et al., 2009; Matthews & Rodin, 1992; Slattery & Neumann, 2008; Wadhwa, 2005). Overall, the evidence in these studies is most consistent for the autonomic nervous system (ANS) relative to HPA reactivity. The lack of consistent results from studies of the HPA axis may be a product of differences in the nature of the challenge presented. For example, chemical and physical stimulation of the HPA axis in late gestation, including a standard corticotrophin releasing hormone (CRH) test (Schulte, Weisner, & Allolio, 1990) and a painful cold pressor task (Kammerer, Adams, Castelberg, & Glover, 2002), failed to produce a robust cortisol response in pregnant women, while the dexamethasone test (Odagiri et al., 1988) produced less suppression of cortisol production compared to non-pregnant women. These studies support the notion of attenuated HPA axis reactivity. Studies involving what are arguably more psychological forms of stimulation, in contrast, have been less consistent. For example, fetal blood transfusion (a potentially potent psychological stressor) did not produce a maternal cortisol response in the 2nd or 3rd trimesters (Gitau, Fisk, Teixeira, Cameron, & Glover, 2001) whereas public speaking and mental arithmetic did (De Weerth, Wied, Jansen, & Buitelaar, 2007; Nierop et al., 2006). Considering the evidence to date, there may be a selective dampening

of the HPA axis during pregnancy such that tasks designed to elicit social and mental stress remain potent activators.

Prospective studies with repeated assessment throughout pregnancy are needed to determine if biobehavioural coherence between psychological distress and the HPA axis is progressively attenuated over the course of pregnancy. At present, there are only two such published reports. Entringer and colleagues (Entringer et al., 2010) used a standard Trier Social Stress Task (TSST) to assess cortisol reactivity among pregnant and non-pregnant women. Modification of the TSST protocol to reduce physical discomfort during the procedures (women were reclined in a comfortable chair), however, led to a failure to produce a cortisol increase in either group. In a field study involving 603 pregnant women, Obel and colleagues (Obel et al., 2005) reported no association between life events / worry and diurnal cortisol among women in the 2nd trimester. In contrast, women in the 3rd trimester with higher self-reported stress had elevated levels of evening cortisol, suggesting that basal cortisol response to stress may increase rather than decrease over the course of gestation.

In the present study we not only assessed the biobehavioural coherence between psychological distress and cortisol but also we tested the specific claim that this coherence is progressively attenuated with advancing gestation (Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001; Glynn, Schetter, Wadhwa, & Sandman, 2004). The study was designed to build upon previous cross-sectional research in which basal levels of cortisol were positively associated with changes in self-reported psychological distress (Giesbrecht, Campbell, Letourneau, Kooistra, & Kaplan, 2012). The goal was to prospectively assess subjective experiences of psychological distress and cortisol within the day-to-day experiences of pregnant women over the course of gestation. Characterizing the biobehavioral coherence between

psychological distress and cortisol during gestation has important implications for understanding the potentially differential effects of psychological distress on developmental outcomes arising from different phases of pregnancy.

Method

Participants

Eighty five pregnant women who were enrolled in an ongoing longitudinal study of nutrition during pregnancy (see www.apronstudy.ca for further details) participated. Women were excluded if they were > 14 weeks gestation or if they reported any of the following: a) taking a steroid medication, b) smoking, c) consuming alcohol or 'street' drugs, d) recent dental work or tendency for oral bleeding (leading to falsely elevated cortisol values (Kivlighan et al., 2004), e) known pregnancy or fetal complications (e.g., preeclampsia, fetal genetic anomalies), or illness during data collection (e.g., fever). GA at each assessment was determined based on last reported menstrual period and confirmed by at least one ultrasound. Prior to data collection, participants provided informed consent to the procedures. The study procedures were approved by the University of Calgary Conjoint Health Research Ethics Board.

Procedures

Participants completed the sampling procedures three times during pregnancy: Time 1 (T1) prior to 14 weeks GA, Time 2 (T2) at 21 weeks GA, and Time 3 (T3) at 32 weeks GA. At T1, participants attended an individualized session where they were instructed on the use of the personal digital assistant (PDA) data collection device and Salimetics Oral Swab (Salimetrics, Pennsylvania, USA) for saliva collection. On each assessment occasion, women self-collected saliva at home over 2 consecutive days (excluding weekends in order to rule out potential weekend-weekday differences in stress and diurnal cortisol (Schlotz, Hellhammer, Schulz, &

Stone, 2004) for a total of 6 days across pregnancy. Samples were obtained on the following schedule: upon waking (allowing for individualized wake times), 30 minutes after waking, and semi-randomly after the anchor times of 1100h, 1530h, and 2000h. The semi-random signals occurred on the PDA once within 15 minutes following the anchor times to reduce the possibility of changes in mood associated with anticipation of the signal. To facilitate adherence to the study protocol, the PDA was programmed to allow a 20 minute response window following the signal, after which data were considered missing.

Each time the PDA rang, it first provided a unique code corresponding to a pre-labeled saliva tube and instructed participants to place the saliva roll under her tongue. Each response to the PDA was marked by a time stamp permitting precise modeling of the diurnal patterns. The PDA administered the psychological distress questionnaire during the saliva collection.

Measures

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 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%. A random 10% of samples were assayed in duplicate to confirm reliability; the intra-assay coefficient of variation and correlation coefficient between the

duplicate tests were 4.16% and $r = .99$, $p < .001$. The mean value from duplicate samples was used for data analysis.

Psychological Distress. Psychological distress at each sampling moment was measured using items from the Profile of Mood States (POMS; McNair & Heuchert, 2003), a multidimensional measure of mood with strong psychometric properties (e.g., coefficient alpha values range .79 - .93; Bourgeois, LeUnes, & Meyers, 2010). Using a procedure adapted from Cranford and colleagues (Cranford et al., 2006), we selected 19 items from the anger, anxiety, depression, fatigue, and vigor/positive affect scales that could be administered via PDA. Participants rated each item on a 5-point Likert scale from *not at all* to *extremely*, based on their feelings during the previous 30 min. The 30 min window was chosen to account for the delay in HPA axis response to psychological experience (Kirschbaum & Hellhammer, 1989). As per standard scoring procedures for the POMS, a psychological distress score was derived for each sampling moment by subtracting the vigor subscale from the sum of the remaining subscales. In two separate ambulatory studies, Cranford et al. (2006) demonstrated that a short version of the POMS had appropriate reliability to detect within-person changes in mood; validity was supported by demonstrating sensitivity to mood changes in participants experiencing a major life stressor.

Statistical Procedures

Multilevel equations were specified at three levels to account for the nested data structure (measurement moments nested within GA and GA nested within persons)[1]. At level 1 (moment-level), the between-moment variability in the log of cortisol for each individual was modeled as a function of psychological distress. In order to isolate within-person covariation between psychological distress and cortisol, psychological distress was person-centered (Blackwell, de

Leon, & Miller, 2006). The primary goal was to determine whether the within-person association between psychological distress and cortisol changed with advancing gestation. Accordingly, the focus of the analysis was on the level-2 submodel for the slope of psychological distress, with GA included as a potential moderator. The following model served as the basis for these analyses:

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

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

Level 3: β_{00k} through $\beta_{41k} = \gamma_{000} + u_{00k}$

where $\underline{\text{logCortisol}}_{ijk}$ is the natural log of salivary cortisol for moment i, GA j, and person k. CAR represents a dummy variable (1 = sample taken 30 min after waking) included to model the initial increase in cortisol after waking.[2] 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. Time was centered at the mean of time since waking (5.1 hours since waking). Momentary psychological distress in the level 1 model refers to POMS total mood disturbance for measurement moment i, GA j, and person k. The model is designed to determine the extent to which changes in mood disturbance and cortisol were coordinated within persons. To facilitate interpretation, gestational age in the level-2 model was grand mean centered at 21 weeks.

Data were analyzed with HLM 7.0 software (Raudenbush, Bryk, Cheong, Condon, & du Toit, 2011). 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.

Results

Missing Data and Adherence

Of the 85 participants with complete data at T1, 78 had data at T2 and 76 had data at T3. Participants who did not provide data at T3 did not differ from participants with complete data on any of the demographic or study variables. Three participants were excluded from the study because they miscarried after the first assessment. Descriptive information for the study sample is shown in Table 1. Out of a total possible 2460 saliva samples (had all of the remaining 82 participants collected complete data at each time point), 1956 valid saliva samples were available for analysis. Approximately half of the missing samples were due to participant attrition; the other half were missing because of insufficient quantity of saliva to conduct the assay (n = 21), PDA failure (n = 14), participant was busy (n = 116), experimenter error (n = 22), illness (n = 5), and other reasons not specified (n = 86).

To estimate adherence to the protocol, self-reported wakeup time was compared to time of the 30 minute post-waking sample recorded on the PDA. If participants had responded immediately to the PDA signal, then all responses would have occurred at 30 minutes post waking. Mean and median response times were 34.4 (SD = 6.7) and 33 minutes respectively, suggesting very good overall adherence. As 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.

The occurrence of teeth brushing and exercising were rare (2.0% and 0.4%, respectively), while consuming food within 30 minutes of sample collection was more common, 11.7%. Preliminary multilevel models suggest a significant increase in cortisol associated with brushing teeth (but not with exercising or eating), however most samples affected were those collected at

30 min post waking leading to the strong probability that the effect of teeth brushing was an artifact of the cortisol awakening response. Analysis conducted with and without the teeth brushing variable yielded identical results and therefore teeth brushing was not included in the analysis.

Descriptive Statistics

Descriptive statistics for study variables are presented in Table 2. As expected, there was diurnal change in cortisol at all time points and increases in cortisol associated with advancing gestational age. Table 2 also suggests diurnal variation in mood that is nonlinear, with highest levels of psychological distress shortly after waking and just before bed. Psychological distress was higher in the 1st and 3rd trimesters compared to the 2nd trimester.

Time-Varying Covariation Between Psychological Distress and Cortisol

As a first step, we modeled the time-varying-covariation between psychological distress and cortisol (Model 1 of Table 3). After adjusting for time of day effects (including the CAR, time since waking, and time since waking squared), psychological distress was positively associated with cortisol, π_{4jk} = .024, 95% confidence interval (CI) = .007 – .040. For each unit increase in psychological distress above a participant's typical level, there was a corresponding 2.4% increase in cortisol.

To assess the amount of unexplained variability in the association between psychological distress and cortisol in Model 1, we included an error term in the level-2 submodel for psychological distress. This parameter estimates the deviations from the average slope for psychological distress. The variance component for psychological distress, ε_{4jk} = .00005, was not significant, χ^2 = 248.13, p >.5, suggesting that all three trimesters share the same average rate of change in cortisol as a function of psychological distress (π_{4jk} in Model 1).

Effects of Gestational Age on the Covariation of Psychological Distress and Cortisol

A direct test of the effect of GA on the psychological distress slope was included in Model 2 (Table 3). In this model, GA was positively associated with waking levels of cortisol, $\beta_{01k} = 0.27$, $p < .001$, 95% CI = .024 – .031, indicating that cortisol levels upon waking (after accounting for the effects of psychological distress) increased with advancing gestation. Effects for GA were also observed for time since waking, $\beta_{21k} = .001$, $p < .05$, 95% CI = .0003 – .002, and time since waking squared, $\beta_{31k} = -.0002$, $p < .05$, 95% CI = -.0016 – -.00003, the combined effect of which is a progressive flattening of the diurnal cortisol pattern over the course of gestation. Together, the effects of GA on the intercept, time, and time squared indicate overall increases in cortisol production over the course of the day as pregnancy progresses. Although we observed a decrease in the CAR over the course of gestation, the effect was not reliable, $\beta_{11k} = -.006$, $p = .17$, 95 CI = -.015 – .003.

Our primary interest, however, was in the slope parameter, $\beta_{41k} = .0009$, $p = .13$, 95% CI = -.0003 – .002, for the effect of GA on the association between cortisol and psychological distress. The data suggest there is insufficient evidence to infer a linear change in the association between psychological distress and cortisol over the course of pregnancy. [3]

Discussion

The purpose of this longitudinal study was to determine whether the association between psychological distress and salivary cortisol is attenuated over the course of pregnancy. The findings suggest that within-person changes in psychological distress are associated with changes in cortisol throughout pregnancy and argue against the notion that psychological experiences become less potent stimuli for the maternal HPA axis as a function of advancing gestation. The prospective design of the current study combined with a multi-level modeling approach to data

analysis represents a strong test of the attenuation hypothesis because they allow direct modeling of the extent to which fluctuations in cortisol from one sampling moment to the next are associated with corresponding changes in psychological distress *within the same woman* (Blackwell et al., 2006), and how this association changes as pregnancy progresses.

The findings are consistent with cross-sectional studies reporting no attenuation of the HPA axis response to psychological distress during pregnancy (de Weerth, Jansen, Vos, Maitimu, & Lentjes, 2007; de Weerth, Wied, Jansen, & Buitelaar, 2007; Giesbrecht et al., 2012; Nierop et al., 2006). Our finding of preserved biobehavioral coherence throughout pregnancy does, however, contrast with other studies demonstrating decreased neuroendocrine response to chemical (Odagiri et al., 1988; Schulte et al., 1990) and combined physical and psychological (Gitau et al., 2001; Kammerer et al., 2002) stimulation in late gestation. It should be noted that these studies assessed HPA axis reactivity to laboratory induction of stress and none of the studies utilized primarily psychological stimuli. Considering the evidence to date, it is possible that there may be a selective dampening of the HPA axis during pregnancy associated with certain categories of stimulation.

The notion that some forms of stimulation may become progressively less potent to the HPA axis during pregnancy is supported by differences in the neural mechanisms by which physical and psychological stimulation activate the HPA axis. Whereas psychological stimulation requires extensive processing that involves rostral cortico-limbic structures, physical stimulation may be processed with little or no conscious effort by more primitive caudal brain regions (Russell et al., 2008). Although it should be emphasized that reciprocal connections between lower and higher brain regions serve to integrate HPA axis responsiveness to stress

(Herman et al., 2003) such differences in the pathways by which stress signals are processed may nevertheless have different trajectories over the course of pregnancy.

Our findings of robust biobehavioural coherence between cortisol and negative psychological experiences throughout pregnancy contrast with reports of attenuated HPA axis responses to stress among animal models which have largely informed our understanding to date (Russell et al., 2008). In rats, for example, HPA axis responses to a range of physical and psychological challenges progressively decrease in the last week of pregnancy (Neumann et al., 1998). Our findings also contrast with outcomes from prospective examinations of ANS responses to stress during human pregnancy (de Weerth & Buitelaar, 2005). For example, Entringer and colleagues (2010) report that heart rate and blood pressure responses to the TSST in a group of pregnant women were lower at 31 compared to 17 weeks of GA. Similar findings for ANS reactivity were reported for the high frequency component of heart rate variability, an index of parasympathetic (vagal) tone, despite the fact that subjective stress was not attenuated even while ANS activity decreased (Klinkenberg et al., 2009). In a study of cardiovascular reactivity that recruited and tested women both prior to and during pregnancy, it was noted that blood pressure responses to mental stress decreased after women became pregnant (Matthews & Rodin, 1992). Unfortunately, that study employed only one assessment during pregnancy (22 weeks GA) precluding evaluation of further attenuation over the course of gestation.

Whereas the evidence supporting attenuation of responses to negative psychological experiences across multiple ANS indicators during pregnancy is strong, the current evidence suggests that our understanding of how and when HPA axis responses to psychological stimulation change requires further elaboration. Taken together, the evidence to date suggests

that there is specific dampening of psychobiological systems rather than global dampening of biobehaviorual responses to negative psychological experiences in pregnant women.

The findings suggest that a modification is required to our understanding of the mechanisms by which the timing of exposure to psychological distress affects fetal development. To the extent that ANS response to negative psychological experience is attenuated, it is reasonable to propose that such attenuation may have a protective effect for the fetus, shielding it from the negative consequences of maternal distress. In light of the current findings, however, the notion that advancing gestation protects the fetus from distress-related increases in maternal cortisol, does not appear plausible. Instead, it is possible that timing of exposure has differential effects on developmental outcomes as a function of period-specific vulnerability of biological systems (e.g., during rapid development; Kapoor et al., 2006; Michael & Papageorghiou, 2008), and/or because maternal experiences of psychological distress in early pregnancy may program placental cortisol metabolism, modulating subsequent exposure of the fetus to active glucocorticoids (Jaquiery et al., 2006).

The findings may also have implications for clinical practice with obstetric patients. Women's mental health is increasingly becoming a focus of public health policy and clinical practice but the focus of these efforts, in the main, has been on postpartum depression and anxiety. The results of this and similar studies (e.g., Giesbrecht et al., 2012; Nierop et al., 2006) suggest that a more inclusive perspective is needed that addresses women's mental health needs both during and following pregnancy.

Repeated prospective assessment of cortisol and ecologically relevant psychological distress in all three trimesters of pregnancy is a unique and significant strength of this study. This study highlights the importance of within-person approaches to the association between

psychological distress and cortisol and replicates a previous cross-sectional study demonstrating biobehavioural coherence during pregnancy (Giesbrecht et al., 2012). Studies reporting a dissociation between cortisol and psychological distress during pregnancy often suffer from methodological limitations (e.g., single time measures) or analytic limitations (e.g., individual difference analyses). Nevertheless, several limitations of the current study require consideration. First, the ambulatory assessments we conducted do not provide a direct comparison to laboratory studies of HPA axis reactivity. Although we assume that observed changes in cortisol represent HPA axis responsiveness to salient negative psychological experiences (and this assumption is supported by experimental studies suggesting that distress causes increases in cortisol (Schmidt, Fox, Goldberg, Smith, & Schulkin, 1999; Wolkowitz et al., 1990) the nature of the data limits our interpretation of the findings to the issue of biobehavioural coherence rather than stress reactivity. Second, peak HPA-axis response to stimulation typically lags acute onset of a stress by approximately 20 min, although clear changes in cortisol are evident within 10 min (Kirschbaum & Hellhammer, 1994). We addressed this issue by asking participants to report on their psychological distress over the past 30 min. To the degree that psychological distress may have changed dramatically during the 30 min immediately prior to momentary assessment this design may have underestimated the true extent of biobehavioural coherence between psychological distress and cortisol. Finally, we note that the sample represents a relatively stable population of highly educated, employed, mature, and White women. It is not known how these results might change in a sample of women with clinically relevant psychopathology or women with significant social or economic disadvantage.

In summary, this study prospectively examined biobehavioural coherence between cortisol and ecologically relevant psychological distress during pregnancy. The data do not

support the claim that the association between psychological distress and cortisol is progressively attenuated with advancing gestation. It seems unlikely, in light of these findings, that pregnancy-related changes in the maternal HPA axis confer a protective advantage to the mother or fetus or that advancing gestation results in a reduced cortisol ‘dose’ transduced to the fetus as a result of psychological distress. Further research focusing on period-specific vulnerability of biological systems and the effects of psychological distress on the placenta is needed to understanding how and when psychological distress can alter fetal development.

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Footnotes

[1] Although the data has four levels (moments within days, within trimesters, within persons) for simplicity we use a 3-level structure because the findings of the 4-level model were substantially the same as those reported here.

[2] Only samples that were collected 20 - 59 min after waking were used to model the 30 minute post waking rise (Okun et al., 2010).

[3] As the addition of GA in Model 2 only tested for linear change over the course of pregnancy, an exploratory analysis was conducted that also included GA squared in the level 2 submodel to allow for the possibility that the association between psychological distress and cortisol may be curvilinear. The effect for GA squared was not reliable, $\beta = .00013$, 95% CI = -.0002 – .0002. Together, the findings for GA indicate no linear or non-linear change for the association between psychological distress and cortisol over the course of pregnancy.

Highlights

- We assess changes in the association between psychological distress and cortisol over the course of pregnancy.
- Psychological distress was associated with cortisol throughout pregnancy.
- Psychological experiences remain potent stimuli for the HPA axis during pregnancy.
- Advancing gestation does not protect the mother or fetus from the psychobiological effects of distress.

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Table Legends

Table 1. Participant Characteristics (n = 85)

Table 2. Descriptive Statistics for Study Variables by Time of Day and Trimester

Table 3. Multilevel Models for Covariation between Psychological Distress and Cortisol (Model 1), Effects of Gestational Age on the Covariation between Psychological Distress and Cortisol (Model 2)

Table 1
Participant Characteristics (n = 85)

	Mean	S.D.	Range
Age (years at T1)	31.7	3.8	23.1-40.6
Gravida	2.1	1.13	1 – 6
	Percentage		
Nulliparous	34.9		
Married or common law	98.8		
Education			
university degree (or higher)	70.6		
high school diploma (or less)	9.4		
Annual household income			
more than $100,000/yr	62.4		
$70,001 to 100,000	23.5		
$40, 000 to $70,000	8.2		
less than $40,000/yr	5.9		
Employment (at T1)			
Working full time	61.5		
Working part time	21.8		
Not working	16.7		
Ethnicity			
White	91.7		
Asian	1.2		
Hispanic	2.4		
African North American	1.2		
Other	2.4		

Table 2. Descriptive Statistics for Study Variables by Time of Day and Trimester

	1st Trimester			2nd Trimester			3rd Trimester		
Variables	**Mean**	**S.D.**	**Range**	**Mean**	**S.D.**	**Range**	**Mean**	**S.D.**	**Range**
Raw Cortisol (ug/dL)									
Waking	.40	.16	.09-1.0	.51	.18	.14-1.16	.49	.18	.09-1.12
Waking + 30 minutes	.49	.20	.12-1.20	.58	.25	.18-1.54	.62	.22	.28-1.43
Mid morning	.18	.10	.07-.81	.22	.10	.03-.81	.32	.18	.14-1.64
Mid afternoon	.13	.07	.04-.43	.16	.07	.06-.56	.22	.09	.07-.71
Evening	.08	.05	.02-.45	.08	.04	.02-.27	.13	.06	.06-.42
Psychological Distress									
Waking + 30 minutes	3.36	2.00	-.33-11.40	2.95	1.84	-.13-9.53	3.46	2.20	-.47-11.47
Mid morning	2.94	2.04	-.67-10.80	2.64	1.95	-.33-11.93	3.35	2.17	-.33-11.13
Mid afternoon	3.28	1.99	-.33-10.07	3.05	1.91	0-9.27	3.28	2.22	-.33-9.40
Evening	3.58	2.19	-1.00-12.53	3.30	1.96	0-10.80	3.94	2.14	0-10.93
Demographic									
Gestational Age	12.9	2.7	5-15.9	19.3	1.2	16.1-24.6	32.4	.92	30.1-36.0

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

Table 3

Multilevel Models for Covariation between Psychological Distress and Cortisol (Model 1), Effects of Gestational Age on the Covariation between Psychological Distress and Cortisol (Model 2)

	Model 1			Model 2		
Fixed Effects	Estimate	*SE*	*p*	Estimate	*SE*	*p*
Momentary (level 1) Effects						
WAKING Levels (π_{0jk})	-1.64	.026	<.001	-1.62	.025	<.001
CAR (π_{1jk})	.32	.049	<.001	.32	.047	<.001
TIME (π_{2jk})	-.12	.006	<.001	-.12	.005	<.001
$TIME^2$ (π_{3jk})	.002	.0007	.001	.002	.0007	.004
PSYCHOLOGICAL DISTRESS (π_{4jk})	.024	.008	.006	.024	.006	<.001
Gestation Age (level 2) Effects						
For WAKING Levels (β_{01k})				.027	.002	<.001
For CAR (β_{11k})				-.006	.005	.17
For TIME (β_{21k})				.001	.0005	.02
For $TIME^2$ (β_{31k})				-.0002	.00007	.02
For PSYCHOLOGICAL DISTRESS (β_{41k})				.0009	.0006	.13

Note: Estimates are presented for the final most parsimonious models. Words in capital letters indicate time-varying (Level 1) variables. CAR = cortisol awakening response; SE = standard error.