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Dysregulation of the cortisol diurnal rhythm following prenatal alcohol exposure and early life adversity

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Abstract

The hypothalamic-pituitary-adrenal (HPA) axis is impacted by a multitude of pre- and postnatal factors. Developmental programming of HPA axis function by prenatal alcohol exposure (PAE) has been demonstrated in animal models and in human infants, but remains understudied in older children and adolescents. Moreover, early life adversity (ELA), which occurs at higher rates in children with PAE than in non-exposed children, may also play a role in programming the HPA or stress response system. In a cohort of children and adolescents with PAE and ELA (PAE + ELA), we evaluated HPA function through assessment of diurnal cortisol activity compared to that in typically developing controls, as well as the associations among specific ELAs, adverse outcomes, protective factors, and diurnal cortisol. Morning and evening saliva samples were taken under basal conditions from 42 children and adolescents (5–18 years) with PAE + ELA and 43 typically developing controls. High rates of ELA were shown among children with PAE, and significantly higher evening cortisol levels and a flatter diurnal slope were observed in children with PAE + ELA, compared to controls. Medication use in the PAE + ELA group was associated with lower morning cortisol levels, which were comparable to controls. Complex associations were found among diurnal cortisol patterns in the PAE + ELA group and a number of ELAs and later adverse outcomes, whereas protective factors were associated with more typical diurnal rhythms. These results complement findings from research on human infants and animal models showing dysregulated HPA function following PAE, lending weight to the suggestion that PAE and ELA may interact to sensitize the developing HPA axis. The presence of protective factors may buffer altered cortisol regulation, underscoring the importance of early assessment and interventions for children with FASD, and in particular, for the many children with FASD who also have ELA.

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Keywords

Prenatal alcohol exposure; Cortisol; HPA axis; Early life adversity; Stress regulation

Introduction

Children with Fetal Alcohol Spectrum Disorder (FASD) experience a range of effects following prenatal alcohol exposure (PAE) including central nervous system (CNS) alterations involving cognitive and behavioral function, and in approximately 10% of diagnosed cases, physical indicators, including characteristic facial dysmorphology and/or growth restriction (Astley, 2010; Chudley et al., 2005; Stratton, Howe, & Battaglia, 1996). Deficits in self-regulation and adaptive functioning can occur across the FASD spectrum (Mattson, Crocker, & Nguyen, 2011; Riley, Infante, & Warren, 2011), and alterations in the activity and regulation of the hypothalamic-pituitary-adrenal (HPA) or stress axis may be a physiological marker of these deficits (Bauer, Quas, & Boyce, 2002). The HPA axis is a key component of the stress response system and is shaped or programmed by both pre- and postnatal early life experiences (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006; Smith & Vale, 2006), including PAE (Haley, Handmaker, & Lowe, 2006; Jacobson, Bihun, & Chiodo, 1999; Oberlander et al., 2010; Ramsay, Bendersky, & Lewis, 1996).

The typical circadian rise and fall of cortisol is necessary to support normal brain growth and to sustain general functioning. Cortisol is released at higher levels in the morning to mobilize physiological resources and self-regulatory processes. Over the course of the day cortisol levels decrease as the body regulates resources. There is some research on the association between PAE and cortisol secretion in humans; however, studies have been largely limited to the neonatal and toddler periods. Ramsay et al. (1996) found higher pretest cortisol levels and blunted cortisol responses to medical inoculation procedures in 2-month-old infants exposed to alcohol and cigarettes prenatally. Similar results were found in neonates with PAE following a heel-lance blood draw procedure (Oberlander et al., 2010). Jacobson et al. (1999) found that heavy PAE was associated with both higher basal levels and greater cortisol reactivity to a heel-lance blood draw in 13-month-old toddlers, indicating that reactivity may increase with age. Sexually dimorphic effects have also been found in stress reactivity studies. For example, Haley et al. (2006) demonstrated higher cortisol reactivity in 5- to 7-month-old infants with PAE in response to a “still face” paradigm, but only among boys born to mothers who were high-frequency drinkers, compared to those born to low-frequency drinkers. Ouellet-Morin et al. (2011) found disrupted patterns of cortisol activity in 19-month-old boys, but not girls, with low-level PAE, including both lower baseline levels and higher post-stress reactivity. To our knowledge, only one study has examined cortisol activity under baseline conditions in older, school-aged children and adolescents with FASD. Keiver, Bertram, Orr, and Clarren (2015) found that 8- to 14-year-old children with FASD had significantly higher afternoon and evening cortisol levels and a trend toward lower morning levels compared to controls, suggesting the possibility of a disturbance in normal basal HPA regulation over the day. Animal studies support and extend these findings, demonstrating HPA hyper-responsiveness following PAE, with increased activation and/or delayed recovery of HPA activity following

both acute and repeated stressors, as well as alterations in basal corticosterone levels over the day and changes in central HPA regulation (Hellemans, Sliwowska, Verma, & Weinberg, 2010; Schneider, Moore, & Adkins, 2011; Schneider, Moore, & Kraemer, 2004; Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002; Weinberg, Sliwowska, Lan, & Hellemans, 2008).

Compounding the impact of PAE, children with FASD often experience both early life adversity (ELA) (e.g., maltreatment, early caregiving disruption and contact with the foster care system, poverty, and familial adversity), and later adverse outcomes (e.g., school failure, contact with the criminal justice system, victimization, and comorbid mental health problems) at high rates (Astley, 2010; Coggins, Timler, & Olswang, 2007; Streissguth et al., 2004; Yumoto, Jacobson, & Jacobson, 2008). Both pre- and postnatal experiences are known to play a key role in early programming of the stress response system and are likely important moderators of fetal programming following both PAE and ELA (Bosch et al., 2012; Entringer, Kumsta, Hellhammer, Wadhwa, & Wüst, 2009; Essex, Klein, Cho, & Kalin, 2002; Glover, O'Connor, & O'Donnell, 2010; Lupien, McEwen, Gunnar, & Heim, 2009; Weinstock, 2008). Factors at the parenting level (stress, psychopathology, high psychosocial risk, ongoing substance abuse, abuse during pregnancy, maternal sensitivity, quality of parent-child interactions), child level (maltreatment), and environmental level (poverty/socioeconomic status, familial adversity) are all linked with later cortisol dysregulation (Hunter, Minnis, & Wilson, 2011). Exposure to early and chronic stress can lead to dysregulation of the HPA axis by middle childhood and has been linked with both short- and long-term physical, behavioral, cognitive, and mental health problems (Brand et al., 2010; Cicchetti, Rogosch, Gunnar, & Toth, 2010; Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Suor, Sturge-Apple, Davies, Cicchetti, & Manning, 2015). Results vary across studies, but generally speaking, acute stressors are associated with hyper-responsiveness of the HPA axis and higher cortisol levels, whereas hypo-responsiveness and lower cortisol levels may be seen as a consequence of chronic stress and overstimulation of the axis (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Gunnar, Fisher, & Early Experience, Stress, and Prevention Network, 2006; Gunnar & Vazquez, 2001). A "dual-vulnerability" theory has also been supported, wherein individuals with multiple vulnerabilities, such as altered stress reactivity and impaired cognitive functioning, may be especially prone to later problems in everyday living (Robinson, Ode, & Hilmert, 2011).

In addition to risk factors linked with poor developmental outcomes, a number of protective factors have been identified as playing important moderating roles in reducing adverse outcomes for children with PAE, including early assessment, diagnosis, intervention, and the quality and stability of the home environment (McLachlan et al., in preparation; Rasmussen, 2012; Streissguth et al., 2004). Critically, evidence from young children (without PAE) suggests that altered cortisol activity and regulation can be ameliorated following improvements in care and interventions targeting self-regulatory deficits (Hunter et al., 2011; Slopen, McLaughlin, & Shonkoff, 2014). Thus, understanding cortisol regulation in children and adolescents with PAE and ELA may provide an important window into both promising opportunities for intervention and possible physiological indices of both ELA and treatment gains.

The adverse effects of ELA parallel in many ways the adverse effects of PAE, both generally, and in particular, in relation to effects on HPA activity and regulation. Moreover, due to the high rates of ELA that frequently characterize the environment for children with PAE, it is often difficult, if not impossible, to separate the consequences of these early life insults. Thus, many studies evaluating the impact of PAE on cortisol regulation may be better characterized as studies of both PAE and environmental stress/adversity. However, few have explicitly focused on specific adversities in children with PAE, or on their combined impacts in older children and adolescents. This is the area where our work fills an important gap in the literature.

Based on the discussion above, we expected that the current cohort of children and adolescents with PAE would have high rates of ELA. Given that both ELA and PAE have demonstrated links with HPA activity and regulation, we sought to explicitly assess rates of ELA to determine if in fact this sample had experienced both impacts on development. Then, in the identified cohort of children and adolescents with PAE + ELA, we evaluated HPA function through assessment of cortisol activity, compared to that in typically developing children, by examining diurnal cortisol patterns. From the findings of Keiver et al. (2015) we postulated that dysregulation might be manifested by higher PM and possibly lower AM cortisol levels. We also sought to extend findings from the infant PAE-cortisol literature and the recent findings by Keiver et al. (2015) by exploring associations among cortisol regulation, ELA, adverse outcomes, and protective factors in children and adolescents with PAE + ELA. Based on evidence from other populations and on the fact that ELA might be acting on a stress system already sensitized by PAE, we hypothesized that the combination of PAE + ELA may be linked with increased HPA dysregulation, manifested as alterations in diurnal cortisol activity. Conversely, we expected that the presence of protective factors may be linked with more typical cortisol patterns.

Material and methods

Participants

Participants included 85 children and adolescents ages 5–18 years (mean = 11.54, SD = 3.09, 44.7% male) in two groups: those with confirmed PAE ($n = 42$), including a subset with a diagnosis of FASD ($n = 29$, 67.4%), and typically developing controls ($n = 43$). Participants with available cortisol data were drawn from the larger NeuroDevNet FASD study cohort (Reynolds et al., 2011) and did not differ in regard to demographic characteristics (age, gender, socioeconomic status) relative to the larger study cohort. Participants with PAE were recruited from FASD diagnostic clinics in British Columbia and Alberta that adhered to FASD assessment approaches based on the Canadian Diagnostic Guidelines for FASD in assigning diagnostic designations (FAS/pFAS/ARND) (Chudley et al., 2005) as well as the Washington FAS Diagnostic and Prevention Network (DPN) 4-digit code ranking system to assess diagnostic features (growth restriction, facial dysmorphology, CNS deficits, and PAE level) (Astley, 2004). Typically developing children were recruited from the same geographic regions and were excluded from the study if they had a neurological, genetic, or psychiatric disorder. In the present subsample, the same exclusions apply.

Measures

Prenatal alcohol exposure—Information about maternal alcohol consumption during pregnancy among children with PAE was collected retrospectively via file review from FASD assessments. All children had a Washington DPN 4-digit ranking for PAE available for review. All children in the PAE + ELA group had confirmed PAE, with 69% ($n = 29$) coded a rank ‘3’ (alcohol use during pregnancy is confirmed, and level of alcohol use is either unknown, or less than rank 4)² and 31% ($n = 13$) were coded a rank ‘4’ (alcohol use during pregnancy is confirmed and exposure pattern is consistent with the medical literature placing the fetus at “high risk”).

Socioeconomic status—Socioeconomic status (SES) was calculated using Hollingshead’s Four-Factor Index of Social Status (Adams & Weakliem, 2011; Hollingshead, 1975). Across both the PAE and control groups, SES scores spanned almost the full gradient, ranging from 13 through 66, with 8 being the lowest possible score and 66 being the highest. The following reference categories describe employment positions associated with each level band: 8–19 characterizes unskilled laborers and service workers; 20–29 characterizes machine operators, semiskilled workers; 30–39 characterizes skilled craftspeople, clerical sales, sales workers; 40–54 characterizes medium business, minor professional, technical; and 55–66 characterizes major business and professionals. These ranges are presented to help meaningfully categorize SES scores, though they should not be confused for any particular family’s employment status because the scores are computed from a combination of both education and employment for either one or two employed cohabitating parent(s) or caregiver(s) for each participant. For the present study, families were classified as falling into a “low SES” classification where their scores were 30 or lower, “moderate SES” comprised scores from 31 to 54, and “high SES” comprised scores from 55 to 66, for descriptive purposes.

Early life adversity—Caregivers of children with PAE completed a structured interview querying the presence or absence of specific ELAs including: contact with child protective services/foster care system, number of home placements, physical and sexual abuse, neglect, exposure to domestic violence, and prior caregiver adversity. A total caregiver adversity score was calculated by summing the number of caregiver adversities endorsed from four categories: serious mental illness, suspected of having FASD, substance-abuse problems, and trouble with the law (higher scores indicate higher caregiver adversity). Finally, we created an ELA total score by summing the presence of each of eight early life adversities, drawn from those coded in the Adverse Childhood Experiences (ACE) study (Dube, Felitti, Dong, Giles, & Anda, 2003), including: not raised by a biological parent; someone in home in trouble with the law; parent figure had serious mental illness; victim of physical abuse; victim of sexual abuse; victim of neglect; exposed to domestic violence.

²Rank 3 also typically requires a clinical interpretation of “some risk” in regard to drinking patterns in pregnancy, wherein either the specific quantity and/or frequency of patterns are unknown, or PAE is judged to be more than minimal or light exposure. Drinking levels, where unknown, could also be commensurate with “high risk” levels if confirmation of the pattern of exposure were available.

Adverse outcomes—Caregivers indicated whether participants had experienced a range of adverse outcomes (yes/no) including previous school punishments, trouble with the law, alcohol abuse, drug dependence, inappropriate sexual behavior, and homelessness.

Protective factors—Caregivers reported on several protective factors, including the age at which participants were assessed for PAE-related difficulties and/or diagnosed with FASD, and whether they had ever lived in a quality, stable home. The duration of quality and stable placements was also queried, and a ratio of years spent in such care situations relative to participant age was calculated.

Salivary cortisol—The diurnal cortisol rhythm was measured to capture basal cortisol levels over the day. Salivary cortisol measurement provides a non-invasive and cost-effective way to evaluate the diurnal cortisol rhythm. Two saliva samples were collected on each of two days, in the morning (AM) and evening (PM), for a total of four samples for each participant. Families were instructed to collect AM samples within 30 min of awakening and before consuming food or beverages or brushing teeth, and PM samples within 30 min of bedtime, at least 45 min after eating, drinking, and before brushing teeth. A passive drool sampling method was used whereby participants “drool” (let saliva flow) into a straw inserted into a tube, allowing saliva to collect. Caregivers were directed to store samples in the refrigerator until they could deliver them to the laboratory at Children’s and Women’s Health Centre of British Columbia either in person or by mail. Samples were stored in the -20°C freezer at the laboratory until sent for analysis at the University of British Columbia. Cortisol was analyzed using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics LLC, Philadelphia, PA). Intra-assay and interassay coefficients of variation were 5.6% and 6.8%, respectively.

Mean AM and PM cortisol levels were calculated by averaging each of the two morning and two evening samples, respectively. A difference score between mean PM and AM values was calculated to capture the diurnal rhythm, or change in cortisol concentration over the day (slope). Two samples could not be analyzed due to poor quality or insufficient saliva for quantification. In total, 12 participants were identified as having an outlying value on one of the four samples (from a total of 336 samples), defined as >2 standard deviations above or below the mean, and in these cases, the remaining value at that AM or PM time point was substituted. As expected, mean PM cortisol values were skewed and log-transformed for subsequent analyses.

A subset of our parents ($n = 35$, 41.2%) was asked to complete diary questionnaires to permit an assessment of factors that could have influenced cortisol findings, indicating sample collection time, last food/liquid intake, activity prior to sample collection, and medication use. Children in the diary and non-diary questionnaire subgroups did not differ on basic demographic characteristics (e.g., PAE, age, gender, SES). Data were screened to ensure participants provided samples within the required time frames (e.g., at least 45 min after eating and drinking) and suggested good adherence to data collection protocols. Average collection times were 08:45 (ranging from 07:00 to 11:10) and 20:57 (ranging from 20:07 to 23:17). This relatively wide range of collection times was not unexpected given our sample’s broad age range. No association was found between time of day at sample

collection and AM or PM cortisol concentrations, controlling for participant age using partial correlations.

Confounders—Several sociodemographic child factors previously associated with cortisol regulation were evaluated, including low socioeconomic status (Essex et al., 2002; Evans & Kim, 2007; Lupien, King, Meaney, & McEwen, 2000, 2001), age (Knutsson et al., 1997; Netherton, Goodyer, Tamplin, & Herbert, 2004), and gender (Gunnar & Quevedo, 2007; Ouellet-Morin et al., 2011).

Procedure

Caregivers provided informed consent and assent was obtained from all children. Caregivers completed a semi-structured interview with a research assistant while children underwent longer testing sessions for the NeuroDevNet study. Study procedures were approved by Research Ethics Boards at the University of British Columbia and University of Alberta and adhered to governing ethical guidelines.

Data analysis

Descriptive data are presented to characterize the sample, including rates of ELAs, adverse outcomes, protective factors, and indicators of cortisol regulation. Group differences on demographic variables were compared using *t* tests and chi-squared analyses for non-parametric data. Basal cortisol levels were compared between the PAE and control group using analysis of covariance (ANCOVA), controlling for age. Exploratory analyses evaluating the effects of SES and gender, controlling for age, on AM, PM, and slope cortisol values were assessed using analysis of covariance (ANCOVA). Partial correlations controlling for age were calculated to examine the association between cortisol indicators and ELAs, adverse outcomes, and protective factors in the PAE group. Effect sizes for *t* tests (Cohen's *d*), chi-square (ϕ , ϕ), and ANOVA (partial eta-squared, η^2_p) analyses are reported to indicate the size of statistically significant differences. Cohen's *d* values range from .2 (small) to .5 (medium) to .8 and above (large), while ϕ and partial eta-squared values range from .1 (small) to .3 (medium) to .5 and above (large) (Cohen, 1988). Study data were collected and managed using REDCap electronic data capture tools (Harris et al., 2009). All analyses were conducted using IBM Statistics 22 for Macintosh OS.

Results

Participant characteristics

Demographic characteristics of the sample are presented in Table 1. The PAE + ELA group had significantly lower average SES scores relative to controls, with 31% ($n = 13$) being classified as “low SES,” versus only three participants (7%) from the control group. Substantial variability in caregiver placement was also observed in the PAE + ELA group, whereas the controls all resided with their biological families. As anticipated, our typically developing control group reported virtually no ELA or adverse outcomes and thus are not included in subsequent analyses of these variables. Among the subset of 35 children with PAE for whom ELA data were available (85%), only one child had not experienced any of the eight identified ELAs from the ACE study (see Table 2). Children with PAE + ELA had

experienced a median of 4 unique ELAs (ranging from 0 to 7 on ACE scores, mean = 3.8, SD = 1.8), supporting the suggestion that the impact of PAE is often compounded by ELA. More than half the children and adolescents in the PAE + ELA group had experienced physical/sexual abuse and/or neglect, and the majority ($n = 30$, 86%) had a parent who had experienced at least one form of adversity (history of trouble with the law, serious mental illness, suspected of having FASD, substance-use problems). Many participants with PAE + ELA also reported experiencing adverse outcomes, including criminal justice system involvement and substance-use problems, despite only just over half (52.4%) the sample having reached age 12 (Table 2). On average, participants reported spending only two-thirds of their lifetime in stable home placements. Average age of assessment for PAE/FASD was between 7 and 8 years, but ranged widely.

Participants with PAE + ELA reported a range of additional prior and current mental health diagnoses, including attention deficit hyperactivity disorder (ADHD), internalizing disorders (e.g., anxiety, depression), and externalizing disorders (e.g., oppositional defiant disorder) (Table 1). Among children with PAE + ELA, 40% ($n = 17$) reported using medication(s) at the time of study enrollment. Stimulant and non-stimulant based medications that are typically prescribed for ADHD (methylphenidate and amphetamine/dextroamphetamine, clonidine, atomoxetine) and atypical antipsychotics (risperidone, quetiapine) were most commonly endorsed. Children in the control group, by design, reported no concurrent mental health diagnoses or medication use at study enrollment.

Cortisol patterns in children with PAE + ELA compared to controls

Cortisol levels in both the PAE + ELA and control groups showed the expected diurnal pattern, with higher AM and lower PM levels (Table 1). This finding supports the validity of our cortisol assessment approach. The PAE + ELA group had significantly higher PM values ($p = .03$) and a flatter diurnal slope ($p = .02$), and showed a trend for lower AM cortisol ($p = .07$) relative to controls (see Fig. 1). Age was significantly correlated with AM cortisol values ($r = .28$, $p = .09$), and controlled for in later analyses. Medication use recorded on saliva collection days was not associated with cortisol levels in PAE + ELA or control participants who completed the diary cards. However, participants who reported medication use at the time of study enrollment had lower morning cortisol levels ($.18 \pm .06 \mu\text{g/dL}$) [mean $\pm$ SD] compared to those who did not ($.29 \pm .15 \mu\text{g/dL}$), $t(40) = 2.68$, $p = .01$, $d = .85$, and smaller difference scores (i.e., flatter slope) from AM to PM ($.13 \pm .09 \mu\text{g/dL}$ vs. $.23 \pm .16 \mu\text{g/dL}$), $t(42) = 2.34$, $p = .02$, $d = .74$. Notably, participants with PAE + ELA who reported no medication use at study enrollment had mean AM cortisol concentrations identical to those of controls (Fig. 2). No differences in PM cortisol levels were seen between children with PAE + ELA who used medication and those who did not. Finally, within the PAE + ELA group, no difference in AM or PM cortisol levels or slope for cortisol were found between those with rank 3, or 4, PAE ($p = .77$ to $.96$). In other words, the impact on cortisol secretion and variation was similar in both PAE groups, regardless of the level of reported exposure.

The effects of gender on AM, PM, and slope cortisol values were next evaluated using a series of ANCOVAs, treating age as a covariate. Main effects for age ($F = 7.79$, $p = .01$, η^2_p

= .09) and gender were found ($F = 5.69, p = .02, \eta^2_p = .07$), but no sex $\times$ study group interactions were found for AM cortisol. *Post hoc* pairwise comparisons revealed that across groups, boys (mean = .23, SD = .11) had significantly lower AM cortisol levels compared to girls (mean = .30, SD = .13), mean difference = .06, SE = .03, $p = .02$. The same pattern was evident for cortisol slope, with main effects of age ($F = 8.76, p = .004, \eta^2_p = .10$), gender ($F = 5.77, p = .02, \eta^2_p = .07$), and group ($F = 4.41, p = .04, \eta^2_p = .05$), but no sex $\times$ study group interaction. *Post hoc* pairwise comparisons indicated that cortisol slope was significantly steeper in girls (mean = .26, SD = .13) than boys (mean = .17, SD = .13), mean difference = .07, SE = .03, $p = .02$. Analyses to assess the impact of SES (continuous score) on basal cortisol levels revealed no significant effects. As noted, only three low SES families were identified for the control group, thereby limiting meaningful group-wise comparisons. However, an exploratory descriptive evaluation of SES (trichotomized) on basal cortisol levels showed that children and adolescents with PAE + ELA residing in low and moderate SES households had directionally higher evening cortisol levels relative to those living in high SES households (Fig. 3). No comparable pattern emerged for either AM cortisol levels or slope.

Cortisol regulation, early life adversities, adverse outcomes, and protective factors

Partial correlations between ELAs, adverse outcomes, and protective factors for the PAE + ELA group, controlling for age, are presented in Table 2. Looking first at ELA, a history of foster care involvement was significantly associated with higher AM cortisol levels and steeper slope, while a history of abuse was associated with lower PM cortisol. Greater caregiver adversity was associated with a steeper cortisol slope. In regard to adverse outcomes, both history of criminal justice system contact and substance abuse problems were associated with higher AM cortisol levels. With respect to indicators of protective factors, a younger age at the time of PAE/FASD assessment was associated with lower PM cortisol levels, which held after controlling for current age using a multiple linear regression, $b = .003, t(32) = 3.16, p = .003, d = 1.11$. Participants who were assessed and/or diagnosed before age 11 ($n = 23, 65.7\%$) had significantly lower AM cortisol levels ($.05 \pm .04 \mu\text{g/dL}$) compared to those diagnosed during adolescence ($.08 \pm .05 \mu\text{g/dL}$), $t(33) = -2.42, p = .02, d = .84$. Lengthier placement in a stable home was associated with lower AM cortisol levels.

Discussion

Children and adolescents with PAE experience a range of difficulties in self-regulation and adaptive functioning, as well as high rates of ELA. Studies of infants and toddlers with PAE suggest possible disruption in HPA axis function, indexed via changes in cortisol activity, as one factor that might underlie some of these behavioral difficulties (Jacobson et al., 1999; Oberlander et al., 2010; Ouellet-Morin et al., 2011; Ramsay et al., 1996). To our knowledge, only one previous study has examined cortisol function in school-age children/adolescents, and that study demonstrated an altered diurnal rhythm following PAE (Keiver et al., 2015). Our data support and extend these findings in two key directions. First, given high rates of adversity that often characterize the experiences of children with PAE, it has been difficult, if not impossible, to separate the consequences of PAE from those of ELA on child outcomes in previous studies. Moreover, the adverse effects of ELA and PAE are in many

ways parallel with respect to HPA activity and regulation. Thus, we focused on the combined impact of PAE and ELA in school-age children and adolescents. Second, in addition to demonstrating basal HPA dysregulation as indexed by the cortisol diurnal rhythm, we elucidate important associations among ELA, adverse outcomes, protective factors, and cortisol regulation in this population.

Our finding that participants with PAE + ELA had significantly higher evening cortisol concentrations, a trend for lower morning cortisol concentrations, and a flatter slope over the day is consistent with the work of Keiver et al. (2015), and with data from human and animal studies showing higher basal and post-stress cortisol levels following PAE (Jacobson et al., 1999; Weinberg et al., 2008). These data lend support to the robustness of effects shown in the present study, and to the hypothesis that diurnal cortisol secretion patterns may provide a biological marker of dysregulated HPA function in children following PAE + ELA. Additional indicators of difficulties with self-regulation and adaptive function seen in children with PAE, such as problems with executive functioning and sleep (Jan et al., 2010; Rasmussen, 2005), are known to be linked with abnormal cortisol levels in typically developing children, children with ADHD, and those from low-income backgrounds (Blair, Granger, & Peters Razza, 2005; Blair et al., 2011; El-Sheikh, Buck-halt, Keller, & Granger, 2008; Scher, Hall, Zaidman-Zait, & Weinberg, 2010). Further research evaluating the complex interplay between HPA axis activity and self-regulatory mechanisms following PAE may offer a more complete understanding of the challenges in daily functioning faced by children with FASD. Importantly, we observed a pattern of flattened slope over the day and elevated evening cortisol levels in children exposed to both low to moderate or unknown (“some risk”) PAE levels, as well as confirmed significant (“high risk”) PAE levels, suggesting that PAE may impact HPA axis regulation in children and adolescents with ELA across the spectrum of exposure.

In keeping with seminal studies in the field, we observed high rates of ELA in our PAE + ELA sample, as well as higher rates of adverse outcomes (e.g., Streissguth et al., 2004). Adverse outcomes included contact with the criminal justice system and substance abuse problems. This finding is particularly concerning given that only just over half the sample had emerged into adolescence, suggesting that rates of adverse outcomes in the current group of children and adolescents will very likely increase with age. Keiver et al. (2015) noted that ELA was likely present at high frequency in their sample and that greater understanding of such experiences might help to further explain their findings. We suggest that the effects of ELA, acting on a system already sensitized by PAE, may further exacerbate the HPA disturbances and contribute to adverse outcomes observed. Increasingly, human studies are attempting to understand the conditions under which low to moderate PAE levels impact functional outcomes (Andersen, Andersen, Olsen, Grønbæk, & Strandberg-Larsen, 2012; Valenzuela, Morton, Diaz, & Topper, 2012), underscoring the importance and broad implications of our findings, as well as the need for replication.

Medication effects reflect a likely confounding factor in all clinical FASD research, and our findings indicate that medication use is a moderating factor for HPA function. In the present study many of the children and adolescents with PAE + ELA reported medication use at study enrollment (primarily stimulant and non-stimulant medications typically prescribed for

ADHD, and atypical antipsychotics, as noted), and these children showed significantly lower morning cortisol levels compared to both children with PAE + ELA who were not taking medications and controls. Findings from studies examining the effects of these types of medications on cortisol levels are mixed, showing either elevated basal cortisol levels or no effects (Isaksson, Hogmark, Nilsson, & Lindblad, 2013; Lee et al., 2008; Wang, Huang, Hsiao, & Chen, 2012). At least one study has shown an opposite pattern of findings in children with combined-type ADHD, with more typical morning cortisol levels in children taking stimulant medications, and depressed morning levels in those untreated (Kariyawasam, Zaw, & Handley, 2002). There is presently limited evidence concerning the efficacy or effect of medications used to treat common problems in children with FASD (Koren, 2015; Ozsarfati & Koren, 2015). These medications could exert physiological and/or behavioral effects that impact diurnal cortisol levels. Conversely, it is possible that children presenting with the greatest degree of behavioral dysregulation, potentially coupled with diurnal cortisol dysregulation, are those who are placed on medication. Our findings highlight the importance of further study in order to better understand the impact of medication on self-regulatory processes and underlying physiological processes, including HPA axis function, in children and adolescents with PAE + ELA.

ELA, adverse outcomes, and diurnal cortisol activity in children and adolescents with PAE + ELA

Although there is clear evidence that ELA can exert adverse effects on HPA axis regulation and diurnal cortisol activity, the reported nature and directionality of changes over the day remain inconsistent across the literature and complicated by effects of moderating and mediating factors on developmental outcomes (Chida & Steptoe, 2009; Cicchetti et al., 2010). Increasingly, studies are finding that HPA axis dysregulation in response to ELA may manifest through both hyper- and hyporesponsive patterns of functioning, depending on the context and vulnerabilities of a given individual under stress. Consequently, the field appears to be moving away from the identification of “optimal” cortisol levels or “optimal” diurnal patterns (Cicchetti et al., 2010; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Heim, Plotsky, & Nemeroff, 2004; McEwen, 2000). Cicchetti et al. (2010) stated:

“Overall, predicting that the measures of the HPA system will reflect dysregulation of the axis, rather than predicting that any given measure will be elevated or suppressed, reflects the current status of knowledge in the field. This is especially true in research on young children from vulnerable populations, where researchers are often limited to assessing basal measures of cortisol at only one or a few times during the day.” (p. 253)

Consistent with this approach, in the present study we found a number of associations between specific ELAs and indicators of diurnal cortisol activity, and these findings support the suggestion that interpreting the meaning of specific morning and evening cortisol levels or patterns remains a challenge. For instance, while children with PAE + ELA had higher evening cortisol levels overall compared to typically developing children, a history of physical and/or sexual abuse was associated with lower evening cortisol levels, more comparable to those in controls. This may suggest a possible blunting of basal cortisol levels in response to a chronic stressor in a sample where PM levels were otherwise elevated

relative to controls, and is consistent with findings from at least one other study evaluating the impact of early abuse on later HPA axis functioning (van der Vegt, van der Ende, Kirschbaum, Verhulst, & Tiemeier, 2009). On the other hand, higher caregiver adversity in the PAE + ELA group was linked with a steeper cortisol slope, characterized by trends toward both higher morning and lower evening cortisol concentrations. The importance of caregiving as both a key variable in healthy neurobiological development of the HPA axis and emotional health, and a buffer against the effects of early life adversity, is well established (Loman, Gunnar, & Early Experience Stress and Neurobehavioral Development Center, 2010). While caregiver adversity is clearly a factor moderating HPA activity in children with FASD in the present study, further work is needed for a full understanding of the directionality of our findings.

Altered cortisol activity was also associated with criminal justice system involvement and substance-abuse problems in our sample, with higher morning cortisol levels in these children and adolescents compared to those who had not experienced these problems. Although there is a general tendency to see decreased or blunted basal and stress-activated HPA hormone levels in children who engage in externalizing and antisocial behavior, little of the research in this area has focused on circadian changes (Alink et al., 2008; Fairchild, van Goozen, Calder, & Goodyer, 2013; Haltigan, Roisman, Susman, Barnett-Walker, & Monahan, 2011; Platje et al., 2013; Shirtcliff, Granger, Booth, & Johnson, 2005; van Goozen, Fairchild, Snoek, & Harold, 2007). One commonly cited hypothesis suggests that children who show externalizing and antisocial behavior have elevated thresholds for stress. Becoming easily bored, they seek to sate this low state of arousal through antisocial behavior (Zuckerman & Neeb, 1979), and blunted HPA function may lead to efforts to “normalize” the stress response. De Bellis et al. (1999) suggest that altered basal cortisol levels, such as those in our PAE + ELA sample, may reflect the activation of a compensatory strategy whereby the stress regulatory system attempts to buffer dysregulation of the HPA axis by “resetting” morning or evening levels to more moderate levels. This may partially explain these findings, and would be in keeping with the theoretical frameworks of allostasis and allostatic load, concepts that are increasingly being applied to cortisol research in the context of ELA (Cicchetti et al., 2010; McEwen, 2000; Suor et al., 2015).

Finally, trends suggested that higher evening cortisol levels were particularly evident under conditions of low SES for children with PAE + ELA compared to those from moderate- and high-SES households, who appeared to have levels more comparable to controls. This is consistent with a sound body of evidence showing that children from lower SES backgrounds have higher cortisol concentrations than those from higher SES households (Essex et al., 2002; Evans & Kim, 2007; Lupien et al., 2000, 2001). In keeping with the stress-diathesis model, we hypothesize that PAE may program or sensitize the developing HPA axis, such that later exposure to stressors and adversity significantly increases the likelihood of not only altered HPA axis function, but also impaired neurobehavioral, physical, and mental health outcomes. In turn, these real world functional deficits may predispose an individual to further stressors and negative outcomes, fueling the stress-diathesis cycle (Hellems et al., 2010). Our findings also suggest that higher SES, as a marker for some combination of improved social, economic, and caregiving circumstances, may be somewhat protective with respect to healthy cortisol regulation and underlying HPA

axis functioning, consistent with a large body of research showing the adverse impact of poverty on cortisol regulation (Desantis, Kuzawa, & Adam, 2015; Lupien et al., 2009).

The present data extend the findings of Keiver et al. (2015) by examining factors that may moderate outcomes in children/ adolescents with FASD, and provide novel information on the associations among a number of specific ELAs and cortisol activity over the day in this population. Together, our findings clearly suggest dysregulation of the diurnal cortisol rhythm, but consistent with Cicchetti et al. (2010), our interpretation of the meaning of specific AM or PM increases or decreases remains cautious. More sophisticated analytic approaches may help to inform future research, such as those employed by Suor et al. (2015), who applied growth-mixture modeling pattern analysis to tease apart the heterogeneity in basal cortisol activity over time in a high-risk sample of young children experiencing both low SES and different types of familial adversity. They identified three patterns (elevated, moderate, and low) of basal cortisol levels, as well as specific family risk factors differentiating cortisol patterns at a subsequent time point. They also showed that both low and elevated basal cortisol levels were associated with lower cognitive functioning when children were 4 years old, consistent with the possibility that both hypo- and hyper-responsiveness may occur following adversity, and demonstrating that different patterns of HPA axis dysregulation may result in similar poor developmental outcomes. Moving toward more sophisticated longitudinal models such as these in future studies with larger sample sizes may facilitate better understanding of the unique contribution of various ELAs in children with PAE.

Protective factors

Early assessment represents a key protective factor against later problems and adversity for children with PAE (Rasmussen, 2012; Streissguth et al., 2004), and our findings complement this work. Children who were assessed for FASD at earlier ages showed more typical cortisol patterns relative to those assessed later, and likely reflect the fact that these children had opportunities to access interventions and services earlier in development. Participants with PAE + ELA who had greater access to stable and quality home environments also had more typical cortisol patterns (lower basal levels in the evening), suggesting that interventions bolstering this area, even following PAE + ELA, may improve biological regulation of the stress system and self-regulation over the day. Interventions targeting self-regulation, relationships, psychosocial functioning, and the caregiving environment following childhood adversity have been shown to result in normalization of cortisol regulation in studies of young children (Slopen et al., 2014). Thus, interventions targeting ELA may have an important physiological impact “under the skin” and should be explored in children with PAE.

Limitations and future directions

The present study provides novel data on associations among cortisol regulation, ELA, adverse outcomes, and protective factors in children and adolescents with PAE + ELA. Results support our hypothesis that the combination of PAE and ELA may be linked with HPA dysregulation, and conversely, that the presence of protective factors may be linked with more typical cortisol patterns. However, several limitations warrant discussion,

including the interpretative challenges raised by the clinical complexity of our PAE + ELA sample. Consistent with other seminal studies (Streissguth, Barr, Kogan, & Bookstein, 1996), in addition to PAE, the children and adolescents in our sample experienced substantial ELA, rendering it difficult to separate direct effects of PAE from those of environmental stressors on cortisol dysregulation. While animal research provides clear evidence that PAE results in HPA dysregulation in the absence of adversity (Hellemans et al., 2010), in human populations, cortisol dysregulation likely reflects a complex interaction between PAE and environmental experiences, in addition to the likely role of genetic and epigenetic influences on HPA function (Chen, Coles, Lynch, & Hu, 2011; Dodge, Jacobson, & Jacobson, 2014; Gilliam & Kotch, 1992; Kobor & Weinberg, 2011; Tunc-Ozcan, Sittig, Harper, Graf, & Redei, 2014). Rather than view this complexity as a limitation, it should be viewed within the real-world context, and as one that has emerged more broadly for the ELA/HPA field as research has moved from animal models toward human studies (Cicchetti et al., 2010).

This complexity also raises challenges in selecting the most appropriate control group. We opted to contrast our PAE sample with typically developing children of similar age and gender who had not experienced PAE. This provides a benchmark for comparison to typical development, given that there are no agreed-upon norms for the absolute concentrations of free cortisol in saliva across the diurnal cycle, and that it can be difficult to compare absolute cortisol values across studies using a range of sampling, assaying, and analytic techniques (Bruce, Fisher, Pears, & Levine, 2009; Clow, Thorn, Evans, & Hucklebridge, 2004). Research using larger samples, longitudinal approaches, and assessment of children with PAE who have experienced little or no adversity, along with children who have high ELA but no PAE, would increase our understanding of the relative contributions of PAE and ELA. However, recruitment of these latter two groups will be challenging.

Our assessment of cortisol function also has some limitations. Although measurement of morning and evening cortisol levels provides an important snapshot of regulation across the day, associations between the cortisol awakening response (CAR) and HPA dysregulation following ELA have been observed in other populations (Gonzalez, Jenkins, Steiner, & Fleming, 2009; Keeshin, Strawn, Out, Granger, & Putnam, 2014). Taking additional samples to capture other time points during the day would help in evaluating the nuances of PAE-induced alterations in HPA axis activity. Evaluating cortisol responses to challenge could provide additional information and extend the current literature on stress responses in infants and toddlers with FASD. However, attempting to solicit multiple salivary cortisol samples at fixed time points among children who often function in “real world” chaotic home environments introduces challenges that may be difficult to overcome. Diary cards were completed by only a subset of our sample, which is another possible limitation. Nevertheless, analyses suggested good adherence to data collection protocols whether or not diary cards were completed, and there was no association between time of day at sample collection and AM or PM cortisol concentrations.

Conclusion

This study provides significant novel insights into HPA axis functioning in children and adolescents with PAE + ELA and the important associations among ELA, adverse outcomes, protective factors, and cortisol regulation in this population. Results complement and extend findings from research on human infants and animal models showing altered cortisol activity and stress responsivity following PAE and have important implications for the daily functioning of individuals with FASD. Due to the widespread effects of glucocorticoid hormones on virtually every system of the body, altered sensitivity of the HPA axis to stressors and the inability to respond appropriately to these stressors may render these individuals particularly susceptible to the impacts of ELA and increase the incidence of adverse outcomes such as depression and metabolic disorders later in life. The relationship between PAE and ELA is likely not linear, and the high rates of adversity commonly seen in this population only further fuel the damaging effects of life stressors or challenges on an already sensitized system. The novel and important findings from this study underscore the need to identify “at risk” children as early as possible, and to provide access to assessment, diagnosis, and support in an effort to offset the damaging interactive effects of PAE and ELA.

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Fig. 1.

Basal cortisol levels for AM and PM in children with PAE + ELA and controls. Note: $n^{\text{FASD}} = 42$, $n^{\text{Control}} = 43$. Data are presented as raw, untransformed values for both AM and PM salivary cortisol values to facilitate interpretation. Lines represent slope scores (difference between mean AM and PM cortisol values). * = $p < .05$.

Fig. 2.

Children with PAE + ELA using medications have significantly lower morning cortisol levels than children with PAE + ELA not using medications. Note: $n^{\text{FASD}} = 42$, $n^{\text{Control}} = 43$. Presented cortisol values are untransformed to facilitate interpretation.

Fig. 3.

Differences in PM cortisol levels evident under low vs. high SES conditions in the PAE + ELA group compared with controls. Note: $n^{\text{FASD}} = 42$, $n^{\text{Control}} = 43$. Only 3 control participants are included in the Low-SES group.

Table 1

Participant characteristics and basal cortisol levels.

	PAE	Control	Statistic	<i>p</i>	Effect size
Age (<i>M, SD</i>)	11.81 (3.33)	11.28 (2.85)	.79 ^a	.43	.17 ^d
SES (<i>M, SD</i>)	35.11 (14.10)	47.10 (8.09)	-4.81 ^a	<.001	1.06 ^d
Low (<i>n, %</i>)	13 (31)	3 (7)			
Moderate	24 (57)	31 (72)	8.27 ^b	<.05	.31 ^e
High	5 (12)	9 (21)			
Gender (<i>n, %</i> male)	22 (52)	16 (37)	1.98 ^b	.16	.15 ^e
Caregiver (<i>n, %</i>)					
Biological family	5 (12)	43 (100)	63.94 ^b	<.001	.87 ^e
Adopted/foster/other	37 (88)	0 (0)	18.65 ^b		
Diagnosis (<i>n, %</i>)					
FAS	2 (5)	—			
pFAS	5 (12)	—			
ARND	22 (52)	—			
None	13 (31)	—			
Other diagnoses (<i>n, %</i>)	30 (71)	—			
ADHD	23 (55)	—			
Internalizing	9 (21)	—			
Externalizing	4 (10)	—			
Psychotic/bipolar	3 (7)	—			
Sleep	5 (12)	—			
Other	5 (12)	—			
Medications (<i>n, %</i>)	17 (40)	—			
Stimulant ADHD	9 (21)	—			
Non-stimulant ADHD	6 (14)	—			
Atypical antipsychotics	6 (14)	—			
Other	10 (24)	—			
Number of home placements (<i>M, SD</i>)	3.97 (2.24)	—			

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	PAE	Control	Statistic	<i>p</i>	Effect size
Range	1–10				
Cortisol (<i>M</i> , <i>SD</i>)					
AM	.24 (.13)	.29 (.12)	3.38 ^c	.07	.04 ^f
PM	.06 (.04)	.04 (.03)	4.92 ^c	.03	.06 ^f
Slope	.19 (.15)	.25 (.13)	5.68 ^c	.02	.07 ^f

^a*r*²_{PAE} = .42; *r*²_{control} = .43. SES = Socioeconomic Status, calculated using Hollingshead's Four-factor Index (Hollingshead, 1975). FAS = Fetal alcohol Syndrome, pFAS = Partial fetal alcohol syndrome, ARND = Alcohol-Related Neurodevelopmental Disorder, PAE = prenatal alcohol exposure. Stability of Home Index = sum of yes/no items pertaining to home stability and quality. Numbers were bolded in the table to visually highlight findings that reached a threshold of statistical significance at or below *p* = .05.

^a*t* statistic.

^b χ^2 statistic.

^c = *F* statistic.

^d Cohen's *d*.

^e ϕ .

^f η^2_p .

Table 2

Basal cortisol levels and ELA in children and adolescents with PAE + ELA.

	Frequency	AM	PM	Slope
	<i>n</i> (%)	<i>r^b</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)
Early life adversities				
Foster care	30 (86)	.41 (.04)	-.27 (.18)	.43 (.03)
Physical/sexual abuse	19 (56)	-.11 (.60)	-.41 (.04)	.08 (.71)
Neglect	20 (57)	.05 (.80)	.07 (.74)	.07 (.74)
Parental adversity, <i>M</i> (<i>SD</i>)	2.38 (.135)	.31 (.13)	-.30 (.14)	.44 (.02)
Adverse outcomes				
School punishments	20 (57)	.15 (.44)	.15 (.42)	.12 (.51)
Trouble with the law	7 (20)	.14 (.46)	.36 (.04)	.01 (.97)
Substance abuse	7 (20)	.18 (.33)	.49 (.01)	-.01 (.97)
Protective factors, <i>M</i> (<i>SD</i>)				
Age at assessment ^a	7.76 (3.18)	.06 (.76)	.46 (.01)	-.03 (.89)
Years in stable home	.65 (.26)	-.44 (.01)	.16 (.39)	-.49 (.01)

n = 35. ELA = Early life adversity. Years in Stable Home = ratio of the number of years spent in a stable caregiving/home situation relative to age. Bolded text is intended to draw attention to statistically significant correlations at the *p*.

^a Assessment dates were not available for three participants thus age at assessment could not be calculated.

^b Partial correlations controlling for participant age.