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journal article

Adverse Childhood Experiences and HPA Axis Function in Pregnant Women

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Adverse childhood experiences (ACEs), such as abuse, neglect and family dysfunction, have devastating potential to influence physical and mental health outcomes throughout the lifespan (Duncan, Yeung, Brooks-Gunn, & Smith, 1998; Wegman & Stetler, 2009). There is also growing research interest in the intergenerational transmission of maternal ACEs to offspring, and specifically how maternal ACEs can increase the risk for poor physical, behavioural, and cognitive outcomes in the offspring (Bowers & Yehuda, 2016). Nevertheless, the specific mechanisms by which parental ACEs exposure is transduced to the offspring have yet to be fully elucidated. The objective of the current study was to examine maternal hypothalamic-pituitary-adrenal (HPA) axis function during pregnancy as a potential mechanism by which maternal early life experiences are transduced to offspring development.

Seminal work by Felitti and colleagues (1998) examining the associations between ACEs and later life health status and behaviors in over 9,000 American adults demonstrated that the prevalence of ACEs in the general population was high, with more than half of the respondents reporting at least one ACE and 6.2% of respondents indicating experiencing 4 or more ACEs. Importantly, this high prevalence rate was present despite the fact that the sample had a low number of sociodemographic risk factors such as being primarily White (80%) and educated (75% of participants reported some post-secondary education). Furthermore, this study found that those individuals reporting 4 or more ACEs demonstrated substantial increases in risk for many of the leading causes of death in adults. These individuals had 4- to 12-fold increases in risk for alcoholism, drug abuse, depression, and suicide attempt, 2- to 4-fold increases in risk for smoking, poor self-rated health, and sexually transmitted disease, and 1.4 to 1.6-fold increases in risk of physical inactivity and severe obesity, compared to those who had no ACEs exposure. Numerous studies since then have confirmed these findings and demonstrated that exposure to

ACEs is associated with increased risk for leading causes of death in adults including heart disease (Dong et al., 2004; Edwards, Holden, Felitti, & Anda, 2003), cancer (Brown, Thacker, & Cohen, 2013; Edwards et al., 2003), and ultimately, reduced life expectancy (Brown et al., 2009). Importantly, contemporary studies have also thoroughly supported the relationship between ACEs exposure and poor mental health outcomes including depression, anxiety, psychosis, as evidenced by a number of recent meta-analyses which examined literature published between 1990-2014 (Li, D'Arcy, & Meng, 2016; Lindert et al., 2014; Varese et al., 2012). Li and colleagues (2016) also calculated population attributable fractions (PAFs), which indicated that over half of global depression and anxiety cases are potentially attributable to childhood maltreatment. This finding was further supported by an investigation conducted by the World Health Organization (WHO), which examined 5,149 individuals in 21 countries and found ACEs to be the strongest predictors of mental health disorders, accounting for approximately 29.8% of cases worldwide (Kessler et al., 2010).

The intergenerational transmission of the health consequences attributed to ACEs exposure has also been observed, indicating that parental ACEs can affect health and development outcomes of the next generation. For instance, adult offspring of Holocaust survivors are at greater risk for depression and anxiety disorders (Yehuda, Bell, Bierer, & Schmeidler, 2008), and poor physical health outcomes, such as hypertension and Type 2 diabetes (Flory, Bierer, & Yehuda, 2011). Children born to mothers with a history of child abuse have also been demonstrated to have an increased risk of asthma and allergy (Tomfohr-Madsen, Bayrampour, & Tough, 2016). Although specific biological pathways by which parental ACEs become embedded in offspring development have been proposed (Buss et al., 2017; Madigan, Wade, Plamondon, Maguire, & Jenkins, 2017; Racine, Plamondon, Madigan, McDonald, &

Tough, 2018; Yehuda, Engel, et al., 2005), until recently the prevailing paradigm suggested that the intergenerational transmission of maternal adversity likely occurs after birth through environmental pathways, such as low quality caregiving, characterized by low sensitivity and responsiveness (Center on the Developing Child at Harvard University, 2010; Lang, Gartstein, Rodgers, & Lebeck, 2010; Plant, Barker, Waters, Pawlby, & Pariante, 2013; Rijlaarsdam et al., 2014). Despite considerable evidence supporting such mother-to-child transmission in the postnatal period, the developmental origins hypothesis suggests that fundamental developmental processes during gestation may also be involved (Buss et al., 2017).

The developmental origins hypothesis postulates that developmental adaptations instigated by early life adversity confer later risk for health and disease (Barker, 1998). More specifically, chronic or repeated exposure to stress, particularly during sensitive periods of development, can alter brain development with potential for long term consequences for cognition and mental health (Lupien, McEwen, Gunnar, & Heim, 2009). Whereas the majority of developmental origins research focuses on maternal stress experienced *during* gestation, and especially how such stress experiences affect maternal HPA axis function (Giesbrecht, Campbell, Letourneau, Kooistra, & Kaplan, 2012), little is known about the potential mechanisms by which maternal stress *prior* to pregnancy may affect offspring development. To the extent that early life adversity may result in dysregulation of the maternal HPA axis during pregnancy, these early experiences may have multigenerational effects through HPA axis signaling from mother to fetus. If an association exists between maternal ACEs and HPA axis function during pregnancy, it would support the hypothesis that change in HPA axis function may be a mechanism by which a history of adverse childhood experiences is transmitted to a second generation.

To our knowledge, associations between ACEs and HPA axis function during pregnancy have previously been assessed by only two research groups (i.e., Bublitz & Stroud, 2012; Shea et al., 2007). The first study evaluated 66 women in late pregnancy and demonstrated that greater early life adversity, as assessed by the Childhood Trauma Scale, was associated with reduced baseline waking cortisol levels (explaining 12% of the variance), but not with the cortisol awakening response (CAR) (Shea et al., 2007) which refers to the rapid increase in cortisol levels that occurs thirty minutes after waking (Fries, Dettenborn, & Kirschbaum, 2009). Although recent stressful life events were assessed, they were not associated with maternal cortisol and were not included in the model to determine whether the association held after adjusting for more proximal stressors (e.g., recent stressful life events).

Bublitz and colleagues have published three papers assessing associations between experiences of childhood sexual abuse and HPA axis function during pregnancy based on a sample of women enrolled in the Behavior and Mood in Mothers, Behavior and Infants (BAMBI) study. In a study of 135 women aged 18-40, Bublitz and Stroud (2012) demonstrated that women who reported experiencing childhood sexual abuse (n = 30), displayed increasing CARs over the second and third trimesters of pregnancy, compared to women with other abuse histories (n = 58) or no such history (n = 47). The remaining two studies evaluated the effects of current life stressors on the association between experiences of childhood sexual abuse and HPA axis function during pregnancy. In a pilot study (n = 41), Bublitz and Stroud reported that stress experienced on the day prior was associated with increased current day cortisol levels at 30 minutes after awakening, and same day stress was associated with increased evening cortisol levels among women with a history of childhood sexual abuse, compared to women with non-sexual abuse or no abuse history (Bublitz & Stroud, 2013). In a follow-up longitudinal study

with a larger sample (n = 185), Bublitz and colleagues (2014) demonstrated interactions between current stressors and HPA axis function. Specifically, they reported that poorer self-perceived current family functioning moderated the association between childhood sexual abuse and the CAR such that pregnant women with more severe histories of childhood sexual abuse and poorer family functioning had increasing CARs with advancing gestation (between 25 and 35 weeks). In sum, previous studies of pregnant women have demonstrated that early life adversity (e.g., trauma and sexual abuse) is associated with lower waking levels of cortisol, increased CAR with advancing gestation, and that proximal stressors interact with childhood adversity to predict increased CAR.

A larger body of work examining ACEs and diurnal HPA axis function in non-pregnant adults confirms the potential for long-term changes in the HPA axis, but has otherwise not produced a consistent set of results. Inconsistencies in the literature may exist because contextual factors such as current psychopathology or the type of early life adversity experienced may also influence current HPA axis function (Gonzalez, 2013). For instance, previous studies have demonstrated that physical abuse (Cicchetti & Rogosch, 2001) and physical neglect (Bruce, Fisher, Pears, & Levine, 2009) were associated with lower morning cortisol levels, whereas emotional abuse was associated with elevated morning cortisol levels (Bruce et al., 2009). Longitudinal studies show that changes in HPA axis function in individuals with ACEs are maintained over time and may increase in divergence from HPA axis function in individuals without ACEs exposure (Trickett, Noll, Susman, Shenk, & Putnam, 2010; van der Vegt, van der Ende, Kirschbaum, Verhulst, & Tiemeier, 2009). For instance, Yehuda and colleagues (2005) demonstrated that geriatric Holocaust survivors with posttraumatic stress disorder had a blunted diurnal pattern of cortisol with lower levels at awakening and at bedtime. Together, these

findings suggest that the effects of ACEs on HPA axis function may depend to some extent on the nature of the adversity, and that the physiological adaptations in response to ACEs may leave an enduring signature on the HPA axis into adulthood.

Special consideration has been given to HPA axis function during pregnancy, not only because cortisol, the end product of the HPA axis, crosses the placenta into fetal circulation (Gitau, Cameron, Fisk, & Glover, 1998), but also because there are significant pregnancy-related changes in the HPA axis. The placenta secretes large quantities of corticotropin-releasing hormone (CRH) (Duthie & Reynolds, 2013; Reis, Fadalti, Florio, & Petraglia, 1999), which stimulates the maternal HPA axis to secrete more cortisol, resulting in a two to fourfold increase in circulating cortisol by the end of the third trimester (Jung et al., 2011). These overall increases in circulating cortisol are essential to facilitate fetal development in preparation for birth (e.g., maturation of fetal lungs; Garbrecht, Klein, Schmidt, & Snyder, 2006). Despite these pregnancy-related changes, the diurnal cortisol pattern, including the CAR and the diurnal slope, which is characterized by a gradual decline in cortisol levels throughout the day (Fries et al., 2009), are largely preserved during pregnancy (Entringer et al., 2010). Nevertheless, individual differences in the size of the CAR, the steepness of the diurnal slope and the overall amount of cortisol increase during pregnancy are associated with 'programming' of the fetal HPA axis (Davis, Glynn, Waffarn, & Sandman, 2011; Giesbrecht, Letourneau, & Campbell, 2017). One previous study also demonstrated that maternal exposure to childhood trauma was associated with altered placental-fetal stress physiology in utero, as evidenced by significant increases in placental CRH secretion towards the end of gestation (Moog et al., 2016). As such, variation in prenatal stress physiology is a putative mechanism by which factors affecting the maternal HPA axis may alter child development outcomes.

The existing literature examining the association between ACEs and HPA axis function during pregnancy has several limitations that impede clear understanding of the mechanisms by which maternal ACEs may confer developmental and health risks in a second generation. First, the samples sizes have been small, limiting the ability to detect more subtle effects and to include multiple covariates or interaction terms that would help to specify the unique and synergistic effects of ACEs on HPA axis function during pregnancy. Second, studies have been limited to the second half of gestation. Data from the first trimester is especially important because the fetal HPA axis is not functional until about mid gestation, meaning that the growth and development of the fetus in the first half of pregnancy is regulated primarily by exposure to maternal/placental cortisol (Kota et al., 2013). Furthermore, previous studies assessing pregnancy-related changes in HPA axis function have been limited to examining only half of pregnancy, and specifically the latter half during which the placenta plays an increasingly important role in regulating maternal HPA axis function (Mastorakos & Ilias, 2003). Third, previous studies have focused on discrete forms of ACEs, such as childhood sexual abuse, which provide only a partial picture of adversity. Although it is informative to determine whether individual forms of adversity are associated with maternal HPA axis function, it is also important to understand how the total experience of adversity may be related to HPA axis function. This is especially important in light of the fact that individuals who experience adversity tend to experience multiple forms of adversity (Dube, Anda, Felitti, Edwards, & Williamson, 2002). Finally, despite the inclusion of daily stress and recent life events in some previous studies, it is not clear whether the associations between ACEs and maternal HPA axis function are unique and independent of more proximal stressors. This is an important consideration because it is necessary to rule out the possibility that individuals with ACEs exposure also experience more stress during pregnancy

and that any effects on the HPA axis may be more reasonably attributed to these more proximal stressors rather than more distal early life stressors. In addition, it may be the case that ACEs potentiate the effects of more proximal stressors such that those with ACEs exposures have relatively larger associations between more proximal stressors and HPA axis function compared to individuals without such exposure. The current study seeks to address these gaps in the literature to determine the potential role of the maternal HPA axis as a mechanism transducing the effects of early life adversity to fetal development.

The objective of the current study was to overcome limitations of previous studies in determining whether associations between maternal ACEs and HPA axis function during pregnancy are a plausible biological mechanism by which maternal ACEs may be transduced to offspring. In order to explore the association between exposure to ACEs and alterations in HPA axis function during pregnancy two primary aims were addressed. ***Primary aim 1*** was to determine whether ACEs are associated with changes in the *diurnal* cortisol pattern during pregnancy. Here we aggregated all samples taken during pregnancy in cross-sectional analyses to gain insight into the overall association. Similar to non-pregnant women with exposure to ACEs, we expected that pregnant women with exposure to ACEs would exhibit higher levels of cortisol at +30 minutes after waking (Bublitz & Stroud, 2012; Weissbecker, Floyd, Dedert, Salmon, & Sephton, 2006) and flatter diurnal slopes (Gonzalez, 2013; van der Vegt et al., 2009). ***Primary aim 2*** was to determine whether ACEs are associated with *longitudinal* pregnancy-related changes in HPA axis function over the course of pregnancy; that is, are ACEs associated with the way that the HPA axis adjusts to pregnancy. Based upon previous studies, we expected that women with exposure to ACEs would exhibit increasing CARs over the course of pregnancy (Bublitz & Stroud, 2012), and flatter daytime slopes (Gonzalez, 2013; van der Vegt et al., 2009).

The ***secondary aims*** were to clarify the unique and/or synergistic effects of ACEs and more proximal life stressors on the maternal HPA axis. Again, based on previous studies, we expected that ACEs would be associated with HPA axis function even after adjusting for more proximal stressors, and that exposure to ACEs would accentuate the effects of more proximal stressors on the maternal HPA axis. Finally, an ***exploratory aim*** was to determine the relative strengths of the associations between individual components of early life adversity (i.e., abuse, neglect, household challenges) and HPA axis function during pregnancy.

Material and Methods

Participants

Participants were 356 women enrolled in an ongoing prospective cohort study, the Alberta Pregnancy Outcomes and Nutrition (APrON) study, (see Kaplan et al., 2012 for study details) which is a community sample recruited from prenatal clinics between 2009 and 2012. Women were included if they had a singleton pregnancy, were less than 22 weeks of gestation at the first study visit, and were 18 years of age or older. Women were excluded if they smoked or consumed alcohol during pregnancy, or were being treated with a synthetic glucocorticoid. The study time points were as follows: Time 1 (T1; 0-13 weeks gestation, mean = 11.4), Time 2 (T2; 14-27 weeks gestation, mean = 17.1), and Time 3 (T3; 28-40 weeks gestation, mean = 32.4). The full range of gestational ages included in the current study sample is 5.9-36.7 weeks.

Descriptive information for the study sample and infant birth data are displayed in Table 1. The study sample represents a relatively low sociodemographic risk population: women were mature (mean age 31.8 years, range = 21 - 43), and the majority were married or in common-law relationships, White, had university-level education, and had annual household income above $100,000.

Procedure

Maternal history of ACEs was assessed using a retrospective self-report measure of adverse experiences prior to the age of 18. Prenatal maternal cortisol was assessed from saliva samples self-collected by mothers over multiple days within each time point, (excluding weekends to rule out potential weekend-weekday difference in stress and diurnal cortisol (Schlotz, Hellhammer, Schulz, & Stone, 2004)). Participants collected saliva samples on 2 consecutive days at T1, T2, and T3. During the first study visit, participants were instructed on the use of a personal digital assistant (PDA), which was used to facilitate saliva collection at waking, 30 minutes after waking, 1100h, and 2100h. To facilitate adherence to the study protocol, the PDA was programmed to allow a 20-min response window following the initial signal, after which the reminder was no longer available to the participant. Participants also kept a paper diary of sample collection and any discrepancies between the PDA and paper diary were resolved by contacting the participant. 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. 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.

Measures

Maternal History of ACEs. The Adverse Childhood Experiences questionnaire consists of 10 yes or no questions (Felitti et al., 1998) that assesses early life adversity in three domains: ***abuse*** (emotional, physical, and sexual), ***neglect*** (physical and emotional), and ***household challenges*** (mother treated violently, substance abuse, mental illness, parental separation/divorce, and incarcerated household member). The ACEs questionnaire is a widely

used measure that has demonstrated good reliability and internal consistency ($\alpha = .81$) (Bruskas & Tessin, 2013) as well as adequate test-retest reliability (weighted $\kappa = .64$) (Dube, Williamson, Thompson, Felitti, & Anda, 2004). In the current study, the occurrence of individual ACEs was summed to create the ACE scores (range: 0-9). Because scores of 4 or more occurred infrequently, and in keeping with previous methods (e.g., Anda et al., 2006), scores above 4 were recoded to a value of 4 so that the final ACEs scores ranged from 0-4.

Prenatal Maternal Cortisol. Maternal cortisol was collected using whole saliva obtained from under the tongue with the Salimetrics Oral Swab (Salimetrics, State College, PA). After self-collection, saliva samples were stored in home freezers until they could be shipped on freezer packs to the lab, where they were stored at -80^{o}C until they were shipped frozen to Salimetrics, State College, PA, for assay. All samples were assayed for salivary cortisol using an enzyme immunoassay that has a lower limit of sensitivity of 0.007 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 serial dilution were 100.8% and 91.7%. A random 25% of samples was assayed in duplicate to confirm reliability; the intra-assay coefficient of variation and correlation coefficient between the duplicate tests were 4.20% and $r = .99$, $p < .001$, respectively. For samples that were assayed in duplicate, the mean value was used for analysis.

Covariates

To control for factors that have previously been shown to be related to both HPA axis function and ACEs, sociodemographic characteristics (i.e., SES, parity, and maternal age) were included as covariates in all models (Cohen, Doyle, & Baum, 2006; Kivlighan, DiPietro, Costigan, & Laudenslager, 2008; Madigan, Wade, Tarabulsy, Jenkins, & Shouldice, 2014;

Metzler, Merrick, Klevens, Ports, & Ford, 2017). Additionally, recent life stressors and psychological distress were assessed to determine whether ACEs accounted for unique variance after adjusting for these more proximal stressors.

SES. A composite SES variable was created by summing z-scores of self-reported family income (1 = <$20,000/year, 2 = $20,000-$39,999, 3 = $40,000-$69,000, 4 = $70,000-$99,999, 5 = ≥$100,000), maternal education (1 = Less than high school, 2 = High school, 3 = Trade or technical school, 4 = Undergraduate, 5 = Graduate degree), and ethnicity (0 = Non-white, 1 = White). The distribution of the composite SES scores for the study sample were negative skewed, thus the scores were reflected and log transformed, as recommended (Tabachnick & Fidell, 2012). Final composite SES scores were then mean centered with higher values indicating higher sociodemographic risk (i.e., lower annual income, less education, non-white).

Stressful Life Events. The Stressful Life Events Questionnaire (SLEQ) is a measure of exposure to stressful life events, adapted from (Barnett, Hanna, & Parker, 1983); we used a 7-item yes or no questionnaire designed for use with pregnant women (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007). Scores on this measure ranged from 0 to 7, with higher scores indicating exposure to more stressful life events. Predictive 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 during pregnancy at each study visit, and the perceived impact of the events.

Maternal Psychological Distress. Maternal psychological distress during pregnancy was evaluated by combining measures of maternal depression and anxiety obtained at each study visit. Maternal depressive symptoms were assessed using the Edinburgh Depression Scale (EDS;

Bergink et al., 2011), a 10-item self-report measure designed to identify depression in the perinatal period. This instrument has demonstrated excellent reliability and validity among pregnant and postpartum women (Bergink et al., 2011; Cox, Holden, & Sagovsky, 1987; Jomeen & Martin, 2007). In addition, maternal symptoms of anxiety were assessed using the 10-item anxiety scale from the Symptom Checklist-90 Revised (SCL-90-R; Derogatis, 1992), a multidimensional self-report measure designed to evaluate a broad range of symptoms of psychopathology. The anxiety scale has demonstrated good convergent and divergent validity (Morgan, Wiederman, & Magnus, 1998) and adequately discriminates between clinical and non-clinical samples (Bonicatto, Dew, Soria, & Seghezzo, 1997; Holi, Sammallahti, & Aalberg, 1998). Scores from the two instruments were z-score transformed and summed to create a composite measure of psychological distress.

Data Analytic Plan

Primary Aim 1: Cross-sectional diurnal cortisol pattern during pregnancy. Multilevel modeling (MLM) was used to determine whether diurnal cortisol patterns during pregnancy were related to a mother's history of ACEs. MLM allows for individual differences in the timing of samples and therefore does not require that all individuals complete assessments on the same schedule. One of the primary advantages of MLM is that it uses all available data to calculate slopes and intercepts, even in the presence of missing data, which are inevitable with intensive longitudinal research designs. Additionally, MLM weights each case according to the amount of data that is present. Multilevel equations were specified at two levels to account for the nested data structure (measurement moments nested within persons). In the Aim 1 models, level of cortisol for each person at each moment was the outcome. Time was parameterized as both time since waking (in hours) as well as time since waking squared to model the curvilinear shape of

the diurnal cortisol slope over the course of the day. Every available cortisol data point for each individual (i.e., up to 8 samples at each sampling time point) was utilized and aggregated in these models to estimate the average diurnal pattern of cortisol during pregnancy. Missing data were estimated using full information maximum likelihood. Gestational age (GA) was included in the model to account for the fact that cortisol increases with advancing gestation. For Aim 1, the final model was as follows:

Level 1: $\text{logCortisol}_{ij} = \pi_{0ij} + \pi_{1i}{}^{+}30\text{min}_{ij} + \pi_{2i}\text{TSW}_{ij} + \pi_{3i}\text{TSW}^2_{ij} + \pi_{4i}\text{GA}_{ij} + \sigma_{ij}$

Level 2: π_{0ij} through $\pi_{3ij} = \beta_{00} + \beta_{01}\text{ACEs}_i + \beta_{02}\text{Covariates}_i + \varepsilon_{0j}$

where $^{+}$30min is a dummy variable that takes a value of 1 for samples taken 30 minutes after waking[1] (all others = 0), TSW is linear time since waking and was the parameter used to estimate the linear diurnal slope, TSW^2 is time since waking squared and was the parameter used to estimate the quadratic diurnal slope, GA refers to gestational age (weeks) at time of sample collection (centered at the sample mean of 22.6 weeks), ACEs refers to scores on the ACEs questionnaire, and Covariates refers to the covariates included in the model. Because cortisol was natural log transformed, interpretation of the beta coefficients is facilitated by expressing each coefficient as a percent change using the formula $\beta\%\text{ change} = (e^{\beta} - 1) \times 100$. The percent change can be thought of as an effect size because it refers to the size of the change in maternal cortisol for each 1-unit change in ACEs (holding all other variables constant).

Secondary Aim 1. To determine whether associations between ACEs and the cross-sectional diurnal cortisol pattern remained after including more proximal stressors, the model in Aim 1 was re-run adding stressful life events occurring during pregnancy and maternal psychological distress as covariates. Similarly, to determine whether ACEs exposure and

[1] Samples were accepted as valid if they were collected between 20 and 50 minutes after waking (see Okun et al., 2010).

proximal stressors may have synergistic effects on diurnal cortisol patterns, the models in Aims 1 were re-run adding interaction terms between ACEs and proximal stressors.

Primary Aim 2: Longitudinal pregnancy-related changes in HPA axis function with advancing gestation. Data analyses focused on associations between maternal ACEs and *changes* in HPA axis function (i.e., the cortisol awakening response, total cortisol secretion, and daytime slope) over the course of pregnancy. To conduct these analyses, we first calculated HPA axis function parameters from the multiple cortisol samples collected from each person. The ***cortisol awakening response*** (CAR) was calculated using the trapezoid method for area under the curve increase (AUCi) as a measure of the morning increase in cortisol (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Because the area under the curve increase is dependent on the amount of time between baseline and the $^{+}$30 minute sample, CAR was standardized to reflect 30 minutes of output in order to account for individual differences in the time between the waking and waking +30 minute samples. ***Total cortisol secretion*** over the day was estimated as area under the curve with respect to ground (AUCg) (Pruessner et al., 2003). As with the AUCi, AUCg is sensitive to the total amount of time between the waking and bedtime samples, and because individuals had different amounts of time between these samples, AUCg was standardized to the average time between waking and 2100h samples (853 min).[2] The ***daytime slope*** was calculated as 2100h – waking / time (in hours) to describe the decline in cortisol concentration over the day (Fekedulegn et al., 2007). Values from the 2 days of sampling at each time point were averaged to create a single estimate for the CAR, AUCg, and daytime slope in each trimester.

[2] Note that the correction factor for both the AUCi and AUCg were applied after using their actual sample times to calculate their AUCi and AUCg values.

Multilevel equations were specified at two levels to account for the nested data structure (time point nested within persons). In the models for Aim 2, CAR, AUCg or the daytime slope (assessed in separate models) for each person in each trimester was the outcome. Time was parameterized as gestational age (weeks). For Aim 2, the final model was as follows:

Level 1: $\text{CAR/AUCg/Slope}_{ij} = \pi_{0ij} + \pi_{1i}\text{GA}_{ij} + \sigma_{ij}$

Level 2: π_{0ij} through $\pi_{1ij} = \beta_{00} + \beta_{01}\text{ACEs}_i + \beta_{02}\text{Covariates}_i + \varepsilon_{0j}$

where CAR/AUCg/Slope refer to aspects of HPA axis function (calculated as above and modeled individually), GA refers to gestational age (weeks) at time of sample collection (centered at 6 weeks gestation to represent the earliest time point in pregnancy for which we had data), and Covariates refers to covariates included in the models.

Secondary Aim 2. As for Secondary Aim 1, models in Aim 2 were rerun with proximal stressors to determine unique and potential synergistic effects.

Exploratory Aim. Post-hoc analyses were conducted for all significant models in Aim 1 and 2 to determine which subcomponents of ACEs (i.e., Abuse, Neglect, and Household Challenges) may be driving the observed associations.

Results

Descriptive Statistics

Descriptive statistics for the primary study variables are presented in Table 2. Of note, approximately 43.8% of participants reported exposure to at least 1 ACE and 8.7% reported experiencing ≥4 ACEs. Some salivary cortisol data were missing: 115 women collected samples during the first trimester, 279 women collected samples during the second trimester, and 308 women collected samples in the third trimester. The first time point sample size is lower due to difficulty enrolling women this early in pregnancy. The majority of women provided data at 2 or

more time points during pregnancy (n = 286), while 70 women provided data at only 1 time point.

Primary Aim 1: Cross-sectional diurnal cortisol pattern during pregnancy

Model 1.1 (see Table 3) revealed that ACEs were not associated with waking cortisol levels, β = -.003, p = .48, but were associated with increased $^{+}$30 minute levels, β = .008, p = .003, with a flatter linear diurnal slope, β = .002, p = .050, and but was not associated with the quadratic diurnal slope, β = -.0001, p = .067. These findings indicate that for each additional ACE exposure, the cortisol concentration of the $^{+}$30 minute sample increased by approximately 1%, and the linear diurnal slope became approximately 0.2% flatter per hour. Compared to women with no exposure to ACEs, women with ≥4 ACEs had a 4% increase in cortisol concentration at +30 min post waking and a 13% flatter diurnal slope over the course of the day. The findings in Model 1.1 are illustrated in Figure 1.

Secondary Aim 1. Maternal ACEs were associated with psychological distress during pregnancy, r(354) = .218, p <.001, but not with stressful life events occurring during pregnancy, r(354) = .101, p = .060. The addition of proximal stressors (Table 3 Model 1.2) reduced the association between ACEs and the linear diurnal slope such that it became non-significant, β = .001, p = .074; however, the association between ACEs and the $^{+}$30 minute sample remained, β = .009, p = .001. These results indicate a unique association for ACEs with the $^{+}$30 minute sample but not the linear diurnal slope. Of note, maternal psychological distress during pregnancy also had a unique association with the $^{+}$30 minute sample, β = -.010, p = .036, such that increased distress was associated with reduced cortisol levels at +30 minutes post waking regardless of ACEs exposure. No significant interactions were observed between ACEs and proximal stressors

(Table 3 Model 1.3), indicating that the association between ACEs and diurnal cortisol patterns is not moderated by exposure to proximal stressors.

Primary Aim 2: Longitudinal pregnancy-related changes in HPA axis function with advancing gestation

Aim 2.1: CAR. The model for the CAR (Table 4 Model 2.1) revealed that ACEs were associated with an increased CAR in early pregnancy (at the intercept), β = .672, p = .001, but not with changes in the CAR with advancing gestation, β = -.016, p = .106. The mean estimated CAR for a woman with no exposure to ACEs was 1.91μg/dL whereas the mean estimated CAR for a woman with exposure to ≥4 ACEs was 4.71 μg/dL, a 2.5-fold increase.

Secondary Aim 2.1. The addition of proximal stressors did not alter the associations between ACEs and CAR (Table 5 Model 2.2) indicating a unique association for ACEs over and above the effects of proximal stressors. There were no interactions between ACEs and proximal stressors, indicating that the association between ACEs and the CAR is not moderated by exposure to proximal stressors (Table 5 Model 2.3).

Aim 2.2: Daytime slope. The model for the daytime slope (Table 4 Model 3.1) indicated an overall negative slope, β = -.020, p < .001, as expected, and a non-significant flattening of the daytime slope as a function of advancing gestation, β = .0001, p = .077. ACEs were not associated with the intercept, which in this case represents the daytime slopes at 6 weeks GA, β = .0007, p = .143. However, there was a significant association between ACEs and the daytime slope as a function of gestational age, indicating that exposure to ACEs reverses the tendency toward flattening as pregnancy progresses (i.e., the daytime slope became more negative with advancing gestation), β = -.00005, p = .043 (Figure 2). Relative to the beginning of pregnancy (i.e., 6 weeks GA), women with no ACEs exposure had a 10% flattening of the daytime slope at

40 weeks GA (note however this is non-significant, as shown above) whereas women with ≥4 ACES had a 17% steeper daytime slope at 40 weeks GA.

Secondary Aim 2.2. With the addition of proximal stressors, the association between ACEs and changes in the daytime slope over pregnancy became non-significant, β = -.00004, p = .134, indicating that ACEs did not have a unique association with the daytime slope after accounting for proximal stressors (Table 5 Model 3.2). Psychological distress during pregnancy was associated with flatter daytime slopes at the beginning of pregnancy, β = .002, p = .031, but was not associated with changes in the daytime slope as a function of advancing gestation, β = -.0001, p = .120. There were no interactions between ACEs and proximal stressors to predict maternal daytime slope, indicating that the association between ACEs and the daytime slope is not moderated by exposure to proximal stressors (Table 5, Model 3.3).

Aim 2.3: Total cortisol. Total daytime cortisol production, as determined by AUCg (Table 4 Model 4.1), increased as pregnancy progressed, β = 3.99, p < .001, however ACEs were not associated with either the level of total cortisol at the beginning of pregnancy, β = 3.31, p = .222, or the increase in total cortisol over the course of pregnancy, β = -.063, p = .617. The only significant predictor was maternal parity, which was associated with lower daytime cortisol levels at the beginning of pregnancy, β = -9.15, p = .003.

Secondary Aim 2.3. The addition of proximal stressors did not alter the associations between ACEs and total daytime cortisol production and the associations remained non-significant (Table 5 Model 4.2). Similarly, there were no interactions between ACEs and proximal stressors to predict maternal total daytime cortisol levels (Table 5 Model 4.3).

Exploratory Analyses

Aim 1. Components of ACEs associated with cross-sectional pattern of diurnal cortisol. Given that significant associations were observed between ACEs and the diurnal cortisol pattern, Model 1.1 was rerun with each of the three subcomponents of the ACEs measure as the predictor to determine which may be driving the associations (Supplementary Table 1). The +30 minute post waking sample was positively associated with Abuse, β = .014, p = .007, and Neglect β = .023, p = .011, but not Household Challenges, β = .007, p = .075. Abuse was also positively associated with the linear diurnal slope, β = .004, p = .006, and negatively associated with the quadratic diurnal slope, β = -.0003, p = .006; these associations were non-significant for Household Challenges and Neglect. These findings suggest that abuse experiences may be the primary driver of the association between ACEs and diurnal cortisol during pregnancy.

Aim 2. Components of ACEs associated with longitudinal pregnancy-related changes in HPA axis function with advancing gestation. Follow-up analyses were conducted with the CAR and daytime slope, for which associations with ACEs were previously observed (See Supplementary Tables 2 and 3, respectively). For the CAR, all three components were associated with an increased CAR at 6 weeks gestation (i.e., the intercept): Abuse, β =.867, p = .023; Neglect, β = 1.96, p = .001; and Household Challenges, β = .774, p = .004. The CAR decreased significantly over the course of pregnancy as a function of Neglect, β = -.058, p = .048, but not Abuse, β = -.007, p = .702 or Household Challenges, β = -.020, p = .150.

The daytime slope became more negative over the course of pregnancy as a function of Household Challenges, β = -.0001, p = .047, but not Abuse, β = -.0001, p = .083, or Neglect, β = -.0001, p = .122. As with the overall ACEs measure, there were no associations between the ACEs components and the intercept (i.e., slope at 6 weeks gestation) for the daytime slope model.

Discussion

The present study examined associations between maternal history of adverse childhood experiences (ACEs) and HPA axis function during pregnancy. The cross-sectional analyses (aggregating all samples collected during pregnancy) showed that maternal exposure to ACEs was associated with increased cortisol concentrations +30 minutes after awakening and flattening of the diurnal slope. The addition of proximal stressors to the model indicated a unique association for ACEs with the 30-minute post waking sample but not the linear diurnal slope, which suggests that the association between ACEs and the cross-sectional diurnal slope during pregnancy was reduced when recent stressors were accounted for. There was no evidence of synergistic effects between ACEs and proximal stressors on diurnal cortisol patterns, as indicated by the non-significant interactions between ACEs and proximal stressors.

As for the longitudinal pregnancy-related changes in HPA axis function over the course of pregnancy, ACEs were associated with steepening of the daytime slope as a function of advancing gestation, but ACEs were not associated with changes in the CAR or total cortisol output as pregnancy progressed. The association between ACEs and steepening of the daytime slope became nonsignificant with the addition of proximal stressors to the model, indicating that ACEs did not have a unique association with changes in the daytime slope with advancing gestation after accounting for proximal stressors. There was no evidence of synergistic effects between ACEs and proximal stressors on changes in HPA axis function over the course of pregnancy, as indicated by the non-significant interactions between ACEs and proximal stressors. Given that maternal ACEs history has been associated with altered placental-fetal stress physiology during pregnancy (Moog et al., 2016) and that maternal HPA axis function during pregnancy is associated with a wide variety of developmental outcomes in children,

including preterm birth (Giurgescu, 2009), reduced birthweight (Diego et al., 2006), negative or difficult temperament (Davis et al., 2007; de Weerth, van Hees, & Buitelaar, 2003; Thomas et al., 2017), alterations in brain development (Buss et al., 2012), and reduced cognitive development (Bergman, Sarkar, Glover, & O'Connor, 2010; Davis & Sandman, 2010), the impact on the developing fetus warrants consideration. These findings support the hypothesis that changes in maternal cortisol consequent to ACEs exposure may be a biological mechanism by which maternal early life adversity may be transmitted to a second generation, and necessitate future studies to evaluate prenatal cortisol as a mediator between maternal ACEs and offspring outcomes.

Cross-sectional analyses of prenatal diurnal cortisol as a function of maternal ACEs history

The pattern of findings observed (i.e., increased waking +30 minute cortisol levels and flattened diurnal slope) may indicate hypersecretion of cortisol throughout the day among pregnant women with ACEs. Similar patterns of diurnal cortisol secretion have been demonstrated in other studies of men and women with a history of ACEs including experiences of low parental care (Engert, Efanov, Dedovic, Dagher, & Pruessner, 2011), maltreatment (Weissbecker et al., 2006), and maternal separation (Kumari, Head, Bartley, Stansfeld, & Kivimaki, 2013). This pattern of cortisol hypersecretion may result from increased sensitivity of the HPA axis to environmental stimulation as a result of exposure to early life adversity (Del Giudice, Ellis, & Shirtcliff, 2011; Laurent, Gilliam, Bruce, & Fisher, 2014). According to the adaptive calibration model of stress responsivity, individuals exposed to dangerous or unpredictable environments in early life may develop highly responsive stress response systems that increase vigilance and readiness to respond to potential threats (Del Giudice et al., 2011). These biological adaptations, evolutionarily suited to be advantageous in harsh and unpredictable

environments, also have associated costs such as major depressive disorder, posttraumatic stress disorder, and anxiety (Cowen, 2010; Ehlert, Gaab, & Heinrichs, 2001).

Alterations in HPA axis functioning, such as flattening of the diurnal slope, have been found to persist through old-age in individuals with a history of ACEs (Gerritsen et al., 2010), indicating that altered HPA axis functioning associated with early childhood experiences may remain relatively stable throughout the lifespan. The current study also demonstrated that these HPA axis alterations are present during pregnancy, and thus are a potential pathway by which maternal childhood history of adversity may be transmitted to her child. It is known that maternal diurnal cortisol patterns during pregnancy are associated with the development of the fetal HPA axis (Giesbrecht et al., 2017), with shorter gestational length (Buss et al., 2009), and lower infant birth weight (Guardino et al., 2016). Nevertheless, studies linking diurnal cortisol as a mediator of the association between ACEs and infant outcomes are needed to determine the role of the prenatal HPA axis in the intergenerational transmission of stress.

The association between ACEs and higher waking +30 minute cortisol levels in the present study is in line with previous investigations examining diurnal patterns of cortisol in perinatal women with a history of childhood adversity. Specifically, increased waking +30 minute cortisol levels have previously been observed in pregnant women with a history of childhood sexual abuse (Bublitz et al., 2014; Bublitz & Stroud, 2012) as well as women in the first 6 months postpartum who experienced childhood maltreatment and/or the early loss of a parent (Gonzalez, Jenkins, Steiner, & Fleming, 2009). However, studies investigating these associations in broader populations (i.e., adult men and non-pregnant women) have yielded mixed results. Although some studies have found that adult men and women who experienced childhood trauma exhibit similar elevations in cortisol at waking +30 minutes (Lu et al., 2013),

others found that adults with a history of various ACEs, including childhood maltreatment (Power, Thomas, Li, & Hertzman, 2012), early life loss (Meinlschmidt & Heim, 2005), childhood trauma (Mangold, Wand, Javors, & Mintz, 2010), and decreased parental affection (Taylor, Karlamangla, Friedman, & Seeman, 2011), exhibit lower or blunted morning cortisol levels in adulthood. Notably, these studies utilized different measures of ACEs, and most focused on individual adverse experiences rather than an overall measure of cumulative adverse experiences, which may explain some of the discrepancies. Furthermore, cortisol levels at 30 minutes post waking are influenced by demographic (e.g., age and gender), physical and psychological health conditions (e.g., cardiovascular, autoimmune, and psychiatric diseases), and health behaviours (e.g., smoking) (see Fries et al., 2009 for a review), which may explain some of the discrepancies across studies. In addition, blunting of the HPA axis has been observed in individuals with post-traumatic stress disorder (Wessa, Rohleder, Kirschbaum, & Flor, 2006), which may help to explain the observation of lower than expected 30 minute post waking cortisol levels in some samples.

The pattern of results obtained when including indicators of recent life stressors and current psychological distress to the statistical models suggest that ACEs have long-term effects on the HPA axis (specifically with regard to cortisol levels at 30 minutes post waking) that cannot be explained by the exposure to more recent stressors. That is, there was some evidence that the effects of ACEs were independent of, and in addition to, the effects of proximal stressors on the HPA axis. The implication is that early life exposures in mothers may constitute a relevant developmental exposure for her fetus, regardless of her current psychological adjustment. There was no indication, however, that ACEs and proximal stressors had synergistic effects on the diurnal cortisol pattern during pregnancy. The lack of interaction effects between ACEs and

proximal stressors suggests that exposure to ACEs does not 'prime' the HPA axis to be more sensitive to recent life stress, contrary to the notion that early life stress calibrates the HPA axis to be more sensitive to life stressors. This is also in contrast to a previous study that found interactions between a history of childhood sexual abuse and proximal stress to predict increased CAR and evening cortisol levels (Bublitz & Stroud, 2013). As this prior study focused on childhood sexual abuse and not the broader construct of ACEs, it is possible that different types of ACEs (and possibly specifically sexual abuse) may prime greater HPA axis dysregulation following stress in adulthood compared to other types of adverse experiences.

Maternal ACEs history and longitudinal pregnancy-related changes in HPA axis function across gestation

Whereas little is known about ACEs-related changes in HPA axis function during pregnancy, more is known about normative pregnancy-related changes in HPA axis function. The most easily detected pregnancy-related change in HPA axis function is the approximately 2-4-fold overall increase in the amount of cortisol secreted at all times of the day (Mastorakos & Ilias, 2003). There is some evidence that the CAR becomes attenuated with advancing gestation (Buss et al., 2009; Entringer et al., 2010). In contrast, Bublitz and Stroud, (2012) found that pregnant women with a history of childhood sexual abuse exhibited higher CARs over the second half of pregnancy compared to women that experienced other forms of abuse or no abuse, suggesting that a history of sexual abuse may impact the HPA axis in such a way that it is less adaptive to pregnancy-related changes. In a subsequent study, Bublitz and colleagues (2014) demonstrated that women who experienced childhood sexual abuse were also more sensitive to experiences of family stress such that women with greater stress exposure had greater increases in the CAR across the later weeks of gestation compared to women without this history. In the

current study, which included data from the first trimester of pregnancy, ACEs were associated with increased CAR at the intercept (i.e., in early pregnancy) but then there was a small (although non-significant) decline in the CAR for women with ACEs. Additionally, we did not observe any interactions between ACEs and proximal life stressors, suggesting that ACEs may leave a 'signature' of stress on the maternal CAR during pregnancy that operates independently of more proximal life stressors.

These contrasting findings are not necessarily incompatible, given the differences in the timing of the assessments. Specifically, there may be a curvilinear association with an overall decrease in the CAR from beginning to end of pregnancy, as suggested by the current sample and previous studies with 'normative' data (see for example Buss et al., 2009), but among women with exposure to childhood sexual abuse there may be an increase in the CAR in later pregnancy. We are unable to adequately test this possibility in the current study because the inclusion of gestational age^2 significantly reduced model fit, suggesting that its inclusion did not account for a significant amount of variance in the data (model not shown). It should be noted that although the current study had the same number of pregnancy time points as the Bublitz studies, their studies had all three data points in the second half of pregnancy, which may improve the ability to detect changes in HPA axis function during this gestational period. Furthermore, Bublitz and colleagues focused on childhood sexual abuse whereas the current findings are based on the more general construct of ACEs. In post hoc analyses, we noted that the overall decrease in CAR over gestation was associated with Neglect, but not Abuse. These findings seem to indicate that exposure to Abuse has different associations with the CAR, compared to Neglect, and may therefore be in keeping with the data from Bublitz and colleagues.

Finally, a significant difference was observed in the daytime slope across pregnancy between women with a history of ACEs and those without such a history. Specifically, women with a history of ACEs exhibited steeper cortisol daytime slopes with advancing gestation, whereas the daytime cortisol slopes of women with no such history appeared to flatten. These findings were not altered by the addition of proximal stressors in the model. This was contrary to our hypothesis, as we expected that women with a history of ACEs would exhibit a flattening of the daytime cortisol slope with advancing gestation. Notably, the effect of ACEs on the diurnal slope as measured in Aims 1 and 2 of this study appear to be conflicting at first glance (i.e., ACEs were associated with a flattened diurnal slope in Aim 1 and with steeper daytime slopes across gestation in Aim 2). However, the questions asked in each aim differ substantially such that Aim 1 examined the *average* diurnal slope during pregnancy, whereas Aim 2 utilized waking and evening cortisol levels to evaluate *changes* in daytime slope across gestation. Table 4 Model 3.1 shows that women with ACEs began pregnancy with slightly flatter daytime slopes, $\beta = .001$, $p = .14$, compared to women with no ACEs, thus the significant steepening of the daytime slope over gestation may reflect a compensatory mechanism to prevent daytime slopes from becoming too flat over the course of pregnancy. Steepening of the daytime slope over gestation may function to downregulate overall cortisol secretion across gestation to protect the fetus from elevated cortisol levels. The trend toward reduction of the CAR with advancing gestation also supports this hypothesis. Ultimately, however, this may not confer advantage to the fetus given that heightened cortisol levels are crucial for fetal development, particularly nearing the end of gestation when cortisol plays a critical role in slowing fetal growth and promoting organ development (e.g., lung development in preparation for birth) (Murphy & Clifton, 2003).

Effect of different types of adverse experiences on maternal cortisol in pregnancy

Given that previous studies with pregnant women have focused on childhood sexual abuse, we wanted to determine what other types of adverse experiences (i.e., Abuse, Neglect, Household Challenges) may be driving the associations with maternal cortisol during pregnancy. Across both the cross-sectional analyses (Aim 1: see Supplementary Table 1) and the longitudinal pregnancy-related changes in HPA axis function (Aim 2: see Supplementary Table 2) models, all three forms of ACEs were associated with the morning increase in cortisol (i.e., waking +30 minute in Aim 1 and CAR in Aim 2). These findings suggest that the morning increase in cortisol may be particularly susceptible to a range of early life adversities, which may explain why it is the most consistently observed association in pregnancy studies to date. Of note, (and as was done in previous studies; Bublitz et al., 2013; 2014), we also ran models in which we used childhood sexual abuse as the predictor (results not shown). Findings were strongly similar to those reported (above) for the Abuse variable.

With regards to the longitudinal pregnancy-related changes in HPA axis function, only Neglect was associated with changes in the CAR over the course of pregnancy (see Model S5, Supplementary Table 2). Women without exposure to ACEs had small increases in the CAR as pregnancy progressed, but these changes were not observed in women with exposure to ACEs; in fact, for women with exposure to more than one experience of neglect, the model actually indicates a decrease in the CAR. These findings suggest that experiences of neglect may uniquely make the HPA axis less responsive to the normal pregnancy-related adjustments in HPA axis function that occur during pregnancy, or if the neglect was severe enough, results in adjustments that are not expected. Pregnancy-related changes in the daytime slope were

associated only with Household Challenges (see Model S9, Supplementary Table 3), suggesting that this effect may be driven by exposure to chaos or disruption in the early life environment.

Taken together, the findings for the cross-sectional analyses (Aim 1: Supplementary Table 1) and longitudinal pregnancy-related changes in HPA axis function models (Aim 2: Supplementary Tables 2 and 3) suggest that the HPA axis response to awakening may be modifiable by many different kinds of early life stressors but the changes in patterns of daytime cortisol are much less affected by exposure to early life adversity. It is not clear, however, why various indicators of HPA axis function and their adjustment over pregnancy may be more vulnerable to certain forms of early life adversity. Epigenetic studies are needed to shed light on the unique effects that different forms of adversity may have on HPA axis function during pregnancy.

Study Limitations

This study has several strengths, including a relatively large sample size, the focus on a multidimensional measure of early life adversity, the availability of cortisol data from the first trimester of pregnancy and repeated assessment of women during pregnancy. These strengths allow for a more complete and comprehensive analysis of maternal HPA-axis functioning during pregnancy to advance understanding of the HPA axis as a potential mechanism by which maternal experience of ACEs are transduced to her children. Additionally, the current study examined unique and synergistic effects between ACEs and more proximal stressors that could be expected to influence HPA axis function during pregnancy. Nonetheless, several limitations require consideration.

First, although we had a diverse sample, the majority of participants in this study represent a relatively low sociodemographic risk sample of women as most participants were

married or in common-law relationships, White, had university-level education, and had relatively high household annual income. As such, it may not be appropriate to generalize the findings to socio-demographically diverse or non-White populations. Despite the demographic homogeneity, a substantial proportion of women reported a history of at least one ACE (43.8%), which, consistent with Felitti et al (1998), indicates that ACEs are pervasive, even among populations of low sociodemographic risk. More importantly, this demonstrates that the effects of ACEs on the HPA axis persist despite the apparent psychosocial advantages of the women in this study. Second, we did not enroll women who reported consuming tobacco, non-prescription drugs, or alcohol during pregnancy, which may have excluded women with exposure to more severe forms of early life adversity, given the well-documented associations between ACEs and poor health behaviours (Anda et al., 1999; Dube et al., 2003; Dube, Anda, Felitti, Edwards, & Croft, 2002). Third, this study relied on retrospective self-reports of ACEs history, which may be subject to recall biases (Hardt & Rutter, 2004). Nevertheless, research indicates that such retrospectives reports yield a significant amount of false negatives while false positive reports are rare (Aalsma et al., 2002; McKinney, Harris, & Caetano, 2009). As such, one might expect smaller effect sizes than would actually be the case if these data were collected without measurement error. It is important to note that the ACEs questionnaire asks about the occurrence or non-occurrence of highly salient life events, and because of this, there is consensus that retrospective recall of ACEs are a valid method to assess early life adversity (Dube et al., 2004; Hardt & Rutter, 2004). Fourth, as is evident from our focus on pregnant women, we did not evaluate the potential sex-specificity of the associations between ACEs and cortisol patterns in adult men. Fifth, given the difficulty of enrolling women early in pregnancy, there was less data available for women in the first trimester compared to the subsequent trimesters. Fortunately, the

maximum likelihood estimators in multilevel models are asymptotically efficient even in unbalanced designs, which makes them invaluable for studies in which a balanced design is very difficult to achieve. Finally, this study used cortisol, the end product of the HPA axis, as an indicator of HPA axis function during pregnancy. Although this is a reasonable approach, it neglects other biomarkers of HPA axis function (such as adrenocorticotropic hormone or corticotrophin-releasing hormone), which could lend insight into specific aspects of HPA axis function that are associated with exposure to ACEs. Further study is also needed specifically with regard to placental corticotrophin releasing hormone to determine what role the placenta may play in transducing the effects of ACEs to the fetus.

Conclusion

In summary, we found support for the notion that maternal history of adverse childhood experiences affects HPA axis functioning during pregnancy. To date, the prevailing paradigm suggests that intergenerational transmission of maternal adversity likely occurs after birth through environmental pathways such as low quality caregiving (Center on the Developing Child at Harvard University, 2010; Lang et al., 2010; Plant et al., 2013; Rijlaarsdam et al., 2014). The present study supports the proposal of a *biological pathway* for the intergenerational transmission of ACEs and is in keeping with recent proposals suggesting multiple biological pathways by which early life experience becomes embedded in offspring development (Buss et al., 2017). Specifically, the current study suggests that changes in maternal HPA axis function are a plausible biological mechanism by which maternal experience of early life adversity may be transduced to the fetus, with the potential for programming of the fetal HPA axis.

These findings contribute to a growing evidence base that increasingly underscores the importance of early intervention efforts for children exposed to ACEs and suggest potential

targets for such efforts. Specifically, our findings support the value of interventions with individuals exposed to early life stress that include a physiological component. For instance, a previous study has demonstrated that, by changing the caregiving environment in which an abused child is placed and creating more predictable surroundings, the alterations to the HPA axis associated with abuse may be reversed (Graham et al., 2012). Studies have also demonstrated that stress reduction interventions during pregnancy may reduce maternal negative affect and anxiety (Vieten & Astin, 2008) as well as reduce morning cortisol levels (Urizar Jr. & Munoz, 2011). These studies provide support for future research to investigate the potential to ameliorate the effects of ACEs on HPA axis functioning in pregnant women and thereby to reduce the intergenerational transmission of stress, which may improve developmental outcomes in future generations.

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Tables and Figures

Table 1
Descriptive statistics for the study sample (n=356).

Variable	%
Infant Sex	
Male	53.1
Female	46.9
Maternal Parity	
Primiparous	50.3
Multiparous	49.7
Marital Status	
Single	1.1
Married	88.5
Common Law	10.4
Maternal Education	
Less Than High School Diploma	1.1
High School Diploma	6.8
Post-Secondary Education	92.1
Ethnicity	
White Caucasian	84.9
Latin American	3.7
Chinese	3.1
Other	8.3
Annual Household Income	
More Than $100,000/Year	57.8
$70,000 - $100,000	24.2
$40,000 - $70,000	10.8
Less Than $40,000/Year	7.2

Table 2

Descriptive statistics for the primary study variables.

Variable	% (n)	Mean	SD	Range
Maternal ACEs score				
0	56.2 (200)			
1	15.7 (56)			
2	11.2 (40)			
3	8.2 (29)			
4	2.8 (10)			
5	2.2 (8)			
6	1.7 (6)			
7	1.4 (5)			
8	0.3 (1)			
9	0.3 (1)			
Stressful life events		.85	.95	0 - 7
Psychological distress		-.04	.88	-1.32 - 4.04

	Time 1 (n = 115)			Time 2 (n = 279)			Time 3 (n = 308)		
Maternal Cortisol (μg/dL)	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Waking	.41	.15	.15-.90	.47	.15	.08-.98	.51	.18	.09-1.25
Waking +30	.49	.19	.10-1.11	.56	.19	.08-1.17	.68	.24	.21-1.48
1100h	.19	.09	.07-.55	.21	.08	.06-.59	.33	.10	.11-.83
2100h	.08	.04	.02-.21	.09	.04	.02-.27	.15	.06	.05-.42
Daytime Slope	-.02	.01	-.05– -.002	-.02	.01	-.05−.01	-.02	.01	-.06−.01
CAR	2.71	4.00	-3.20–19.86	2.70	3.39	-6.65−17.65	3.12	3.38	-6.29−22.71
AUCg	157.38	54.07	80.49−426.64	168.55	40.16	91.83−404.18	235.93	57.66	140.01−736.50

Note. CAR = cortisol awakening response; AUCg = area under the curve from ground.

Table 3

Aim 1. Cross-sectional patterns of maternal diurnal cortisol during pregnancy.

	Model 1.1 (Diurnal cortisol)		Model 1.2 (Adjusted for proximal stressors)		Model 1.3 (Interactions with proximal stressors)	
Fixed effects	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
SES	.002 (.004)	.557	.002 (.004)	.569	.002 (.004)	.591
Maternal Age	.0003 (.001)	.657	.0002 (.001)	.698	.0003 (.001)	.638
Parity	-.006 (.003)	.032	-.006 (.003)	.039	-.006 (.003)	.036
GA	.004 (.0002)	<.001	.004 (.0002)	<.001	.004 (.0002)	<.001
Waking cortisol	.392 (.006)	<.001	.388 (.008)	<.001	.390 (.009)	<.001
ACEs	-.003 (.004)	.477	-.002 (.004)	.591	-.004 (.005)	.459
Stressful life events			.005 (.005)	.392	.002 (.007)	.750
Psychological distress			-.006 (.006)	.275	-.006 (.008)	.415
Stressful life events*ACEs					.002 (.004)	.596
Psychological distress*ACEs					-.0003 (.004)	.945
$^{+}$30 Min	.091 (.004)	<.001	.084 (.006)	<.001	.088 (.006)	<.001
ACEs	.008 (.003)	.003	.009 (.003)	.001	.005 (.004)	.227
Stressful life events			.006 (.004)	.141	.001 (.005)	.914
Psychological distress			-.010 (.005)	.036	-.012 (.006)	.035
Stressful life events*ACEs					.005 (.003)	.112
Psychological distress*ACEs					.002 (.003)	.481
TSW (Linear Diurnal Slope)	-.042 (.001)	<.001	-.040 (.002)	<.001	-.040 (.002)	<.001
ACEs	.002 (.001)	.050	.001 (.001)	.074	.001 (.001)	.244
Stressful life events			-.001 (.001)	.344	-.001 (.001)	.386
Psychological distress			.001 (.001)	.250	.002 (.002)	.196
Stressful life events*ACEs					.0002 (.001)	.831
Psychological distress*ACEs					-.001 (.001)	.542
TSW^2 (Quadratic Diurnal Slope)	.002 (.0001)	<.001	.001 (.0001)	<.001	.001 (.0001)	<.001
ACEs	-.0001 (.0001)	.067	-.0001 (.0001)	.086	-.0001 (.0001)	.271
Stressful life events			.0001 (.0001)	.394	.0001 (.0001)	.414
Psychological distress			-.0001 (.0001)	.370	-.0001 (.0001)	.259
Stressful life events*ACEs					-.00001 (.0001)	.800
Psychological distress*ACEs					.00004 (.0001)	.508

Note. TSW = time since waking.

Table 4

Aim 2. Longitudinal pregnancy-related changes in maternal cortisol across gestation.

	Model 2.1 (CAR)		Model 3.1 (Daytime Slope)		Model 4.1 (AUCg)	
Fixed effects	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Intercept	1.78 (.361)	<.001	-.020 (.001)	<.001	133.5 (5.07)	<.001
SES	.362 (.270)	.180	.001 (.001)	.378	7.57 (4.31)	.080
Maternal Age	.026 (.042)	.540	.0002 (.0001)	.065	-.170 (.668)	.799
Parity	-.206 (.191)	.283	-.001 (.001)	.094	-9.15 (3.07)	.003
ACEs	.672 (.195)	.001	.001 (.001)	.143	3.31 (2.71)	.222
Gestational age	.051 (.017)	.002	.0001 (.00004)	.077	3.99 (.210)	<.001
ACEs	-.016 (.010)	.106	-.00005 (.00002)	.043	-.063 (.125)	.617

Note. CAR = cortisol awakening response; AUCg = area under the curve from ground.

Table 5

Secondary Aim 2. Interactions between ACEs and proximal stressors predicting longitudinal pregnancy-related changes in maternal cortisol across gestatio

	Model 2.2 (CAR Adjusted)		Model 2.3 (CAR Interactions)		Model 3.2 (Daytime Slope Adjusted)		Model 3.3 (Daytime Slope Interactions)		Model 4.2 (AUCg Adjusted)		(A
Fixed effects	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estima
Intercept	1.47 (.444)	.001	1.71 (.501)	.001	-.020 (.001)	<.001	-.019 (.001)	<.001	129.0 (6.19)	<.001	129.9
SES	.366 (.275)	.183	.346 (.274)	.208	.001 (.001)	.467	.0005 (.001)	.520	8.17 (4.40)	.064	8.13
Maternal Age	.022 (.042)	.601	.027 (.042)	.526	.0002 (.0001)	.071	.0002 (.0001)	.077	-.209 (.671)	.756	-.172
Parity	-.193 (.191)	.312	-.207 (.190)	.277	-.001 (.0005)	.087	-.001 (.0005)	.082	-8.89 (3.08)	.004	-8.97
ACEs	.616 (.202)	.002	.387 (.278)	.164	.0005 (.0005)	.343	.0002 (.001)	.738	2.88 (2.79)	.303	2.02
Stressful life events	.416 (.296)	.161	.127 (.394)	.748	-.0003 (.001)	.692	-.0005 (.001)	.597	5.36 (4.06)	.185	4.24
Psychological distress	.087 (.328)	.791	-.004 (.443)	.993	.002 (.0005)	.031	.001 (.001)	.287	-1.16 (4.61)	.801	-1.78
Stressful life events*ACEs			.220 (.195)	.262			.0002 (.0005)	.729			.907
Psychological distress*ACEs			.055 (.226)	.808			.0004 (.001)	.477			.309
Gestational age	.057 (.021)	.008	.057 (.024)	.019	.0001 (.00005)	.147	.0001 (.0001)	.285	4.15 (.268)	<.001	4.28
ACEs	-.012 (.010)	.223	-.011 (.014)	.440	-.00004 (.00002)	.134	-.00001 (.00003)	.688	-.026 (.129)	.843	-.142
Stressful life events	-.012 (.015)	.452	-.010 (.020)	.611	-.00002 (.00004)	.599	.000002 (.00005)	.963	-.231 (.193)	.234	-.372
Psychological distress	-.015 (.016)	.351	-.003 (.022)	.892	-.0001 (.00004)	.120	.000002 (.0001)	.970	-.107 (.208)	.609	-.055
Stressful life events*ACEs			.0005 (.010)	.960			-.00001 (.00002)	.543			.121
Psychological distress*ACEs			-.010 (.011)	.361			-.00005 (.00003)	.065			-.046

Note. CAR = cortisol awakening response; AUCg = area under the curve from ground.

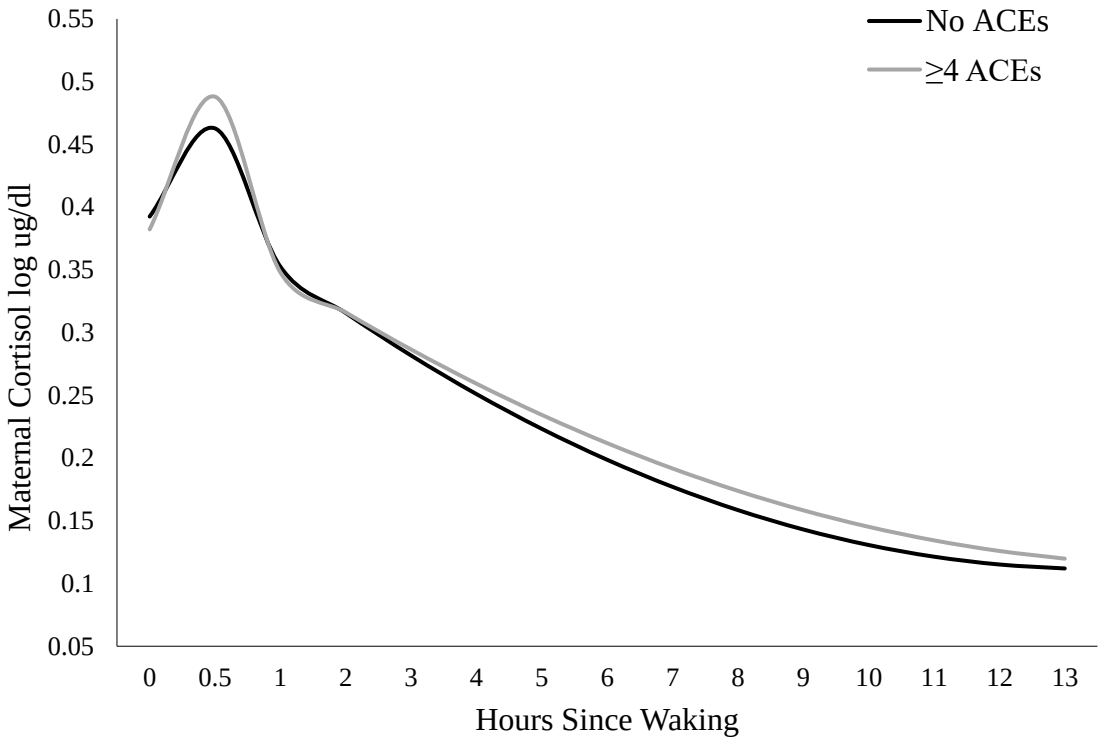

Figure 1. Cross-sectional maternal diurnal cortisol during pregnancy as a function of ACEs exposure. Women with a history of ≥4 ACEs exhibited higher CARs and flatter diurnal slopes compared to women with no ACEs (Model 1.1).

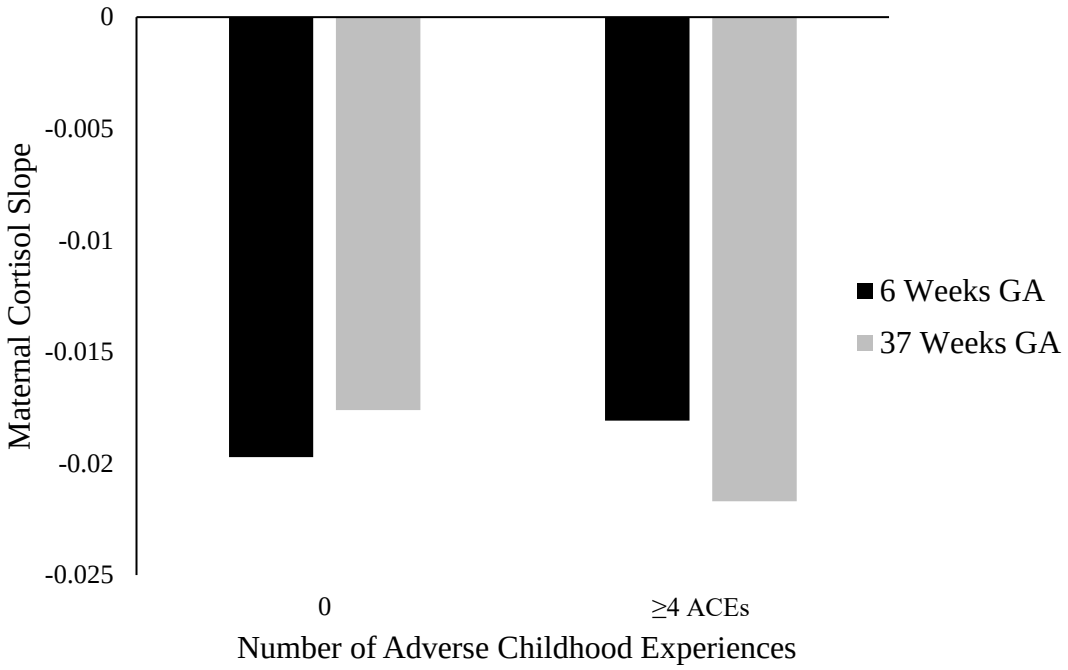

Figure 2. Longitudinal changes in maternal daytime cortisol slope over the course of pregnancy (Model 3.1). In contrast to the trend toward flatter slopes in women with 0 ACEs, women with ≥ 4 ACEs demonstrated increasingly steeper slopes as pregnancy progressed. The difference in daytime slopes at the intercept (6 weeks gestation) was not significant.

Supplementary Tables

Table 3.6

Subcomponents of ACEs predicting cross-sectional maternal diurnal cortisol during pregnancy.

	Model 5.1 (Abuse)		Model 5.2 (Neglect)		Model 5.3 (Household Challenges)	
Fixed effects	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
SES	.005 (.006)	.435	.004 (.006)	.447	.005 (.006)	.365
Maternal Age	-.0004 (.001)	.692	-.0003 (.001)	.752	-.0003 (.001)	.754
Parity	-.005 (.004)	.271	-.005 (.004)	.246	-.005 (.004)	.269
GA	.004 (.0002)	<.001	.004 (.0002)	<.001	.004 (.0002)	<.001
Waking cortisol	.391 (.005)	<.001	.390 (.005)	<.001	.392 (.005)	<.001
Abuse	-.006 (.006)	.312				
Neglect			.001 (.009)	.927		
Household Challenges					-.004 (.004)	.293
$^{+}$30 Min	.093 (.004)	<.001	.094 (.004)	<.001	.094 (.004)	<.001
Abuse	.014 (.005)	.007				
Neglect			.023 (.009)	.011		
Household Challenges					.007 (.004)	.075
TSW (Linear Diurnal Slope)	-.041 (.001)	<.001	-.040 (.001)	<.001	-.040 (.001)	<.001
Abuse	.004 (.002)	.006				
Neglect			.001 (.003)	.614		
Household Challenges					.0003 (.001)	.754
TSW^2 (Quadratic Diurnal Slope)	.001 (.0001)	<.001	.001 (.0001)	<.001	.001 (.00001)	<.001
Abuse	-.0003 (.0001)	.006				
Neglect			-.0001 (.0002)	.602		
Household Challenges					.00001 (.0001)	.920

Note. TSW = Time since waking

Table 3.7

Subcomponents of ACEs predicting longitudinal pregnancy-related changes in maternal CAR across gestation.

	Model S4 (Abuse)		Model S5 (Neglect)		Model S6 (Household Challenges)	
Fixed effects	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Intercept	2.13 (.332)	<.001	2.12 (.317)	<.001	1.98 (.345)	<.001
SES	.380 (.270)	.160	.441 (.270)	.102	.428 (.270)	.114
Maternal Age	.025 (.042)	.549	.033 (.042)	.433	.026 (.042)	.542
Parity	-.161 (.191)	.400	-.200 (.193)	.302	-.196 (.193)	.310
Abuse	.867 (.381)	.023				
Neglect			1.96 (.590)	.001		
Household Challenges					.774 (.271)	.004
Gestational age	.036 (.015)	.016	.043 (.014)	.002	.046 (.016)	.004
Abuse	-.007 (.019)	.702				
Neglect			-.058 (.029)	.048		
Household Challenges					-.020 (.014)	.150

Note. CAR = cortisol awakening response.

Table 3.8

Subcomponents of ACEs predicting longitudinal pregnancy-related changes in maternal daytime cortisol slope across gestation.

	Model S7 (Abuse)		Model S8 (Neglect)		Model S9 (Household Challenges)	
Fixed effects	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Intercept	-.020 (.001)	<.001	-.020 (.001)	<.001	-.020 (.001)	<.001
SES	.001 (.001)	.397	.001 (.001)	.335	.001 (.001)	.422
Maternal Age	.0002 (.0001)	.060	.0002 (.0001)	.068	.0002 (.0001)	.067
Parity	-.001 (.0005)	.087	-.0008 (.0005)	.099	-.001 (.0005)	.094
Abuse	.001 (.001)	.160				
Neglect			.001 (.001)	.475		
Household Challenges					.001 (.001)	.072
Gestational age	.00005 (.00003)	.156	.00004 (.00003)	.231	.0001 (.00004)	.096
Abuse	-.0001 (.00004)	.083				
Neglect			-.0001 (.0001)	.122		
Household Challenges					-.0001 (.00003)	.047