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Disaster-related prenatal maternal stress, and childhood HPA-axis regulation and anxiety: the QF2011 Queensland Flood Study

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Highlights

- Examined toddler cortisol indexes as mediator of PNMS effect on 4-year child anxiety
- Greater toddler CAR mediated effect of subjective PNMS on child anxiety
- Independent of PNMS, greater daily cortisol output was related to child anxiety
- Maternal separation paradigm did not elicit a stress (cortisol) response in toddlers
- HPA-axis functioning: a biological correlate of disaster-PNMS and anxiety symptoms

Background: The fetal programming hypothesis suggests that prenatal maternal stress (PNMS) influences aspects of fetal development, such as the Hypothalamic Pituitary Adrenal (HPA) axis, enhancing susceptibility to emotional problems. No study (to our knowledge) has investigated this pathway considering development of preschool anxiety symptoms.

Using data from the Queensland Flood study (QF2011), our objective was to determine whether toddler HPA-axis functioning mediated the association between aspects of flood-related PNMS and child anxiety symptoms at 4-years, and whether relationships were moderated by the timing of the stressor in utero or by the child's sex.

Methods: Women, pregnant during the 2011 Queensland floods (N = 230), were recruited soon afterwards and completed questionnaires regarding their objective hardship (e.g., loss of personal property), subjective distress (post-traumatic-like symptoms) and cognitive appraisal of the disaster. At 16 months, indexes of the child's diurnal cortisol rhythm (awakening response, total daily output, diurnal slope [N = 80]), and stress reactivity (N = 111), were obtained. At 4-years, N = 117 mothers reported on their own mood and their children's anxiety symptoms; of these, N = 80 also had valid child cortisol reactivity data, and N = 64 had diurnal cortisol rhythm data.

Results: A greater cortisol awakening response at 16 months mediated the relationship between subjective PNMS and anxiety symptoms at 4-years. Greater toddler daily cortisol secretion predicted more anxiety symptoms, independent of PNMS. The laboratory stressor did not elicit a cortisol response. PNMS effects were not dependent upon child sex nor on gestational timing of flood exposure.

Conclusions: Indexes of diurnal cortisol in toddlerhood may represent vulnerability for anxiety symptoms in preschoolers, both independent of, and following, exposure to disaster-related prenatal maternal subjective distress.

Keywords: *childhood anxiety, prenatal maternal stress, disaster, Hypothalamic pituitary adrenal axis, stress reactivity, cortisol awaking response*

1. Introduction

Anxiety is one of the most common childhood mental health disorders (Polanczyk et al., 2015). Research shows that prenatal maternal distress (pregnancy anxiety, depression, psychological distress) is associated with offspring vulnerability for anxiety in young people (McLean, Cobham, & Simcock, 2018). Despite linking prenatal maternal stress (PNMS) to offspring psychopathology, biological correlates of these relationships are poorly understood. The fetal programming hypothesis (Gluckman & Hanson, 2006) posits that development of fetal biological systems, including the hypothalamic pituitary adrenal (HPA) axis – a key biological stress response system – are 'programmed' by in-utero exposure to elevated maternal stress hormones that increase vulnerability for poorer childhood socio-emotional functioning, such as anxiety symptoms.

The HPA-axis releases cortisol in response to stress. Cortisol levels follow a diurnal pattern, with levels high upon waking and peaking 30-40 minutes later followed by a steady decline across the day (Stalder et al., 2016). The pattern of secretion in the first hour after waking is called the cortisol awakening response (CAR). Basal cortisol levels support the capacity for freeze/fight/flight responses to threat, whereas short, stress-related elevations help regulate behavioral and sympathetic reactivity. Generally, research suggests exposure to elevated stress in utero is associated with heightened stress-responses in childhood including greater cortisol secretion in reaction to the stress of waking (CAR; O'Connor et al., 2005), variation in total cortisol output across the day (Neuenschwander et al., 2018; O'Donnell et al., 2013), or in their reactions to a stressor (Tollenaar et al., 2011; Yong Ping et al., 2015). Yet, others find no association between PNMS and cortisol levels (e.g. Davis, Glynn, Waffarn, & Sandman, 2011), suggesting further investigation is warranted.

Identification of PNMS-driven biological correlates helps to progress our understanding of pathways through which PNMS is related to childhood behavior problems. HPA-axis functioning is considered a key biological system as PNMS-exposure has been linked to children's cortisol levels, and stress-reactivity and diurnal cortisol profiles are associated with concurrent or subsequent anxiety

symptomatology. Generally, in non-clinical populations, greater responsiveness to stressors (Gunnar et al., 2009, 2011), higher CAR, and attenuated decreases across the day (Greaves-Lord et al., 2007; Saridjan et al., 2014) are associated with internalizing behaviors (anxiety, depressive symptoms) and anxiety symptoms. Yet, investigations of the hypothesized pathway are scant. Building on animal studies (Weinstock, 2008), altered diurnal cortisol profiles in humans may contribute to the link between prenatal maternal mood and executive functioning in boys (Neuenschwander et al., 2018) and adolescent depression in girls (Van den Bergh et al., 2008). Regarding stressor-reactivity, Martinez-Torteya et al. (2016) showed in-utero exposure to intimate-partner violence was associated with greater cortisol reactivity and internalizing behaviors at 10 years. However, a recent study failed to establish 'programming' effects of prenatal maternal anxiety and depression on 12-month-old infants' behavior (Galbally et al., 2019). Whether variation in diurnal and/or reactive cortisol profiles following disaster related PNMS are associated with anxiety symptoms during preschool years has yet to be determined.

Utilizing an independently-occurring stressor in pregnancy enables examination of different components of PNMS (e.g., objective hardship, subjective distress and cognitive appraisal) on biological systems and behavioral outcomes, independent of mother-child heritability factors. Studies examining PNMS in relation to a random stressor such as a sudden-onset natural disaster enables researchers to specify the stressor's onset during gestation and determine how the timing of this stressor affects child development. According to the fetal programming hypothesis, maternal stress-related hormones (e.g., glucocorticoids) cross the placental barrier affecting aspects of fetal neurodevelopment at sensitive periods in gestation (Gitau et al., 2001). In particular, fetal organs that are in a period of rapid growth or differentiation at the time of the stress are thought to be most vulnerable to maternal glucocorticoids (Gitau et al., 2001). This hypothesis also suggests that, maternal glucocorticoids may moderate fetal neurodevelopment given that male and female placentas respond differently to in utero exposure to maternal stress hormones (Clifton, 2010). Consistent with the fetal programming hypothesis, Yong Ping, Elgbeili, Laplante & King et al. (2015) showed that greater objective hardship and later pregnancy

exposure to the 2008 Iowa floods predicted higher toddler cortisol responses to a maternal-separation, and that greater maternal subjective distress (post-traumatic stress–like symptoms) predicted higher cortisol reactivity in girls only. Continued investigation of disaster-related PNMS in relation to fetal sex and timing of PNMS in-utero will illuminate mechanisms through which PNMS affects anxiety.

Past research has examined the associations between PNMS-related HPA-axis functioning and children's internalizing behaviors as indicative of anxiety symptoms (Galbally et al., 2019; Martinez-Torteya et al., 2016). While comorbidity of anxiety and depressive symptoms are high, during the preschool years anxiety symptoms are more easily identified (Whalen et al., 2017). Moreover, endogenous and exogenous correlates of anxiety and depressive symptoms vary (Hopkins et al., 2013; Ryan & Ollendick, 2018; Whalen et al., 2017). For these reasons, we isolated anxiety symptoms from other internalizing behaviors. We recently showed that flood-related PNMS due to the 2011 Queensland floods predicts childhood anxiety symptoms (McLean, Cobham, Simcock, et al., 2018). Specifically, higher flood-related objective hardship during pregnancy and earlier in utero exposure to the floods predicted increased anxiety symptoms in 4-year-olds, independent of maternal subjective stress and child sex. Following from this study (McLean, Cobham, Simcock, et al., 2018) here we examine whether toddler (16-month-old) HPA-axis functioning, (i.e., diurnal cortisol; cortisol reactivity), mediates associations between flood-related PNMS (objective hardship, subjective distress, cognitive appraisal) and preschoolers' (age 4-years) anxiety symptoms.

We hypothesized that:

1. The association between PNMS and preschooler' anxiety will be explained in part by toddler physiological measures of stress reactivity (elevated cortisol levels and greater total cortisol secretion) following maternal separation.
2. The association between PNMS and preschooler' anxiety will be explained in part by toddler diurnal cortisol patterns.

Mediation effects may be moderated by child sex (stronger effects for girls) and timing of exposure in gestation (exploratory analyses).

2. Methods and Materials

2.1. Participants and Procedure

The QF2011 study included English-speaking women over 18 years of age who were pregnant with a singleton on January 10, 2011 during the 2011 Queensland floods. Enrolment in the study commenced upon receiving ethical approval in April 2011 and continued until 12 months post-flood (January 2012). Most women were recruited into the study by midwives during antenatal visits at a public tertiary hospital in the flood-affected region, while other women responded to advertisements located in the reception areas of local hospitals and medical practices. A total of 68 women were pregnant at recruitment and 162 women had already given birth. At the time of survey completion for those who had given birth, their children were on average 14 weeks old (range 0 – 43 weeks). At recruitment into the study, and at 12 months post-flood, mothers completed surveys regarding their demographics, flood-related experiences (objective hardship) and reactions (subjective stress and cognitive appraisal). The mean delay between the flood and completing the intake survey, was 42 weeks (range 13 – 67 weeks). At 16 months, mother-toddler dyads completed the laboratory visit and stress-paradigm protocol and/or the daily saliva cortisol sampling protocol. At 4-years postpartum, mothers also completed surveys on their own mental health and on their child's anxiety symptoms.

Full eligibility, recruitment and procedural details can be found elsewhere (King et al., 2015). Figure 1 provides a flow-chart of QF2011 participation, specific to the assessments outlined in the current study including exclusion criteria and final sample sizes. The study had ethical approval from the study site's ethical review board (Mater Mothers' Hospital # 1844M) and the affiliated university ethics committee (The University of Queensland, #2013001236). All participants provided written, informed consent at each time point in the study.

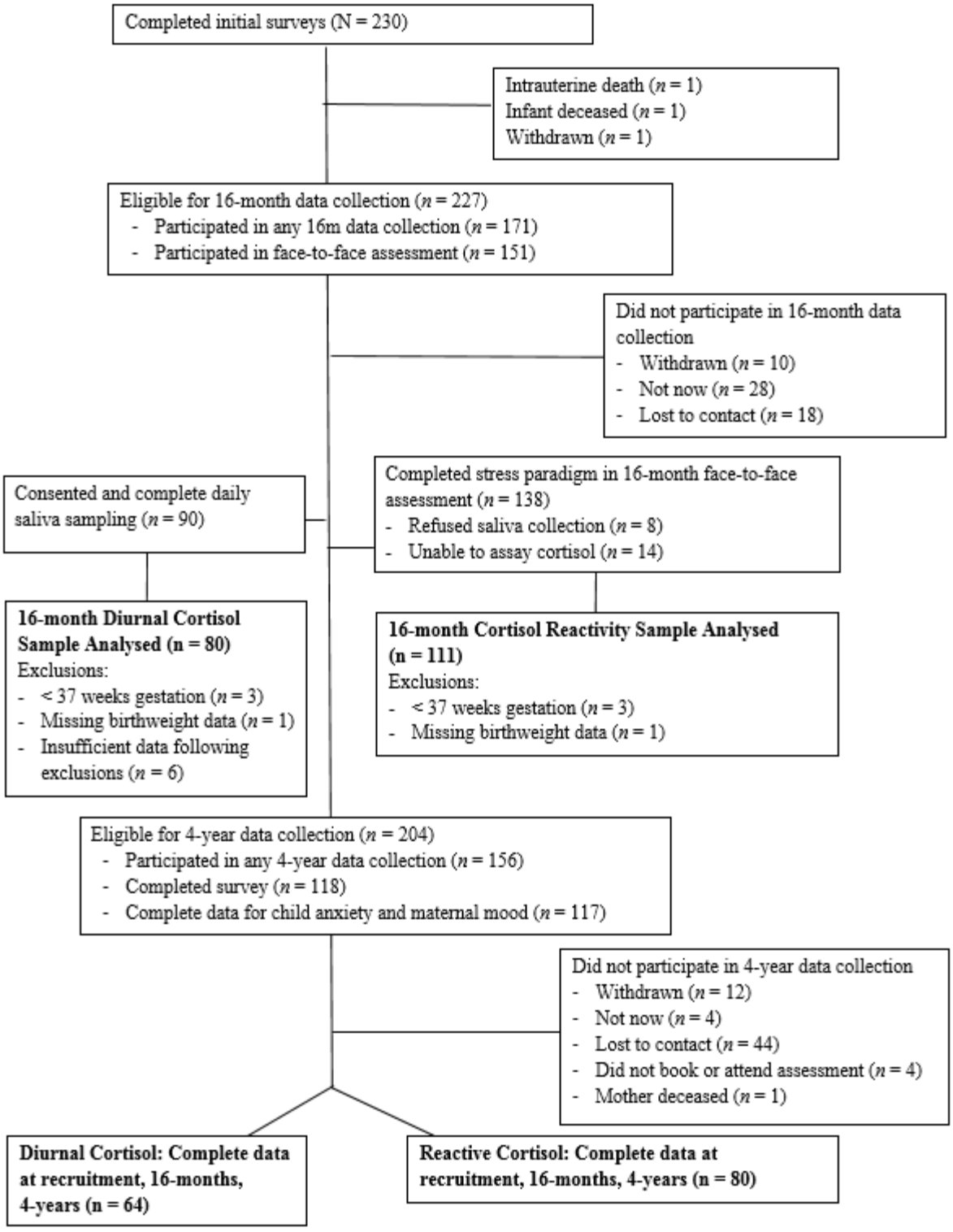

Figure 1. Flow diagram of participation, criteria for inclusion in the current study and final samples at each stage of QF2011 included in the current study (bolded).

2.2 Maternal Flood-Related Variables

2.2.1. O***bjective hardship*** due to flood-exposure was assessed using a questionnaire tailored specifically for the Queensland flood event (King et al, 2015), similar to one used in the Iowa Flood Study (Yong Ping et al., 2015). The items assessed four key dimensions of the women's disaster exposure: Threat (e.g., "Were you injured?"), Loss (e.g., "Did you experience loss of personal income?", Scope (e.g., "How many days were you without electricity?") and Change (e.g., "Did you spend any time in a temporary shelter?"). Points were attributed to each item according to a pre-determined protocol, with points for each scale ranging from 0 (no impact) to 50 (extreme impact), giving a total possible Queensland Flood Objective Stress Score (QFOSS) of 200. Higher scores indicated higher levels of flood-related hardship.

2.2.2. S*ubjective stress* reactions to the Queensland flood were assessed using three questionnaires. The women's initial reactions to the flood were assessed using the 13-item Peritraumatic Distress Inventory (PDI; Brunet et al., 2001) and the 10-item Peritraumatic Dissociative Experiences Questionnaire (PDEQ; Marmar, Weiss, & Metzler, 1997). These tools capture the immediate reactions to the event, recalled retrospectively, including panic-like physical reactions. The women rated statements on each questionnaire on a 5-point rating scale ('not true' to 'extremely true'). The women's emotional responses to the disaster were also assessed using the Impact of Event Scale – Revised (IES-R; Weiss & Marmar, 1997), which includes scores for post-traumatic stress (PTS) symptoms, including intrusion, hyperarousal and avoidance. The women rated the severity of 22 items over the past seven days in relation to the Queensland flood on a 0 (not true) to 4 (extremely true) Likert scale; scores of 32 or above indicate possible PTSD.

Principal Component Analysis (PCA), on the IES-R, PDI and PDEQ total scores from the N=230 participants who provided PNMS data, was used to calculate the COmposite Score for MOthers' Subjective Stress (COSMOSS). The PCA-derived algorithm was: COSMOSS = 0.36*IESR + 0.40*PDI +

0.39*PDEQ, explaining 76.27% of the overall subjective stress variance. The COSMOSS variable is standardized with mean of 0.0 and standard deviation of 1.0, so a positive score represents a level of subjective stress that is higher than the mean.

2.2.3. ***Cognitive appraisal*** of the overall impact of the flood was assessed using a single item. Women responded to the question "If you think about all of the consequences of the 2011 Queensland flood on you and your household, would you say that the flood has been…" on a 5-point Likert scale from -2 (very negative) to +2 (very positive). The scores were dichotomized into 'negative' versus 'neutral/positive', due to the limited range of responses and to differentiate the women who considered the consequences of the flood to be negative from those who did not.

2.2.4. ***Timing of flood in pregnancy*** was calculated by subtracting the number of days between the flood peak date (January 10, 2011) and each woman's date of conception. Date of conception was estimated from the woman's expected due date. The larger the number, the later in pregnancy the flood occurred.

2.3. Toddler Cortisol Variables (16 months)

2.3.1. ***Diurnal salivary cortisol*** was collected on two consecutive days by the toddlers' mothers at home**.** Two weeks prior to her toddler's 16-month birthday, the study research assistant (RA) contacted the mother and described the protocol for the home cortisol collection, addressing any queries. The RA mailed a home cortisol collection kit to the mothers for saliva samples to be taken upon toddler awaking (M = 6:44 a.m., SD = 57 minutes), awakening + 30 minutes (M = 7:15 a.m., SD = 58 minutes) and bed-time (M = 7:14 p.m., SD = 52 minutes). The kit included instructions for the toddler to avoid medication, food or drink 30 minutes prior to collection; a collection diary for relevant notes, dates and the time of collection; and nine Salimetrics Children's Swabs with pre-labelled conical storage tubes. The mother was instructed to hold the swab under her toddler's tongue for up to one minute or until it was damp. The wet swab was immediately placed in a conical tube and sealed with a cap and stored in the home freezer.

The diary and samples were returned, either by mail or in person, to the study site hospital and the samples were stored in a -70˚C freezer. The samples were later shipped on dry ice to Montreal, Canada, where they remained frozen until assayed.

We computed the CAR, AUC with respect to ground (AUCg), and increase from baseline (AUCi) and the diurnal cortisol slope (rate of change in diurnal cortisol output between waking and bedtime. When the participants' 30 minutes post-awakening score was measured at more than 45 minutes post-awakening, the scores were excluded (day 1 n = 10, day 2 n = 10). Values greater than 3 SD above or below the mean were excluded from subsequent analyses (awakening day 1 n = 5, 30 minutes post-awakening day 1 n = 3, evening day 1 n = 2, awakening day 2 n = 2, 30 minutes post-awakening day 2 n = 1, evening day 2 n = 1). Following exclusions, 6 participants were removed due to insufficient data (Figure 1). All measures were taken as an average across two days. When data from only one day were available, cortisol values were calculated from one day rather than two days for the CAR (n = 27 of the 71 children) and for the slope, AUCg, and AUCi (n = 29 of the 62 children). Internal consistencies for awakening (α = .63), 30 minutes post-awakening (α = .58) and evening (α = .69) cortisol samples were acceptable.

2.3.2 ***Reactive salivary cortisol*** was collected by the RA during a 2-hour assessment of the toddlers' cognitive and behavioral functioning in a lab playroom at the study site hospital. The stressor involved a brief maternal separation, whereby the toddler was left alone in the lab playroom while their mother exited and observed their toddler via a one-way window. The separation lasted up to three minutes, or until the mother terminated the separation due to toddler distress (M = 106.27 sec, SD = 51.47 sec). The RA took the toddlers' saliva samples immediately prior to the mothers exiting the lab for the separation, and again 20 minutes post-reunion of the mother-toddler dyad, using Salimetrics Children's Swabs. Immediately after collection of each sample, the RA placed the swab into the conical storage tube and, following the assessment, stored them in the -70˚C freezer until shipment to Canada for assaying. Assaying procedures were identical to those used for the diurnal saliva samples.

While many measures of child cortisol reactivity, across many paradigms, are regularly used in the literature, two key components have been identified: total cortisol production, and reactivity (change in cortisol levels over time; Khoury et al., 2015). Cortisol values 3.29 standard deviations or more from the mean (20 minutes post stressor $n = 2$) were removed prior to computation of outcome variables. In the current study, we computed reactivity (peak [20min] - baseline) to assess "change in cortisol over time". To assess total cortisol production due to the stressor, we computed the Area Under the Curve with respect to ground (AUCg; Khoury et al., 2015; Pruessner et al., 2003). Similar measures were used by Yong Ping et al. (2015) in assessing Iowa flood-related PNMS effects on toddler cortisol reactivity to the same maternal separation paradigm.

2.3.3. Salivary cortisol assays: Salivary cortisol levels were assayed via competitive enzyme immunoassay (EIA) using kits provided by Salimetrics. Each sample was assayed twice and an average concentration level was obtained. The inter- and intra-assay coefficients of variation for the diurnal cortisol vales were 8.6 and 6.1, respectively. The inter- and intra-assay coefficients of variation for the reactive cortisol vales were 8.6 and 5.6, respectively.

2.4. Outcome Variables

2.4.1 ***The Spence Preschool Anxiety Scale, SPAS*** (Spence et al., 2001). This 34-item questionnaire provides information on five subscales corresponding to the DSM-IV criteria (generalized anxiety, social anxiety, obsessive compulsive disorder, physical injury fear, and separation anxiety). For the 28 initial items, the mother reports the frequency at which the statement is true for her child ranging from 1 ("not at all") to 5 ("very often true"). Items 29-34 (not scored) pertain to traumatic events the child may have experienced. The total anxiety symptoms score was used for the analyses. SPAS cut-off scores classified the children as within the normal (< 33) and elevated (> 34) ranges. The total anxiety score is highly correlated with measures of internalizing behaviors, indicating good construct validity (Spence et al., 2001).

2.5. Demographics and Control Variables

2.5.1 Maternal characteristics included factors known to influence child development, such as maternal education level (years of schooling), socioeconomic status (Socio-Economic Index For Area scores [SEIFA] based on Australian postcode and census data, $M = 1000$, $SD = 100$) and marital status (married or de facto vs divorced, separated or single). Maternal mood at 4-years was controlled for using the composite score of the three scales of the Depression, Anxiety and Stress Scale 21 (DASS; Lovibond & Lovibond, 1995).

2.5.2 Child characteristics included gestational age at birth (weeks), child sex and birth weight (grams).

2.5.3 Time of day of reactivity separation cortisol sampling was recorded for each participant: 35.1% of baseline samples were taken before 1100h, 29.8 % between 1101h and 1359h and 35.1% between 1359h and 1659h.

2. 6 Statistical analysis

We first conducted a series of hierarchical linear regressions to determine if there was a linear or curvilinear association between PNMS and cortisol data (first path of the mediation). Separate hierarchical linear regression analyses were run for each combination of PNMS and cortisol variables by first only including the linear component of PNMS, then adding the curvilinear component (PNMS squared). In analyses with subjective distress or cognitive appraisal, objective hardship was adjusted for. If sex or timing of exposure were significantly associated with the cortisol variables, they were also included in the model. Then, to determine if the effect of PNMS on cortisol levels is conditional on sex or timing of in-utero exposure to the flood, or if types of PNMS interact to influence cortisol, PNMS x Sex, PNMS x timing or PNMS x PNMS interaction terms were included in the models.

Next, to determine if there was a linear or curvilinear association between cortisol and child anxiety at 4-years old (the second path in the mediation), hierarchical linear regression analyses were run to regress SPAS at 4-years of age on each cortisol variable, first only including the linear component of cortisol, then adding the curvilinear component. In all analyses, the concurrent maternal mood was included in the model to consider both any effects of a mother's mood on her pattern of response on the SPAS, and any effect of maternal mood on child anxiety. Objective hardship was also adjusted for, as well as PNMS variables significantly associated with the cortisol measure, to be consistent with mediation models.

Lastly, to determine if cortisol significantly mediated the effect of PNMS on child anxiety, controlling for potential confounding variables, version 3.2 of PROCESS for SPSS (Hayes, 2018) was used to run mediation or moderated mediation analyses when both the first (PNMS to cortisol) and second path (cortisol to anxiety) were at least marginally significant ($p < 0.1$), creating confidence intervals around the indirect effects. This approach was used to accurately assess curvilinear PNMS effects on cortisol measures and address Hypothesis 1. It also served to avoid running a large number of mediation analyses, ultimately reducing the risk of false positives.

All analyses were run on SPSS v24 with an a priori α=0.05.

3. Results

3.1 Attrition analyses

Participants differed from non-participants on only a single variable: dyads who participated in the laboratory stress paradigm and/or returned daily cortisol samples at 16 months and/or responded to questionnaires at 4-years (N = 160) were exposed to the floods later in gestation (M = 124.94 days (18 weeks); SD= 76.30 days) than non-responders (N = 70, M =97.02 days (14 weeks); SD= 64.67 days; t (153.87) = 2.85, p = .005). Participants with at least one measure of diurnal cortisol and 4-year data (N =

64) and those with complete reactive cortisol and 4-year data (N = 80) did not differ from those with data at only 4-years (N = 117) on flood-related or socio-demographic factors.

3.2 Descriptive statistics

Table 1 presents the descriptive analyses for all PNMS, cortisol, sociodemographic and anxiety variables for each sub-sample used in the subsequent analyses. Across samples, most children within the current cohort displayed normal levels of anxiety.

Table 2 presents the matrices of correlations among PNMS, cortisol and anxiety variables for diurnal cortisol and reactive cortisol (grey shading) samples respectively. Neither sociodemographic factors (SES, maternal education) nor child weight or gestational age at birth were associated with 16 month or 4-year child outcomes. Maternal mood at 4-years was positively correlated with child anxiety symptoms and was therefore included in models examining this outcome.

Table 1
Cohort descriptive statistics for the outcome, predictor variables and covariates.

		Diurnal Cortisol		Reactive Cortisol	
Variables		16-month sample N = 80	4-year sample N = 64	16-month sample N = 111	4-year sample N = 80
4-year Anxiety symptoms	M (SD)	-	11.84 (9.24)	-	12.35 (10.15)
SPAS Normal Range[a]	% (N)	-	98.40 (1)	-	96.30 (3)
Objective hardship	M(SD)	22.40 (17.66)	22.94 (18.95)	20.0 (16.28)	20.81 (16.60)
Post-traumatic stress	M(SD)	6.52 (10.52)	6.21 (9.28)	6.13 (10.80)	5.73 (9.33)
Peritraumatic distress	M(SD)	11.84 (7.60)	12.24 (7.01)	11.77 (7.81)	11.96 (7.33)
Peritraumatic dissociation	M(SD)	5.64 (6.54)	5.37 (6.02)	5.64 (7.27)	5.13 (6.47)
Composite subjective stress	M(SD)	-0.013 (0.876)	-0.189 (0.748)	-.029 (.96)	-.060 (.821)
Cognitive Appraisal: Neg	N(%)	27 (33.80)	21 (32.80)	34 (30.90)	23 (28.70)
Cognitive Appraisal: Neutral/Positive	N(%)	53 (66.30)	43 (67.20)	76 (69.10)	57 (71.30)
Timing of exposure (days)	M(SD)	133.50 (77.73)	142.66 (78.58)	125.77 (78.69)	133.97 (80.55)
Timing of exposure (trimester, %)	1st	31.3	26.6	37.8	33.8
	2nd	42.5	42.2	36.9	36.3
	3rd	26.3	31.3	25.2	30
Cortisol awakening response	N	71	57	57	48
	M(SD)	0.002 (0.006)	0.002 (0.006)	0.002 (0.006)	0.002 (0.006)
Daily Cortisol Slope	N	75	62	62	53
	M(SD)	-0.821 (0.951)	-0.785 (0.999)	-0.835 (0.998)	-0.792 (1.047)
Diurnal AUCg[b]	N	62	52	49	43
	M(SD)	0.253 (0.230)	0.259 (0.248)	0.268 (0.255)	0.273 (0.269)
Diurnal AUCi[c]	N	62	52	49	43
	M(SD)	-0.049 (0.171)	-0.039 (0.174)	-0.050 (0.179)	-0.038 (0.181)
Reactive Cortisol Response AUCg[d]	N	66	55		
	M(SD)	0.032 (0.167)	0.016 (0.163)	0.027 (0.169)	0.020 (0.176)
	N	66	55		
	M(SD)	0.176 (0.106)	0.177 (0.110)	0.174 (0.10)	0.178 (0.110)
Infant sex(boys)	N(%)	45 (56.30)	43 (67.20)	62 (55.90)	44 (55.00)
Gestational age at birth (wks)	M(SD)	39.53 (1.14)	39.52 (1.14)	39.42 (1.17)	39.49 (1.17)
Birthweight (grams)	M(SD)	3634.51 (448.61)	3643.44 (450.71)	3603.39 (454.10)	3615.23 (461.06)
4-year Maternal mood	M(SD)	-	19.23 (15.80)	-	19.62 (18.59)
Socio-Economic Index	M (SD)	1059.43 (54.60)	1059.53 (49.79)	1056.65 (54.39)	1058.51 (51.32)
Mother's number years of schooling	N	61	48	85	59
	M (SD)	15.01 (1.66)	14.92 (1.75)	14.78 (1.75)	14.88 (1.67)

Note. Untransformed scores are used for the measures of maternal stress; [a] SPAS = Spence Preschool Anxiety Scale; [b] Area under curve ground with respect to time awake; [c] Area under curve increase with respect to time awake; [d] Area under curve ground with respect to duration between cortisol measures in stress paradigm.

Table 2. *Intercorrelations among study variables for participants with diurnal cortisol (lower matrix) and those with reactive cortisol (upper).*

Correlations within the Cortisol Reactivity Sub-samples

Correlations within the Diurnal Cortisol Sub-samples

Cortisol Reactivity Samples

	Variables		1	2	3	4	5	6	7	8	9	10	11	12	13	14
		N	80	57	62	49	49	111	111	111	111	111	111	111	111	80
1	SPAS [a] Total Score	64	-	.19	.22	.26^	.13	.05	.04	.06	.27*	.17	-,05	-.21^	-.21^	.27*
2	Cortisol Awakening Response	71	.27*	-	.57**	.83**	.56**	-.25^	.00	-.02	-.03	.27*	.15	.04	-.28*	-.13
3	Diurnal AUCg [b]	62	.22	.53**	-	.53**	.46**	-.25^	.16	-.07	-.11	.17	.13	.07	-.09	-.18
4	Diurnal AUCi [c]	62	.27^	.83**	.51**	-	.90**	-.36*	-.03	.05	.13	.24	.05	-.15	-.07	-.13
5	Diurnal Cortisol Slope	75	.13	.54**	.44**	.89**	-	-.20	-.07	.03	.11	.13	.02	-.13	-.07	-.05
6	Reactive Cortisol Response	66	.01	-.25^	-.25^	-.36*	-.20	-	.22*	-.06	.09	.04	-.07	-.02	.02	.25*
7	Reactive AUCg [h]	66	-.01	.00	.16	-.03	-.07	.14	-	-.14	.02	-.12	.03	.10	.08	.02
8	Stressor assessment time of day	66	.14	.01	-.07	.03	.00	-.07	-.07	-	-.14	-.11	-.05	-.09	-.09	-.08
9	Objective Hardship [d]	80	.17	.02	-.07	19	.17	.16	-.02	-.18	-	.54**	-.48**	-.12	-.09	-.01
10	Composite Subjective Stress	80	.19	.25*	.17	.27*	.14	.04	-.11	-.09	.47**	-	-.37**	-.10	-.15	.09
11	Cognitive Appraisal [e]	80	-.14	.09	.13	.00	-.03	-.21^	.04	-.12	-.49**	-.39**	-	.12	.01	.05
12	Timing of Exposure (days)	80	-.23^	.10	.07	-.10	-.12	-.09	.09	-.07	-.02	.06	.08	-	-.02	-.15
13	Child sex [f]	80	-.23^	-.27*	-.09	-.09	-.09	-.07	.02	-.03	-.04	.03	-.06	-.15	-	.05
14	4 yr Concurrent Maternal Mood [g]	80	.37**	-.17	-.20	-1.8	-.09	.19	-.04	-.08	-.01	.09	-.03	-.07	-.05	-

Diurnal Cortisol Samples

[a] Spence Preschool Anxiety Scale; [b] Area under curve ground with respect to time awake; [c] Area under curve increase with respect to time awake; [d] log transformed scores used in correlation; [e] Coding for cognitive appraisal: 0 = negative/very negative, 1 = neutral/ positive/very positive; [f] Coding for child sex: 0 = male, 1 = female; [g] Depression Anxiety Stress Scale (DASS-21) composite score; [h] Area under curve ground with respect to duration between cortisol measures in stress paradigm ** $p < 0.001$ * $p < 0.05$ ^ $p = .051$ - 0.99

3.3 Reactive cortisol

Results showed that the mother-child separation stressor failed to significantly increase cortisol levels from pre-stress (M = 0.16, SD = 0.15) to 20 minutes post-stressor (M = 0.19, SD = 0.15; t (110) = 1.68, p = .096), an increase of 0.2 SDs. Change from pre- to post- stressors were not dependent upon child sex, time of cortisol sampling, or the level of flood exposure (p's >.1). These results suggest that our maternal-child stress paradigm failed to elicit physiological reactivity in the children. Therefore, we did not test Hypothesis 1.

3.4 Diurnal cortisol

Across the two days assessed, cortisol levels followed a usual diurnal rhythm. Across the two days, there was a significant increase from cortisol awakening (M = 0.30, SD = 0.19) to 30 minutes post-awakening (M = 0.36, SD = 0.22; t (74) = 3.014, p = .004), an increase of 0.30 SDs. A significant decrease from 30 minutes post-awakening (M = 0.36, SD = 0.23) to evening cortisol levels (M = 0.17, SD = 0.23; t (71) = -11.20, p < .001), a decrease of 0.83 SD, was also observed. Average waking cortisol level was not related to average cortisol awakening response (r = -.19, p > .1) for boys (r = -.03, p > .1) with a marginal, negative association for girls (r = -.34, p = .064).

3.4.1 The direct effect of PNMS on diurnal cortisol profiles. Hierarchical linear regression analyses showed no curvilinear associations between PNMS and diurnal cortisol measures (CAR, daytime slope, AUCg, AUCi) after accounting for linear terms. Quadratic terms were, thus, removed from reported models.

Child sex was significantly associated with child CAR (Table 2), with boys having higher CAR than girls, and included as a covariate in all subsequent analyses examining this outcome. As presented in Table 3 panel a, a significant linear effect of composite subjective stress on CAR was detected (p=0.013), after adjusting for objective hardship and the child's sex, such that higher subjective stress was associated with a steeper CAR. Independent of PNMS effects, a significant sex difference was detected (p=0.012), such that, overall, males had a steeper CAR than females.

Subjective stress explained 8.2% of the variance in CAR not already accounted for by objective hardship. The whole model explained 15.6% of the variance in child CAR.

Controlling for objective PNMS, which explained <1% of the variance, composite subjective stress also had a marginal effect on the diurnal area under the curve with respect to ground (AUCg; p=0.077), after adjusting for objective hardship (Table 3 panel b): higher maternal subjective stress was associated with greater total cortisol secretion across the day. Subjective stress explained 5.7% of the unique variance in AUCg, which was all of the variance explained by the full model.

A similar trend was detected for the area under the curve increase with respect to baseline (AUCi; Table 3 panel c). Subjective stress explained 4.6% of the unique variance in AUCi, with the full model explaining 8.0% of the variance (p=0.093).

Cognitive appraisal, controlling for QFOSS did not significantly predict diurnal cortisol measures, nor did any PNMS variables affect diurnal slope. Neither child sex nor the timing of flood exposure in pregnancy moderated any PNMS effects.

3.4.2 The direct effect of toddler diurnal cortisol measures on 4-year anxiety. A significant linear effect of CAR on child anxiety at 4-years was detected (p = 0.015), adjusting for concurrent maternal mental health, as well as objective and subjective stress (see Table 4 panel a): a steeper CAR at 16 months predicted greater four-year anxiety. Maternal mental health was also significantly associated with child anxiety (p=0.009), such that worse mental health was associated with greater child anxiety symptoms. The CAR explained 9.3% of the unique variance in anxiety, with the whole model explaining 22.9% of the variance.

As presented in Table 4 panels b and c, diurnal AUCg and AUCi were also found to have significant effects on anxiety (p=0.043 and p=0.039, respectively), such that greater cortisol secretion was associated with greater anxiety. The same maternal mental health effect was found here as in the CAR analysis. AUCg and AUCi explained 7.2% and 7.5% of the unique variance in anxiety, with the whole models explaining 22.2% and 22.5% of the variance, respectively.

No other linear of curvilinear effects between diurnal cortisol measures and anxiety outcomes were significant.

3.4.3 The mediating role of diurnal cortisol on the relationship between PNMS and child anxiety. Following initial regressions reported above, the CAR was found to significantly mediate the effect of subjective stress on anxiety at 4-years (Figure 2), such that higher subjective stress led to higher anxiety through a steeper CAR (standardized mediation effect: 0.1365; 95% CI: [0.0125, 0.3132]).

Neither toddler diurnal AUCg nor AUCi mediated the effect of subjective stress on anxiety (AUCg: standardized mediation effect: 0.0882; 90% CI: [-0.0317; 0.1736]; AUCi: standardized mediation effect: 0.0584; 90% CI: [-0.0261; 0.1260]).

Table 3.

Hierarchical regression summary for the significant effects of PNMS on diurnal cortisol.

Predictor Variables	*β*	*B*	*Std. Error*	*R*	R^2	ΔR^2	*F*	ΔF
a) Cortisol Awakening Response (CAR)								
Step 1				.271	.073	.073	5.456*	5.456*
Child's sex	-.271*	-.003*	.001					
Step 2				.271	.074	.0003	2.701§	0.023
Child's sex	-.271*	-.003*	.001					
Objective hardship	.018	1.413E-04	.001					
Step 3				.394	.156	.082	4.115**	6.506*
Child's sex	-.290*	-.004*	.001					
Objective hardship	-.130	-.001	.001					
Composite subjective stress	.323*	.002*	.001					
b) Diurnal area under the curve Ground (AUCg)								
Step 1				.074	.005	.005	.330	.330
Objective hardship	-.074	-.021	.037					
Step 2				.239	.057	.057	1.792	3.242§
Objective hardship	-.189	-.055	.041					
Composite Subjective Stress	.255^	.081^	.045					
c) Diurnal area under the curve Increase (AUCi)								
Step 1				.186	.035	.035	2.147	.2.147
Objective hardship	.186	.040	.027					
Step 2				.283	.080	.046	2.568	2.921§
Objective hardship	.078	.017	.030					
Composite Subjective Stress	.239§	.056§	.033					

Note. ^ $p < .1$; *$p < .05$; **$p < .01$. Sex: 0=boy; 1=girl.

Table 4.

Hierarchical regression summary for the significant effects of cortisol on anxiety at 4-years.

Predictor Variables	*β*	*B*	*Std. Error*	*R*	R^2	ΔR^2	*F*	ΔF
a) Cortisol Awakening Response (CAR) on 4y anxiety								
Step 1				.368	.136	.136	2.770^	2.770^
Objective hardship	.094	1.095	1.708					
Composite subjective stress	.135	1.715	1.871					
Maternal mood	.304*	.182*	.077					
Step 2				.479	.229	.093	3.861**	6.304*
Objective hardship	.178	2.072	1.674					
Composite subjective stress	-.027	-.347	1.964					
Maternal mood	.376**	.225**	.075					
CAR	.341*	545.472*	217.260					
b) Diurnal area under the curve Ground (AUCg) on 4y anxiety								
Step 1				.388	.150	.150	2.828*	2.828*
Objective hardship	.049	.564	1.769					
Composite subjective stress	.222	2.787	1.905					
Maternal mood	.299*	.195*	.087					
Step 2				.471	.222	.072	3.348*	4.322*
Objective hardship	.132	1.527	1.772					
Composite subjective stress	.130	1.626	1.925					
Maternal mood	.366**	.239**	.087					
AUCg	.288*	10.857*	5.223					
c) Diurnal area under the curve Increase (AUCi) on 4y anxiety								
Step 1				.388	.150	.150	2.828*	2.828*
Objective hardship	.049	.564	1.769					
Composite subjective stress	.222	2.787	1.905					
Maternal mood	.299*	.195*	.087					
Step 2				.474	.225	.075	3.406*	4.518*
Objective hardship	.025	.295	1.712					
Composite subjective stress	.161	2.012	1.875					
Maternal mood	.350**	.228**	.086					
AUCi	.288*	15.440*	7.264					

Note. ^ $p < .1$; *$p < .05$; **$p < .01$.

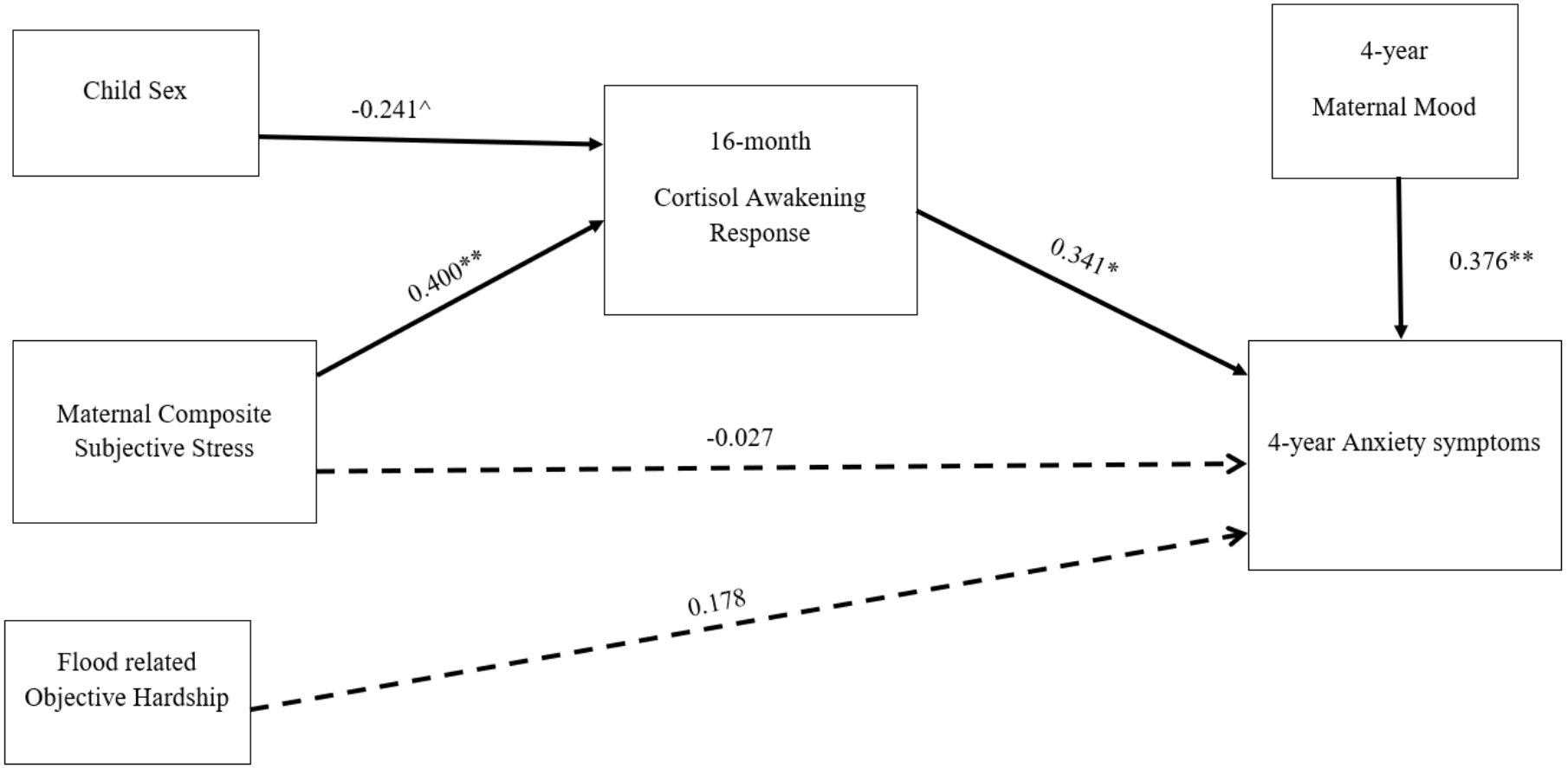

Figure 2. Mediation model linking maternal subjective stress in pregnancy to greater anxiety symptoms at 4-years, via 16-month CAR. All coefficients are standardized. Dashed lines represent non-significant paths. **p < .01, *p < .05, ^ p < .10

4. Discussion

In the present study we found that a heightened daily CAR in toddlerhood mediated the association between greater maternal subjective distress in relation to the 2011 Queensland floods and greater childhood anxiety symptoms at 4-years. However, this mediation was not significant with reactive cortisol levels. To our knowledge, these findings are the first evidence of a potential relationship between disaster-related PNMS and a feature of toddler diurnal cortisol rhythm (the cortisol awakening response) which was associated with a heighted vulnerability towards early child anxiety symptoms. Results should be interpreted in light of the relatively small sample size available and methodological limitations discussed below.

4.1 HPA-axis as a biological correlate of PNMS effects on childhood anxiety

Previous Developmental Origins of Health and Disease (DOHaD) research has established HPA-axis activity as a mechanism through which pregnancy stressors may impact childhood internalizing behaviors (Martinez-Torteya et al., 2016), adolescent depression (Van den Bergh et al., 2008), and fearful, anxious behaviors in controlled animal studies (Weinstock, 2008). Our findings extend this prior work by showing one aspect of the circadian cortisol cycle – the CAR - to be a potential biological correlate of PNMS, associated with anxiety symptoms in early childhood. Unlike former work (e.g. Martinez-Torteya et al., 2016), by utilizing a natural disaster study design, our study enables examination of which specific aspect(s) of PNMS contribute to this developmental pathway, controlling for sociodemographic confounds. Results demonstrate that the level of maternal subjective stress in pregnancy, independent of the level of hardship endured or maternal mood in childhood, 'programs' the fetus and is associated with relatively higher CAR and greater anxiety symptoms. Although there were no associations between PNMS and other indexes of daily cortisol rhythm (total cortisol output, diurnal slope) further research needs to determine the specificity of indexes of HPA-axis functioning as mechanisms that PNMS may affect subsequent child anxiety symptoms.

A steeper CAR has previously been associated with anxiety symptoms and disorders both longitudinally (Saridjan et al., 2014) and concurrently (Boggero et al., 2017). The CAR is thought to

be a distinct marker of HPA-axis functioning, responsive to stress perception and anticipation of daily stressors (Contreras & Gutierrez-Garcia, 2018; Fries et al., 2009) and related to the process of awakening (Wilhelm et al., 2007). We speculate that a child exposed to PNMS, with relatively higher CAR, may be 'programmed' via sensitization of central stress response systems, to adapt and survive in expectation of a dangerous and stressful postnatal environment (Giudice, 2014). This sensitization leads to enhanced neuroendocrine, autonomic and behavioral responsiveness to anticipated stressors which may include a relatively higher CAR. This anticipatory functioning may precede the development of anxiety by increasing stress-reactivity in the face of future stressors (double hit diathesis-stress hypothesis; Monroe & Simons, 1991), wear and tear on the system (allostatic overload; McEwen & Stellar, 1993) or increase one's susceptibility to the postnatal parenting environment (Hartman & Belsky, 2018; Monroe & Simons, 1991). ~~Our interpretation of 'hyper-reactivity' of the HPA-axis function is cautionary, however. Due to a lack of norms for the CAR, it is unknown whether particular CAR values represent hyper-responsiveness, normal responsiveness or hypo-responsiveness (Boggero et al., 2017).~~

4.2 HPA-axis functioning and childhood anxiety

In line with previous work in child, adolescent and adult non-clinical populations (Greaves-Lord et al., 2007; Laurent et al., 2015; Saridjan et al., 2014) we showed that both greater toddler CAR and total cortisol secretion across the day were each associated with greater 4-year anxiety symptoms, independent of PNMS exposure. While a steeper CAR may indicate a relatively higher reactivity to anticipated stressors, greater daily cortisol output may be indicative of a currently stressed HPA-axis. Above and beyond the effects of PNMS, individual differences in cortisol profiles could lead to the development of greater anxiety symptoms via interaction with child sex (Daoust et al., 2018) or postnatal factors including stressful life events and the parental-rearing environment (Kopala-Sibley et al., 2017; Monroe & Simons, 1991). Similar to work in adults (Adam et al., 2017) we failed to establish a prospective association between diurnal slope and child anxiety. While not examined here, child depressive symptoms and broader internalizing behaviors seem to be more strongly related to variation in diurnal slope (Saridjan et al., 2014; Van den Bergh et al., 2008). As recently argued by

Koss and Gunnar (2018), associations between HPA functioning and child psychopathology are far from conclusive, with more research needed to understand the nature of associations across outcome, age, sex and varied demographic populations.

4.3 Aspects of PNMS and Child Anxiety

Examining PNMS within a prospective longitudinal natural disaster study design affords investigation of the unique effects of various aspects of the stress exposure and response on child development. Here we established a relationship between maternal subjective distress and the development of anxiety symptoms at age 4 via a steeper cortisol awakening response at 16 months. In line with our previous findings of a direct link between flood-related objective hardship and childhood anxiety at 4-years (McLean, Cobham, Simcock, et al., 2018), here objective hardship was associated with 4-year anxiety symptoms in the reactive but not diurnal subsample. Despite this, correlations between objective hardship and child anxiety reported here (Table 2) are within the possible range of values for the larger subsample previously reported (r = .20, CIs [0.016, 0.369], McLean, Cobham, Simcock, et al., 2018). Given that no differences in flood-related or socio-demographic factors were found across samples (see attrition analyses), the lack of an association with objective harship in the diurnal sample is likely due to the smaller sample size (N = 64) and resulting lack of statistical power. Taken together, our findings suggest that maternal objective hardship and subjective distress in response to a disaster are uniquely related to the development of childhood anxiety.

Importantly, our work extends current research establishing long-term effects of stress-related fetal programming on child outcomes (van den Bergh et al., 2018), to suggest that aspects of PNMS may alter fetal neurodevelopment via different maternal-child mechanisms (Dunkel Schetter & Tanner, 2012; Graignic-Philippe et al., 2014), at least when considering child vulnerability towards anxiety. The role of several stress-induced physiological indicators that may affect fetal neurodevelopment are being widely investigated but to-date are poorly understood (van den Bergh et al., 2018). These include maternal HPA axis (Graham et al., 2019), diet (Lindsay et al., 2019; Monk et al., 2013), inflammation and the immune system (Hantsoo et al., 2018), epigenetics (Cao-Lei et al.,

2017) and gut microbiome (Jašarević et al., 2018; Jašarević & Bale, 2019). Across natural disaster PNMS studies our team has previously shown links between maternal subjective stress and placental glucocorticoid-promoting gene expression (St-Pierre et al., 2018) while maternal diet (Dancause et al., 2017) and DNA methylation (Cao-Lei et al., 2015) were both found to mediate associations between objective hardship infant outcomes. Whether any of these pathways help to explain how different aspects of PNMS are related to child HPA-axis functioning and anxiety behaviors requires further investigation. Indeed, the inability for studies of maternal mood in pregnancy to disentangle the level of exposure to a stressor from that of maternal subjective distress limits research investigating these nuances, with more work needed.

Rather than in-utero exposure to PNMS, associations between maternal subjective distress, child CAR and later anxiety may reflect genetic heritability or the influence of maternal postnatal mood or behaviors on child stress reactions and anxiety. However, mothers reported their distress and their enduring post-traumatic symptoms specifically in response to the floods, and not on their general stress or anxiety during pregnancy. As such, our measure of subjective PNMS is indicative of flood-related PNMS, rather than maternal mood. Maternal mood at 16-months was unrelated to indexes of child cortisol (all *p's* >0.1) and statistical models presented accounted for maternal mood at 4 years given its positive relationship with child anxiety at this age. Maternal mood at 4-years was independent of their subjective PNMS scores and other demographic factors. Our prior research finds no association between observed maternal overinvolvement and negativity and child anxiety; maternal mood accounted for 4% of the variance in maternal negativity at age 4 years (McLean, 2019). In sum, evidence from our current cohort supports a prenatal pathway: intrauterine effects of PNMS on fetal neurodevelopment that impact toddler stress-sensitivity and later anxiety symptoms.

Continued research utilizing novel study designs as that employed here, will help improve our understanding of the many varied prenatal and potentially postnatal pathways through which various aspects of PNMS may be associated with child anxiety.

4.4 Child Sex and Timing of Flood Exposure

Neither child sex nor gestational timing of flood exposure moderated the effects of PNMS on children's diurnal cortisol profiles. This differs to several studies that established sex-specific differences in HPA-axis activity due to disaster-related PNMS, particularly in girls. (Sandman et al., 2013; Yong Ping et al., 2015). The small sample size may have hindered detection of moderation findings, although previous QF2011 papers also failed to find moderating effects of sex and/or timing of PNMS on toddler and child emotional outcomes (Lequertier et al., 2019; McLean et al., 2018).

Surprisingly, we established an effect of child sex on toddler CAR, independent of PNMS exposure, such that boys displayed a steeper CAR than girls. Limited research has investigated sex differences in diurnal cortisol profiles in children. Sex differences in HPA-axis functioning is largely dependent upon sex hormones and generally arise in puberty (Juster et al., 2016). A recent systematic review suggested that, during childhood, girls may display a higher CAR than boys although findings were largely equivocal with few studies eligible for inclusion in the review (Hollanders et al., 2017). Differences in childhood may be related to genetics, organizational differences of brain structures involved in stress reactivity and regulation (e.g., amygdala, hippocampus), and the developmental programming of gonadal steroids (Bale & Epperson, 2015). More research is clearly needed to understand the nature of sex-specific HPA-axis functioning across the life-span.

4.5 Reactive Cortisol

Our maternal-separation stressor paradigm failed to elicit a significant change in cortisol levels from pre- to 20 minutes post-stressor. Separation paradigms may more reliably elicit physiological stress responses during infancy than toddlerhood, by which time children may have developed the coping skills needed to manage the maternal separation (Gunnar et al., 2009). Still, Yong Ping et al. (2015), established significant mean increases in 30-month toddler cortisol levels for girls with a similarly brief (< two-minute) maternal separation. Limitations in our salivary assessment protocol, taken at baseline and 20 minutes post-stressor, may have affected our ability to detect a significant cortisol response. Study feasibility and participant burden as part of a 2-hour toddler assessment (King et al., 2015) meant we could not implement lab-entry and post 40 min saliva

sampling that may more accurately capture baseline assessment and post-stressor cortisol peaks (Goldberg et al., 2003; Khoury et al., 2015). We administered the cortisol reactivity protocol at various times of the day to accommodate family availability. Although not ideal, we found no association between time of day of cortisol reactivity sampling and change in cortisol from pre- to post- stressor. However, given the sharper decline of the cortisol circadian curve towards midday, it is possible that effects of stress on cortisol concentrations are obscured in those children assessed in the morning (Miller et al., 2016). Assessment of cortisol reactivity in the afternoon when diurnal fluctuations are less influential is recommended (Miller et al., 2016).

4.6 Strengths and Limitations

Several limitations should be noted. Our relatively small sample size, our relatively homogenous high SES, the healthy population, and the small effect sizes attributable to PNMS, may have limited our power to detect the effects of PNMS on HPA-axis indexes and on child anxiety. The current study began prior to the development of guidelines and exemplary protocols for CAR assessment (Baumler et al., 2013; Stalder et al., 2016). Actigraphs, which can be used to establish the young child's moment of waking, were not used due to the age of participants and financial constraints. Mothers recorded the child's time of awakening and time of cortisol sampling on a standardized form. While maternal anxiety and child awakening cortisol were uncorrelated, it is possible that a mother's level of concurrent and/or trait anxiety influenced the timing of saliva sampling upon awakening. In the current study, a third to a half of participants had valid data collected from one but not two days. However, one study found no differences in diurnal cortisol slope associations with health outcomes with one versus two days of salivary cortisol collection (Adam et al., 2017).While maternal mood at 4-years was controlled for in analyses in order to mitigate confounding of potential maternal reporter bias of child behavior, observational measures of child behavior may have strengthened study methodology.

There are, however, several strengths to the current study. Although most PNMS studies include different types of stressful life events that occur during pregnancy, QF2011 participants all

experienced the same stressor but to different degrees, providing a natural experiment with a dose-response component. The sudden-onset nature of the flood offered the ability to accurately time the onset of PNMS; which is difficult to do for life events that have gradual onsets, such as relationship breakdown or even the death of a relative. The collection of both lab and daily saliva to examine diurnal and reactive cortisol as indexes of HPA-axis functioning is a clear strength of the study.

4.7 Future Research

Our findings support, and thus encourage extension of, current DOHaD research. Exposure to a range of PNMS indicators (variations in maternal cortisol, depressive symptoms, maternal anxiety) have been associated with alterations in the structure and function of brain regions involved in regulating HPA-axis activity in offspring (Buss et al., 2012; Graham et al., 2019; Rifkin-Graboi et al., 2013). There is a plethora of glucocorticoid receptors located in the hippocampal and prefrontal cortex regions of the brain (Reul & Kloet, 1985) which are associated with emotion regulation, negative emotionality and anxiety disorders (Fonzo & Etkin, 2017) including the limbic structures (amygdala, hippocampus) and prefrontal cortex. Alterations to these key brain regions may lead to a connected neural network failing to adapt to, or compensate for, altered physiological outflow system responses (i.e. HPA-axis including the CAR; Fries et al., 2009) and, therefore, behavior (Graham et al., 2019; Hakamata et al., 2019). Examining how multiple biological systems interact in the context of PNMS-exposure will help elucidate a more complete picture of the role of PNMS in development of childhood anxiety. Finally, given the small sample of participants with valid cortisol data across both days, it is important that our findings are replicated within larger cohort studies.

4.8 Conclusions

Our findings provide support for the sensitivity of the developing HPA-axis, a key stress response system, to variation in disaster-related maternal subjective stress in pregnancy. Within a methodologically rigorous natural experiment study design, we identified one potential biological correlate of PNMS exposure associated with a heightened vulnerability for the development of anxiety symptoms in early childhood. Our work advances research in DoHAD research, as well as

biopsychosocial models of childhood anxiety development. A greater understanding of the etiological processes through which childhood anxiety symptoms may arise will ultimately enable the development of targeted intervention for at-risk children.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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