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Prenatal maternal stress predicts stress reactivity at 2½ years of age: The Iowa Flood Study

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Summary Prenatal maternal stress (PNMS) predicts psychosocial development in offspring. It has been hypothesized that during PNMS, glucocorticoids pass the placenta, reaching the foetus, leading to a long-term reprogramming and dysregulation of the foetal hypothalamic-pituitary-adrenal (HPA) axis. However, results are inconsistent across PNMS studies. One problem may be the confounding of objective degrees of hardship due to the stressor and subjective degrees of distress in the mother. The present study investigated the association between objective and subjective PNMS due to a natural disaster, the June 2008 Iowa floods, and stress reactivity in the offspring at 2½ years of age. Women who were pregnant during the floods were recruited, on average, within three months of the floods and their stress levels assessed. Mothers and their toddlers ($n = 94$ dyads) participated in a brief mother—toddler separation to induce physiological stress responses in the offspring. Salivary cortisol samples were collected four times during the procedure. We computed absolute change in cortisol (baseline to 20-minute post-stressor; baseline to 45-minute post-stressor) and Area Under the Curve with respect to increase and ground (AUCi; AUCg). Objective and subjective PNMS were positively correlated with AUCi, as was timing in gestation: the later in pregnancy the exposure occurred, the greater the cortisol increase. Controlling for objective hardship and other covariates, sex-by-subjective PNMS interactions showed a significant and positive association between subjective PNMS and

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Absolute Increase (45 min) and AUCi in females only, with little effect in males. These results suggest that PNMS leads to long-term alterations in the functioning of the HPA axis, evident as early as 30-months of age.

1. Introduction

Prenatal maternal stress (PNMS) affects birth outcomes (Mulder et al., 2002), and subsequent cognitive and psychosocial development in infants (Buitelaar et al., 2003) and children (O'Connor et al., 2003). Studying disaster-related PNMS in Project Ice Storm, our group has replicated many of the above-mentioned findings. For example, the severity of subjective and/or objective PNMS predicts birth outcomes (Dancause et al., 2011), more immature play styles (Laplante et al., 2007), deficits in cognitive and language functioning (Laplante et al., 2008), higher rates of obesity (Dancause et al., 2012), and minor physical anomalies, such as fingerprint asymmetry (King et al., 2009). Overall, these results suggest that PNMS has long-lasting effects on infant development that persist into late childhood and early adolescence, and that objective and subjective PNMS have differential effects depending on the outcome of interest.

The growing body of literature linking PNMS to negative outcomes has lead researchers to focus their attention on the biological mechanisms of PNMS. The hypothalamic—pituitary-adrenal (HPA) axis is often implicated in PNMS effects since altered HPA axis activity is commonly associated with many of the observed outcomes (Hunter et al., 2011). Maternal stress is transmitted to the foetus via high levels of glucocorticoids (GCs) (Barbazanges et al., 1996); maternal and foetal cortisol levels are positively correlated (Gitau et al., 2001). While the foetus is normally protected from maternal cortisol via 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2), a placental enzyme that converts cortisol to its inactive form, cortisone, evidence of 11β-HSD2 downregulation in times of increased maternal stress has been reported (Mairesse et al., 2007).

Studies have investigated the impact of PNMS on the development of the foetal HPA axis, and how this impact presents itself throughout postnatal development. In rodents and nonhuman primates, PNMS has been associated with higher basal levels of GCs in offspring, compared to controls (Clarke et al., 1994; Fameli et al., 1994; Coe et al., 2003; Emack et al., 2008). In humans, prenatal and postnatal stress exposure, in the form of higher maternal morning cortisol levels, greater worries during pregnancy, parenting stress, and PTSD, have been associated with higher basal and diurnal cortisol levels in infants and children (Gutteling et al., 2004; Saridjan et al., 2010), as well as lower basal cortisol levels (Yehuda et al., 2005).

PNMS also affects the HPA axis with respect to stress reactivity in animal offspring (Weinstock et al., 1992); rats and nonhuman primates exposed to maternal stress in utero release more GCs in response to stress than controls (Fride et al., 1986; Takahashi and Kalin, 1991; Clarke et al., 1994; Coe et al., 2003). However, offspring whose mothers were exposed to stress prenatally have also been found to exhibit lower or blunted GC levels in response to stressful stimuli (Fameli et al., 1994; Emack et al., 2008). In humans, different experimental paradigms have been used to activate the HPA axis, with the aim of eliciting a change in cortisol levels in infants (noise burst, arm restraint, bathing, vaccination, still-face procedure, and maternal separation-reunion stress), children (vaccination), and adults (Trier Social Stress Test) (Gutteling et al., 2004; Brennan et al., 2008; Entringer et al., 2009; Grant et al., 2009; Tollenaar et al., 2011; O'Connor et al., 2013). From these studies, higher levels of prenatal anxiety, psychosocial stress, maternal cortisol levels, and pregnancy-related anxiety have been associated with increases (Brennan et al., 2008; Entringer et al., 2009; Grant et al., 2009), decreases (Tollenaar et al., 2011), dampened or blunted changes (O'Connor et al., 2013), or no change (Gutteling et al., 2004) in cortisol levels in offspring in response to stress. Collectively, these results suggest that PNMS leads to dysregulated functioning of offspring's HPA axis. Moreover, the inconsistencies across findings, possibly attributable to differing methodologies, highlight the need for further research in this field.

Timing of PNMS exposure and offspring sex have been found to affect the association between PNMS and offspring HPA axis functioning. Huizink et al. (2008) reported second trimester exposure to the Chernobyl disaster of 1986 was associated with higher basal levels of salivary cortisol in adolescent offspring, as was confirmed by Van den Bergh et al. (2008). With respect to sex differences, while female offspring have commonly been reported to be more susceptible to alterations in their physiological and behavioural development than males as a result of prenatal stress exposure (Sandman et al., 2013), little literature exists specifically examining the moderating effect of offspring sex on the relationship between PNMS and cortisol reactivity in human offspring. This is an important area of study as related findings may provide a possible mechanism for the sex difference seen in prevalence rates of psychiatric illness (Fernandez-Guasti et al., 2012). As such, the effects of timing of PNMS exposure and offspring sex were investigated in the present study.

The present study aimed to build on the existing body of literature by examining the association between PNMS and toddler cortisol reactivity. The Iowa Flood Study differentiates itself from other studies in that it examines both subjective and objective levels of PNMS associated with a natural disaster, and subsequent in utero effects that these dimensions of stress can have on the development of exposed offspring. Natural disasters provide unique opportunities to study PNMS because they are ''independent'' events that randomly distribute objective degrees of hardship to a population, irrespective of socioeconomic status, race, age, and/or maternal characteristics, thereby inherently controlling for external factors that may influence a woman's predisposition to stress. Examining stress associated with a natural disaster also allows for the study

of subjective distress while controlling for the objective degree of hardship to which the pregnant woman was exposed.

The present study examined the role of objective and subjective PNMS caused by the Iowa flooding of 2008 on stress reactivity in a sample of 2½ year old toddlers. In 2008, Iowa experienced its worst flooding in more than 50 years. In total, 24 people were killed and 38,000 were driven from their homes; the flood was ranked among the top 10 disasters in U.S. history. It was hypothesized that greater subjective and/or objective PNMS would predict greater dysregulation of toddlers' cortisol response to maternal separation stress. Given that past research has found evidence of hypo- and hyper-cortisol response patterns to stress provocation, and that this study is the first to test the relative effects of objective and subjective PNMS, we did not hypothesize a specific direction of cortisol change. The moderating effects of offspring sex and the timing of in utero flood exposure were also tested.

2. Methods

2.1. Participants

This project is part of the Iowa Flood Study: a prospective longitudinal study that is investigating the effects of PNMS associated with the Iowa flooding of 2008 on child development. Mothers were recruited into the Iowa Flood Study from an ongoing study at the University of Iowa investigating maternal psychosocial characteristics and pregnancy outcomes. A description of that study can be found elsewhere (Nylen et al., 2013). Additional women were also recruited from three severely flood-affected counties: Linn, Johnson, and Blackhawk. Women were eligible to participate if they were 18 years of age or over at the time of recruitment, English-speaking, and pregnant at the time of the flood (June 15, 2008).

In total, 104 mother—toddler dyads successfully completed the protocol outlined in this paper and met all eligibility criteria for the current project as noted above. Ten dyads were removed from the sample due to providing insufficient quantities of toddler saliva for assay ($n = 9$) or invalid cortisol assay results ($n = 1$).

Ninety-four women and their toddlers comprised the final sample. At recruitment into the Iowa Flood Study, these mothers ranged in age from 18 to 41 years of age ($M = 29.3$; SD = 5.1) and described their marital status as married ($N = 81$; 86.2%), divorced ($N = 2$; 2.1%), not married and living with partner ($N = 2$; 2.1%), or single (never married) ($N = 9$; 9.6%). Our sample consisted predominately of Caucasian women ($N = 87$; 92.6%), in addition to American Indian or Alaskan Native ($N = 1$; 1.1%), Asian or Pacific Islander ($N = 3$; 3.2%), Black/African descent ($N = 2$; 2.1%), or Hispanic of any origin ($N = 1$; 1.1%). Total family income was found to range from less than $10,000 to over $70,000: 3.2% indicated a net family income of less than $10,000, 16% between $10,000 to $30,000, 17% between $30,001 to $50,000, 28.7% between $50,001 to $70,000, and 35.1% greater than $70,000. Over half of the sample (68%) was university educated, having completed a bachelor, master, or doctorate degree, 12.8% of the women had a vocational, technical, or associate's degree, while 16% had completed some college, and 3.2% a high school diploma.

Toddlers had been exposed to the flood at all trimesters: first trimester ($N = 24$; 25.5%), second trimester ($N = 37$; 39.4%), and third trimester ($N = 33$; 35.1%). At birth, the sample of toddlers ranged in gestation age from 35 to 42 weeks ($M = 39.2$; SD = 1.2). At the time of the assessment toddlers ranged in age from 29 to 34 months ($M = 29.9$; SD = 1.1), and of these, 48 were female (51.1%).

2.2. Flood-related (predictor) variables

At recruitment into the Iowa Flood Study, participants completed a series of questionnaires about psychosocial and demographic characteristics, and about their objective exposure and subjective distress levels related to the flooding. The majority of women completed the flood-related questionnaires within three months of exposure to the peak of the Iowa flooding ($M = 9.7$ weeks; range 4.7—24.9 weeks). In total, 66 women answered the recruitment questionnaire while still pregnant, and 28 women answered the questionnaire after delivery; moreover, 24 of the 28 women answered within 3 months of giving birth.

2.2.1. Objective PNMS (IF100)

We estimated objective maternal hardship using mothers' responses to questions associated with four categories of exposure that have been used in other disaster studies: threat, loss, scope, and change (Laplante et al., 2007). Each dimension was scored on a scale of 0—25, ranging from no exposure to high exposure (see Table 1). Points for each response on each item were attributed following discussion and consensus among the IF100 committee members, taking into account each participant's write-in comments on the questionnaires. Because there was no theoretical basis to believe that any one of the four dimensions was more predictive than another, and on the basis of McFarlane's study of Australian firefighters (McFarlane, 1988), each dimension was weighted equally and summed to obtain the total score of our scale: The Iowa Flood 100 (IF100). Total IF100 scores in the present sample ranged from 0-50 out of a possible 100 points. Because the distribution of objective PNMS was positively skewed, a natural log transformation was conducted to normalize the data.

2.2.2. Subjective PNMS (IES-R)

Mothers' subjective distress was assessed using the Impact of Event Scale-Revised (IES-R; Weiss and Marmar, 1997). The 22-item scale describes symptoms from three categories relevant to PTSD: intrusions (thoughts and images), hyperarousal, and avoidance. Scale items were modified to reflect symptoms relative to the Iowa flooding disaster. Participants responded on a five-point Likert scale, from ''not at all'' to ''extremely'', the extent to which the item in question described symptoms experienced during the preceding 7 days in response to the floods. The total score range in the present study was 0—60 out of a possible 88 points; IES-R scores greater or equal to 22 indicate possible PTSD. Because the distribution of subjective PNMS was positively skewed, a natural log transformation was conducted to normalize the data.

Table 1 Scoring of the Iowa Flood 100 (IF100).

1	Variable Name	Max. points	Label	Scoring rules
	THREAT	25		Sum(Hurt, Hurtso, Danger, Drown, waterlevel, Shocks, Bridge, Drink, Sewage, Food, Isolation, Otherdanger, time)
1.1	Hurt	1	Were you physically hurt?	Yes = 1, no = 0
1.2	Hurtso	1	Someone close to you got physically hurt?	Yes = 1, no = 0
1.3	Danger	1	Were you in any kinds of dangers?	Dangers = sum (drown, waterlevel, shocks, building, bridge, drink, sewage, food, isolation, otherdanger), recode 1-highest to 1
1.4	Drown	2	Were you in danger of drowning?	Yes = 2, no = 0
1.5	Waterlevel	4	Were you in danger of increasing water level?	Yes = 4, no = 0
1.6	Shocks	1	Were you in danger of electrical shocks?	Yes = 1, no = 0
1.7	Bridge	1	Were you in danger of collapse of bridges?	Yes = 1, no = 0
1.8	Drink	3	Were you in danger of lack of safe drinking water?	Yes = 3, no = 0
1.9	Sewage	1	Were you in danger of exposure to raw sewage?	Yes = 1, no = 0
1.10	Food	1	Were you in danger of lack of food?	Yes = 1, no = 0
1.11	Isolation	1	Were you in danger of isolation?	Yes = 1, no = 0
1.12	Otherdanger	1	Were you in danger of other dangers?	Yes = 1, no = 0
1.13	Time	7	How much time were you given to leave home?	RECODE time (1 = 7) (2 = 4) (3 = 2) (4 = 1)

Table 1 (*Continued*)

2	LOSS	25		Sum(Damage, Heirloom, Property, Investment, Vehicle, Lossincome, Totalloss, Busiloss, damVSinsur)
2.1	Damage	4	Was your home damaged?	RECODE damage (1 = 0) (2 = 1) (3 = 2) (4 = 3) (5 = 4)
2.2	Heirloom	2	Was there a loss of family heirlooms?	Yes = 2, no = 0
2.3	Property	2	Was there a loss of personal property?	Yes = 2, no = 0
2.4	Investment	2	Was there a loss of personal investments?	Yes = 2, no = 0
2.5	Vehicle	1	Was your vehicle damaged?	RECODE vehicle (1 thru 4 = 1)
2.6	Loss income	2	Did you experience loss of personal income?	Yes = 2, no = 0
2.7	Total loss	5	How much is your total loss?	RECODE total loss (1 = 0) (2 = 1) (3 = 2) (4 = 3) (5 = 4) (6 = 5)
2.8	Busiloss	5	The total loss of your personal business?	RECODE busiloss (1 = 0) (2 = 1) (3 = 2) (4 = 3) (5 = 4) (6 = 5)
2.9	DamVSinsur	2	Damage of your home VS flood insurance (home was damaged and no flood insurance get 2 points)	IF ((damage gt 1) and (flood insurance = 0)) damVSinsur = 1
3	SCOPE	25		Sum(Daysaway, Neighbour, Electricity, phone)
3.1	Daysaway	9	Days people were evacuated from home	RECODE daysaway (1 thru 2 = 1) (4 thru 5 = 2) (6 thru 7 = 3) (8 thru 9 = 4) (10 thru 11 = 5) (14 thru 20 = 6) (42 thru 60 = 7) (61 thru 92 = 8) (208 thru 722 = 9)
3.2	Neighbour	6	To what extent was your neigh-bourhood affected?	RECODE neighbour (1 = 0) (2 = 1) (3 = 2) (5 = 6)
3.3	Electricity	5	Days people were deprived from electricity	RECODE electricity (0.04 thru 0.5 = 1) (1 thru 2 = 2) (3 thru 7 = 3) (16 thru 30 = 4) (40 thru 140 = 5)
3.4	Phone	5	Days people were deprived from phone	RECODE phone (0.08 thru 0.5 = 1) (1 thru 3 = 2) (4 thru 9 = 3) (15 thru 17 = 4) (42 thru 68 = 5)

Table 1 (*Continued*)

4	CHANGE	25		Sum(Familyapart, Residence, Shelterdays, House, Changework, Changehour, Commute, Prenatalcare, Birthplan, Work, Spousework, Dietchange, skipmeal)
4.1	Familyapart	3	Did your family stay together? Days apart?	RECODE familyapart (1 thru 5 = 1) (7 thru 10 = 2) (18 thru 45 = 3)
4.2	Residence	2	How many times were you required to change residence because of flood?	RECODE residence (0 = 0) (2 thru 4 = 2)
4.3	Shelterdays	1	Days stay in shelter	RECODE shelterdays (0 = 0) (1 thru 20 = 1)
4.4	House	4	Did you house people? (number of people, number of days)	House = num of people housed * days house people, RECODE house (1 thru 6 = 1) (8 thru 14 = 2) (24 thru 42 = 3) (90 thru 450 = 4)
4.5	Changework	2	Experience decrease/increase in physical work?	Yes = 2, no = 0
4.6	Changehour	1	Experience decrease/increase in work hours?	Yes = 1, no = 0
4.7	Commute	2	Experience longer commuting time?	Yes = 2, no = 0
4.8	Prenatalcare	2	Experience difficulty in accessing prenatal care?	Yes = 2, no = 0
4.9	Birthplan	1	Experience change in birth plan?	Yes = 1, no = 0
4.10	Work	1	Was your place of work damaged?	Yes = 1, no = 0
4.11	Spousework	1	Was the place of work of your spouse damaged?	Yes = 1, no = 0
4.12	Dietchange	2	Experience change in diet?	Yes = 2, no = 0
4.13	Skipmeal	3	Did you skip meals and how many?	RECODE skipmeal (1 thru 2 = 1) (3 = 2) (4 = 3)
Total	IF100	100		Sum(Threat, Loss, Scope, Change)

2.3. Timing of in utero flood exposure

Timing of in utero flood exposure was defined as the number of days between estimated date of conception and June 15th, 2008, the date at which the flooding was recorded to peak; higher flood exposure days indicated flood exposure later in pregnancy. To calculate estimated date of conception, we subtracted 280 days (40 weeks) from women's due date, which was first calculated using their babies' gestational age and date of delivery.

2.4. Maternal separation protocol and toddler saliva sampling

At age 2½ years, toddlers were assessed in a laboratory at the University of Iowa. To assess stress reactivity, four saliva samples were collected from each toddler to assess cortisol levels throughout the procedure: a buffer sample taken 10 minutes after arriving in the laboratory; a baseline sample taken approximately 45 minutes after the buffer sample and 15 minutes before maternal separation (stressor); and two samples taken 20 and 45 minutes post-stressor (after the toddler was reunited with his or her mother). Maternal separation commenced when a research assistant knocked on the two-way mirror, signalling for mothers to leave their toddlers alone in the monitored testing room for 2 min ($M = 1.89$ min, SD = 0.49). No instructions on how to leave were given. Collection of saliva involved placing a Sorbette (Salimetrics, product number 5029) under the toddler's tongue and allowing it to absorb for 45 s to 1 min. After absorption, the Sorbette was removed and placed in a conical tube, and sealed with a cap. Directly after collection, samples were stored in a −80 °C freezer until shipping to the Douglas Institute Research Centre, Quebec, Canada, where they remained frozen until assayed.

2.5. Outcome variables

2.5.1. Toddler salivary cortisol

Salivary cortisol levels were assessed via competitive enzyme immunoassay (EIA) using kits provided by Salimetrics. The inter- and intra-assay coefficients of variation were 11.3 and 9.6, respectively. Cortisol values two or more standard deviations from the mean (buffer $n = 1$; baseline $n = 3$; 20-minute post-stressor $n = 6$; and 45-minute post-stressor $n = 3$) were winsorized, a technique for reducing the effect of extreme outliers outlined by Tabachnick and Fidell (1989). Absolute Cortisol Change (the absolute change in cortisol levels from baseline to 20- or 45-minute post-stressor) was computed to thoroughly assess participants' acute cortisol response, as well as their return to baseline, after administration of the stressor. Area Under the Curve (AUC) with respect to increase (AUCi) and with respect to ground (AUCg) were also analyzed. AUCi and AUCg were computed using the baseline, 20-minute post-stressor, and 45-minute post-stressor cortisol samples collected throughout the assessment and reflecting reactive and total cortisol secretion, respectively (Pruessner et al., 2003). All AUC values were standardized by dividing the total cortisol output by the amount of time elapsed between the first and last sample.

2.6. Control variables

2.6.1. Maternal characteristics

At recruitment, we obtained demographic information, including socioeconomic status (SES), smoking and alcohol habits, and relationship status. We computed SES based on maternal and paternal education and occupation, and coded it as per Hollingshead criteria (Hollingshead, 1973).

We assessed the remaining maternal covariates at the time of the child assessment, when toddlers were 2½ years of age. Stressful life events and the participant's perception of stress were assessed using the Life Experience Survey (LES) and Perceived Stress Scale (PSS), respectively (Sarason et al., 1978; Cohen et al., 1983). Parenting-related stress was assessed using the Parenting Stress Index (PSI; Loyd and Abidin, 1985). The presence of help and/or support in a participant's environment was assessed with the Social Support Questionnaire (SSQ; Sarason et al., 1983). Finally, maternal symptoms of depression and anxiety were measured by the Inventory of Depression and Anxiety Symptoms (IDAS; Watson et al., 2007).

2.6.2. Child characteristics

Toddler birth outcomes, such as birth weight, birth length, and gestational age, were obtained from hospital records.

2.6.3. Time of day of cortisol sampling

The time at which baseline cortisol samples were collected from toddlers was recorded for each participant. Sixty-six percent of samples were taken before 1200 h, 23% between 1200 h and 1659 h, and 11% of samples between 1700 h and 1922 h.

2.7. Procedure

All phases of the Iowa Flood Study were approved by the Institutional Review Board at the University of Iowa. Written informed consent was collected from all mothers who agreed to participate. At recruitment, mothers were asked to complete a questionnaire packet that included the assessment of objective and subjective PNMS, as well as demographic information.

Mother—toddler dyads were invited to participate in the present phase of the study when their children reached 2½ years of age. The study protocol consisted of cognitive, motor, and stress assessments for the toddlers, self-report questionnaires for the mothers, and physical measurements (e.g., height and weight) and biological samples (e.g., saliva samples) taken from both the toddlers and mothers. However, for the purpose of this study, only results relating to cortisol levels and the stressor paradigm are presented.

2.8. Data analysis

First, descriptive analyses (mean, range, standard deviation) were conducted on outcome, predictor, and control variables. To assess acute changes in cortisol levels in

response to maternal separation, paired samples *t*-tests were run between baseline and 20-minute post-stressor and 45-minute post-stressor cortisol levels; these were rerun for males and females separately. Independent samples *t*-tests were also run comparing male and female toddler cortisol levels across the three sampling times. Next, bivariate Pearson correlation coefficients were computed between all variables. Hierarchical multiple linear regression analyses were conducted for each of the four outcome variables: baseline to 20-minute post-stressor absolute change, baseline to 45-minute post-stressor absolute change, AUCi, and AUCg. To assess the effects of PNMS, the level of objective hardship (LogIF100) and subjective distress (LogIES-R) were entered into the model during step 1 and step 2, respectively. The ordering of these steps allowed for the control of objective hardship in the assessment of the effect of subjective distress. Timing of the flood exposure in utero and sex were entered into the model in steps 3 and 4, respectively, to examine their respective main effects on the outcome, and to determine if controlling for these covariates changed the magnitude and significance of the prenatal stress effects. For both absolute change outcome variables, baseline cortisol values were entered into the model during step 5 to adjust for the law of initial values (Wilder, 1962). Next, covariates that significantly correlated with the outcome variable at $p < .05$ were allowed to enter, Stepwise, into the following step of the model (*p*-value for entry .05 and exit .10). This method allowed for the conservation of power considering the limitations imposed by our sample size. Subsequently, to assess the moderating effects of timing of exposure and toddler sex on the relationship between PNMS and cortisol reactivity, we entered an interaction term (IF100 × Timing of Exposure, IES-R × Timing of Exposure, IF100 × Sex, or IES-R × Sex) into the model individually in the final step. Repeated measures mixed model analyses were also conducted that allowed for the retention of all participants, regardless of missing cortisol values. The same predictor variables as those entered in the AUC regression models were used for these analyses. All of the predictor variables were tested as fixed effects. All analyses were conducted using SPSS 20.0.

3. Results

3.1. Descriptives

Descriptive statistics for outcome, predictor, and control variables are presented in Table 2. On average, toddler cortisol levels were found to increase from baseline to 20-minute post-stressor sampling (by 40% on average; $t(78) = -2.093$, $p < .05$), as well as from baseline to 45-minute post-stressor sampling (by 63% on average), $t(81) = -2.796$, $p < .01$. When split by sex, results remained significant for females from baseline to 20-minute post-stressor sampling ($t(40) = -2.019$, $p < .05$) and to 45-minute post-stressor sample ($t(42) = -2.386$, $p < .05$), but not for males (data not shown). Independent samples *t*-tests revealed that cortisol levels at baseline, 20-minute post-stressor, and 45-minute post-stressor did not differ significantly between males and females.

A correlation matrix between predictor and outcome variables is presented in Table 3. Lower toddler birth weight ($r = -0.300$, $p < .01$) and baseline cortisol levels ($r = -0.599$, $p < .01$) were related to greater increases in cortisol from baseline to 20-minute post-stressor. Higher objective PNMS scores ($r = 0.258$, $p < .05$) and subjective PNMS scores ($r = 0.246$, $p < .05$), and later timing of in utero exposure ($r = 0.330$, $p < .01$) were related to greater cortisol secretion across the assessment relative to baseline (AUCi). Time of day of baseline cortisol sampling was also related to AUCi, such that earlier testing was associated with a smaller increase in cortisol.

3.2. Baseline to 20-minute post-stressor absolute change

Results of the hierarchical multiple regression are presented in Table 4a. Objective and subjective PNMS, timing of in utero flood exposure, and toddler sex were unrelated to the 20-minute absolute cortisol change, collectively explaining only 4.3% of the variance. Baseline cortisol levels explained an additional 36.6% of the variance ($p < .01$) of the 20-minute absolute cortisol change: higher baseline levels were related to smaller increases in 20-minute post-separation cortisol levels. Given that birth weight was found to significantly correlate with the outcome variable, the covariate was allowed to enter Stepwise in the next step, and explained an additional 3.7% of the variance. None of the interaction terms explained additional variance. The final model explained 45.2% of the variance of the toddlers' 20-minute post-separation absolute cortisol change levels.

3.3. Baseline to 45-minute post-stressor absolute change

Results of the hierarchical multiple regression are presented in Table 4b. The association between objective PNMS and 45-minute absolute cortisol change was marginally significant, explaining 4.5% of the variance ($p < .1$). Subjective PNMS, timing of in utero flood exposure, and toddler sex were not significantly associated with the 45-minute absolute cortisol change, collectively explaining an additional 4.3% of the variance. Baseline cortisol levels explained an additional 4.0% of the variance ($p < .1$). The subjective PNMS × sex interaction was significantly related to the 45-minute absolute cortisol change and accounted for an additional 6.9% of the variance ($p < .05$): higher subjective PNMS scores predicted a greater change in cortisol levels in response to stress in females, whereas subjective PNMS scores were not related to cortisol change in males (Fig. 1a). By probing the interaction to find the regions of significance, absolute cortisol change values between males and females were found to significantly differ when subjective PNMS log-transformed levels were equal to or greater than 1.4194 (non-transformed IES-R level of 3.1346). Overall, the model explained 19.7% of the variance of the toddlers' 45-minute post-separation absolute cortisol change levels.

Table 2 Descriptive analysis of variables.

Variables	*N*	Mean	Range	SD
Buffer cortisol (μg/dL)	84	0.105	0.01—0.48	0.084
Baseline cortisol (μg/dL)	85	0.097	0.01—0.38	0.070
20-minute post-stressor (μg/dL)	87	0.108	0.01—0.25	0.062
45-minute post-stressor (μg/dL)	91	0.130	0.01—0.54	0.124
Percent change cortisol (baseline to 20 min post-stressor)	78	39.844	−58.06—477.23	107.086
Percent change cortisol (baseline to 45 min post-stressor)	81	63.048	−95.37—1039.89	165.734
Absolute change cortisol (baseline to 20 min post-stressor)	79	0.017	−0.17—0.24	0.073
Absolute change cortisol (baseline to 45 min post-stressor)	82	0.038	−0.25—0.46	0.123
AUC ground (μg/dL)	71	0.176	0.06—0.60	0.090
AUC increase (μg/dL)	71	−0.002	−0.50—0.23	0.113
Objective PNMS (LogIF100)	94	1.850	0—3.93	0.889
Objective PNMS (IF100)	94	8.57	0—50	10.665
Subjective PNMS (LogIES-R)	94	1.268	0—4.11	1.066
Subjective PNMS (IES-R)	94	5.453	0—60	8.799
Timing of exposure in utero (days)	94	148.266	0—280.98	81.999
SES	92	53.77	21—66	9.852
Number of cigarettes/day	92	0.27	0—14	1.529
Alcohol units/week	92	0.07	0—2	0.252
Perceived Stress Scale	90	13.50	1—29	5.075
Life Experience Survey	90	4.43	0—14	2.888
Parenting Stress Index	90	66.44	40—97	13.758
Social support	87	4.39	1—9	1.805
Depression and anxiety (IDAS)	89	32.73	22—70	8.005
Birth weight (g)	92	3546.12	2220—4740	444.292
Birth length (cm)	83	51.70	45—58	2.517
Gestational age at birth (weeks)	92	39.24	35—42	1.249
Time of baseline cortisol sampling	94	12:12	8:55—19:22	2:58

Note: AUC = Area Under the Curve. PNMS = Prenatal Maternal Stress. SES = Socioeconomic Status (higher scores indicate higher SES). IDAS = Inventory of Depression and Anxiety Symptoms.

3.4. Area under the curve increase (AUCi)

Results of the hierarchical multiple regression are presented in Table 5a. Greater objective PNMS predicted greater AUCi in step 1, explaining 6.7% of the variance ($p < .05$): higher objective PNMS scores were associated with a greater increase in cortisol following the stressor. The addition of subjective PNMS in step 2 increased explained variance to 8.8%. When timing of in utero flood exposure entered in step 3 it explained 7.3% of unique variance ($p < .05$), with exposure later in pregnancy associated with a greater increase in cortisol. Toddler sex did not explain additional variance. Next, time of day of the baseline cortisol sampling was allowed to enter Stepwise, but was not retained in the model. The subjective PNMS × sex interaction was significant in the final step and accounted for 7.0% of additional variance ($p < .05$): as subjective PNMS scores increased, so did total cortisol secretion in females; conversely, subjective PNMS levels were not related to total cortisol secretion in males (Fig. 1b). By probing the interaction for the regions of significance, AUCi values between males and females were found to differ significantly when IES-R log-transformed levels were less than 0.393 or greater than 3.463 (non-transformed IES-R levels of 0.482 and 30.919, respectively). Overall, the final model explained 23.4% of the variance of AUCi.

3.5. Area under the curve with respect to ground (AUCg)

Table 5b presents the results of the hierarchical multiple regression analyses conducted for AUCg. Neither the predictor nor the control variables were significantly related to total cortisol secretion during the assessment. Only 5.5% of the variance in AUCg was explained by the final model.

3.6. Additional analyses

A repeated measures mixed model analysis was conducted to further explore the significant interaction between subjective PNMS and toddler sex on cortisol reactivity. A three-way interaction between cortisol sampling time (i.e., minutes since start of procedure), subjective PNMS, and toddler sex was found to be marginally significant ($t(193) = -1.72$, $p = .08$; see Fig. 2): in females, the magnitude of the slope of cortisol levels across time increased as a function of maternal subjective distress exposure, while no differences in slope as a function of subjective PNMS was found for males.

4. Discussion

The goal of the present study was to determine the association between disaster-related PNMS and toddler cortisol

Table 3 Pearson correlation coefficients for all variables (*n*).

Variable	Absolute change (baseline to 20 min post-stressor)	Absolute change (baseline to 45 min post-stressor)	Area under the curve (*increase*)	Area under the curve (*ground*)
Objective PNMS (LogIF100)	**0.190 (79)**†	**0.213 (82)**†	**0.258 (71)***	0.053 (71)
Subjective PNMS (LogIES-R)	0.150 (79)	**0.193 (82)**†	**0.246 (71)***	−0.030 (71)
Timing of exposure in utero (days)	0.080 (79)	0.157 (82)	**0.330 (71)****	−0.100 (71)
Toddler sex (0 = boy; 1 = girl)	0.127 (79)	0.123 (82)	−0.076 (71)	0.124 (71)
Time of day of baseline cortisol sampling	−0.043 (79)	−0.179 (82)	**−0.240 (71)***	−0.005 (71)
SES	**−0.221 (77)**†	**−0.188 (80)**†	0.072 (70)	0.097 (70)
Number of cigarettes/day	0.141 (77)	0.058 (80)	−0.027 (69)	0.075 (69)
Alcohol units/week	−0.103 (77)	−0.122 (80)	−0.024 (69)	−0.030 (69)
Perceived Stress Scale	0.102 (76)	0.139 (79)	0.024 (68)	−0.014 (68)
Life Experience Survey	0.130 (76)	0.114 (79)	0.160 (68)	−0.005 (68)
Parenting Stress Index	−0.031 (76)	0.046 (79)	−0.023 (68)	−0.107 (68)
Social support	−0.005 (75)	−0.086 (76)	−0.009 (67)	−0.155 (67)
Depression and anxiety (IDAS)	0.094 (76)	0.063 (78)	0.099 (68)	−0.127 (68)
Birth weight (g)	**−0.300 (77)****	0.022 (80)	0.137 (69)	0.055 (69)
Birth length (cm)	−0.198 (70)	−0.102 (73)	0.094 (64)	−0.044 (64)
Gestational age at birth (weeks)	−0.070 (77)	−0.031 (80)	−0.047 (69)	0.047 (69)
Baseline cortisol (μg/dL)	**−0.599 (79)****	−0.196 (82)†	−0.028 (71)	**0.790 (71)****

Note: PNMS = Prenatal Maternal Stress. SES = Socioeconomic Status (higher scores indicate higher SES). IDAS = Inventory of Depression and Anxiety Symptoms.

† $p < .1$.

* $p < .05$.

** $p < .01$.

reactivity in response to brief maternal separation. Our method included the ability to distinguish between the degree of objective exposure of the mother to the hardship from the flooding and her degree of subjective distress, and to test the relative contributions of these two aspects of the PNMS experience. Given the sudden onset of the flooding, compared to other forms of prenatal ''stress'' such as anxiety, we were also able to test, and control for, the effects of timing of the stressor in utero. Finally, we also tested the moderating effects of toddler sex.

On average, the stressor paradigm used in the present study was found to elevate toddler cortisol levels for up to 45 minutes following the stressor. Our finding is in accordance with previous studies documenting a significant increase in infant and toddler cortisol levels following exposure to an experimental stressor, including maternal separation, physical examination, and inoculations (for review see Gunnar et al., 2009). Females in the present sample exhibited, on average, a significant increase in cortisol levels following the brief maternal separation, whereas males did not. This difference suggests that the overall elevation in cortisol in response to maternal separation was largely influenced by the females in our sample.

We tested the effects of PNMS on four aspects of toddler stress reactivity: absolute cortisol change from baseline to 20 or 45 minutes post-stressor, and total cortisol secretion relative to ground (AUCg) or to increase from baseline (AUCi). The broad scope of our analyses allowed for a thorough investigation of cortisol reactivity, including acute change, return to baseline, total secretion, and reactive secretion with respect to baseline. We analyzed the extent to which objective (e.g., amount of financial loss, length of power outage) and subjective (PTSD symptoms) aspects of PNMS would explain variance in these cortisol measures. More severe objective PNMS from the floods correlated with greater AUCi and, at the trend level, with absolute levels of change 20 and 45 minutes post-stressor. Similarly, subjective PNMS was positively correlated with AUCi and, at the trend level, with greater absolute change at 45 minutes. Although the effects of objective PNMS applied to the group as a whole, the effect of subjective distress on AUCi and 45-minute change was limited by sex: as seen in Fig. 1a and b, the effect of subjective PNMS on 45-minute absolute cortisol change and AUCi was significant in females but not in males, a finding that was supported by the repeated measures mixed model analysis (Fig. 2). Thus, toddler sex significantly moderated the relationship between subjective PNMS levels and these two aspects of cortisol reactivity: greater subjective PNMS levels predicted greater cortisol secretion in response to maternal separation and across the entire assessment in females; conversely, subjective PNMS levels did not predict cortisol levels or the degree of cortisol change in response to stress in males. While sex differences in basal cortisol levels have been documented over the last decade (for review see Jessop and Turner-Cobb, 2008), with girls commonly having higher baseline cortisol concentrations than boys (Watamura et al., 2003; Gutteling et al., 2004; O'Connor et al., 2013), few studies have documented sex differences in cortisol reactivity in response to experimental stressors in children (Jessop and Turner-Cobb, 2008).

Table 4 Results of hierarchical multiple regression for (a) absolute change baseline to 20-minute post-stressor ($n = 77$) and (b) absolute change baseline to 45-minute post-stressor ($n = 82$).

Predictor variables	β	B	Std. error	R	R^2	ΔR^2	F	ΔF
(a) Absolute change baseline to 20-minute post-stressor ($n = 77$)								
Step 1				.141	.020	.020	1.531	1.531
Objective PNMS	.141	.011	.009					
Step 2				.151	.023	.003	.864	.213
Objective PNMS	.118	.009	.010					
Subjective PNMS	.058	.004	.009					
Step 3				.164	.027	.004	.670	.298
Objective PNMS	.105	.008	.010					
Subjective PNMS	.052	.004	.009					
Timing in utero	.065	5.797E−05	.000					
Step 4				.208	.043	.017	.815	1.244
Objective PNMS	.123	.010	.010					
Subjective PNMS	.035	.002	.009					
Timing in utero	.081	7.231E−05	.000					
Sex (0 = boy; 1 = girl)	.131	.019	.017					
Step 5				.640	.409	.366	9.826**	43.926**
Objective PNMS	.116	.009	.008					
Subjective PNMS	.004	.000	.007					
Timing in utero	.000	−2.553E−07	.000					
Sex (0 = boy; 1 = girl)	.156†	.022†	.013					
Baseline cortisol	−.613**	−.677**	.102					
Step 6				.668	.446	.037	9.410**	4.740*
Objective PNMS	.073	.006	.008					
Subjective PNMS	.028	.002	.007					
Timing in utero	.026	2.356E−05	.000					
Sex (0 = boy; 1 = girl)	.096	.014	.013					
Baseline cortisol	−.584**	−.645**	.101					
Birth weight	−.208*	−3.171E−05*	.000					
Step 7				.672	.452	.005	8.124**	.672
Objective PNMS		.006	.008					
Subjective PNMS		−.002	.009					
Timing in utero		1.093E−05	.000					
Sex (0 = boy; 1 = girl)		.001	.021					
Baseline cortisol		−.650**	.101					
Birth weight		−3.082E−05*	.000					
Subjective PNMS × Sex		.011	.013					
(b) Absolute change baseline to 45-minute post-stressor ($n = 82$)								
Step 1				.213	.045	.045	3.810†	3.810†
Objective PNMS	.213†	.029†	.015					
Step 2				.238	.057	.011	2.367	.929
Objective PNMS	.157	.021	.017					
Subjective PNMS	.119	.014	.015					
Step 3				.262	.069	.012	1.912	1.001
Objective PNMS	.135	.018	.017					
Subjective PNMS	.115	.014	.015					
Timing in utero	.112	.0001	.0001					
Step 4				.297	.088	.020	1.865	1.675
Objective PNMS	.153	.021	.017					
Subjective PNMS	.100	.012	.015					
Timing in utero	.120	.0001	.0001					
Sex (0 = boy; 1 = girl)	.142	.035	.027					

Table 4 (*Continued*)

Predictor variables	β	B	Std. error	R	R^2	ΔR^2	F	ΔF
Step 5				.358	.128	.040	2.231†	3.455†
Objective PNMS	.155	.021	.017					
Subjective PNMS	.089	.011	.014					
Timing in utero	.116	.0001	.0001					
Sex (0 = boy; 1 = girl)	.165	.040	.027					
Baseline cortisol	−.201†	−.349†	.188					
Step 6				.444	.197	.069	3.074*	6.487*
Objective PNMS		.024	.016					
Subjective PNMS		−.018	.018					
Timing in utero		.0001	.0001					
Sex (0 = boy; 1 = girl)		−.039	.040					
Baseline cortisol		−.392*	.182					
Subjective PNMS × Sex		.064*	.025					

Note: PNMS = Prenatal Maternal Stress.
† $p < .1$.
* $p < .05$.
** $p < .01$.

Nevertheless, the finding of greater female cortisol reactivity in the present project is in accordance with findings of increased diversity of physiological outcomes in females exposed to early life adversity compared to males (Sandman et al., 2013). In animal studies, PNMS has been associated with increased abnormal brain morphology and hippocampal neurogenesis in female offspring compared to males (Fameli et al., 1994; Hillerer et al., 2013). Collectively, these results suggest that the sexes differ in susceptibility to prenatal insults, which have the potential to modify development of integrated brain systems, including the hippocampus and HPA axis. The reason for these observed sex differences is currently unknown; however, biological foetal factors, such as androgen levels and activity and expression of 11β-HSD2, may be implicated (for review see Charil et al., 2010). Exposure to elevated maternal GC levels associated with prenatal stress may modify foetal androgen levels, such as testosterone, for which levels and timing of release are critical to brain development. With respect to 11β-HSD2, the activity level of this enzyme has been found to differ between sexes, with females commonly having higher levels than males. While the exact mechanism by which males and females differ in their response to PNMS exposure is unknown, the results from the present study support the notion that dysregulated HPA activity may underlie the sexual dimorphism seen in psychiatric illness prevalence rates, which places females at a greater risk for developing mood disorders (Fernandez-Guasti et al., 2012). Further investigation is warranted to determine whether the sex difference reported in this paper exists beyond toddlerhood. This is especially important given that animal models of prenatal stress have reported greater susceptibility in male offspring (Schopper et al., 2012), suggesting that sex-specific susceptibility may change depending on experimental variables, such as type of stressor or timing of exposure. Given that the present project is part of a longitudinal study, participants will continue to be followed throughout childhood to determine whether the findings persist and, moreover, to examine whether cortisol reactivity is associated with later cognitive and behavioural developmental outcomes.

The results presented here lend support for the foetal *programming hypothesis*, the process by which the external environment reprograms foetal development during critical periods (Glover et al., 2010). Given that exposure to high levels of disaster-related PNMS was associated with increased activity of the HPA axis, evident through elevated cortisol secretion in response to stress, this suggests that elevated maternal GCs, or other physiological pathways, accompanying prenatal stress led to reprogramming of foetal HPA axis, affecting the axis' functioning throughout childhood.

PNMS exposure later in pregnancy was also associated with greater cortisol secretion in toddlers. As seen in Table 5a, timing of foetal exposure to PNMS was found to explain 5.4% of the variance in AUCi values unexplained by objective and subjective PNMS. Therefore, the second half of pregnancy may represent a critical period for foetal brain development, including the HPA axis. Our reported timing effect is supported by past studies where exposure to prenatal stress mid- to late-pregnancy was associated with greater changes in cortisol levels in infants born to mothers with PTSD (Yehuda et al., 2005), and predicted higher basal levels of salivary cortisol in pre-adolescent and adolescent offspring exposed to prenatal maternal anxiety (O'Connor et al., 2005; Huizink et al., 2008; Van den Bergh et al., 2008). Reprogramming of foetal development may possess evolutionary value if resulting modifications lead to offspring with increased resiliency and efficient coping skills, and offspring that are more likely to survive and reproduce in their external environment (Glover et al., 2010).

There are several strengths of the present study. The first is the fact that all of the women in the study experienced the same stressor, a flood, to degrees that varied from very little to extreme. Secondly, the stressor itself was completely independent of the women's own influence. Thirdly, these facts allowed us to distinguish between the

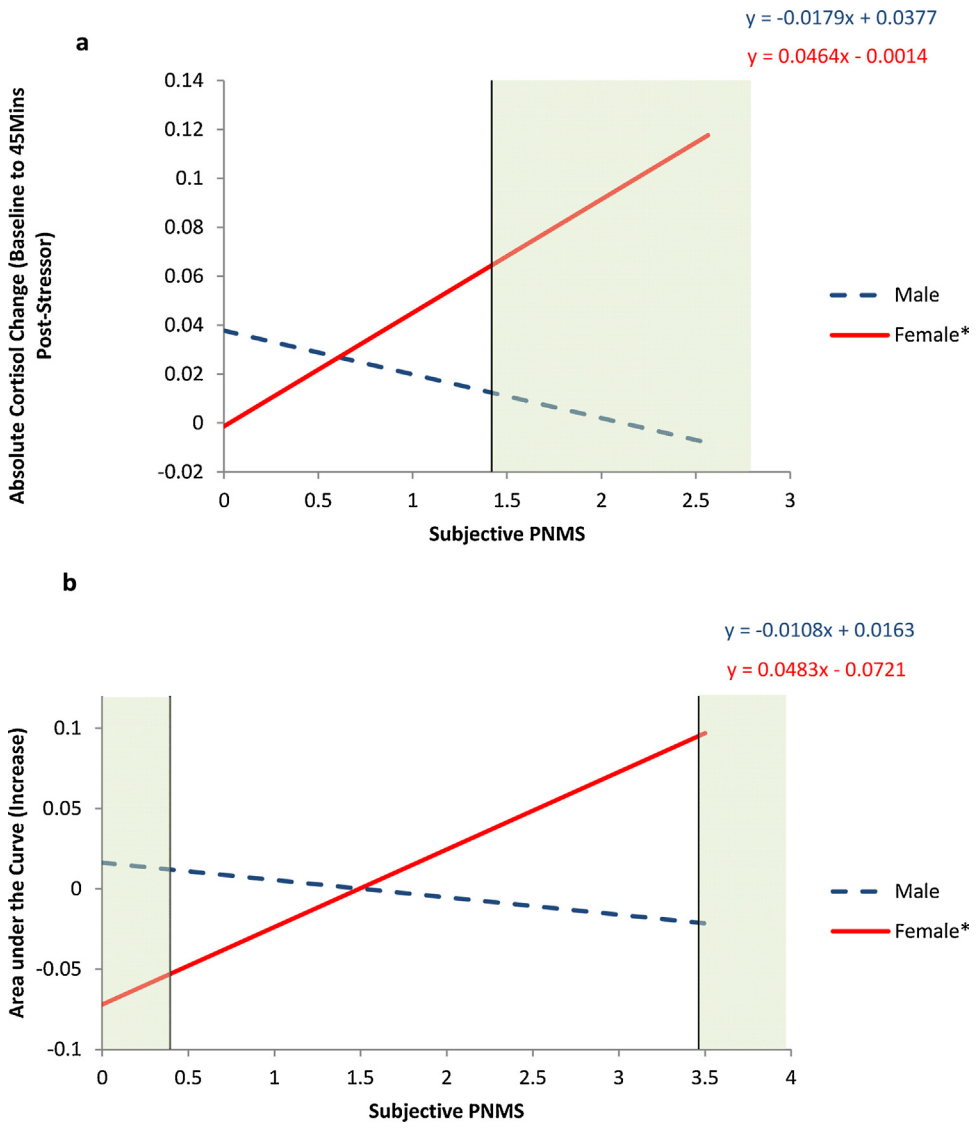

Figure 1 (a) Depiction of sex-by-subjective PNMS interaction on absolute cortisol change (baseline to 45-minute post-stressor) showing region of significance in shaded area. (b) Depiction of sex-by-subjective PNMS interaction on AUC (increase) showing region of significance in shaded area. * indicates significant slope at $p < .05$.

mothers' objective exposure to hardship from the floods and their level of distress. Although the objective and subjective PNMS measures were relatively uncorrelated in Project Ice Storm ($r < 0.30$), they are more highly correlated in the Iowa Flood Study ($r = 0.55$) and operate as overlapping risk factors (Kraemer et al., 2001). Objective exposure is uncorrelated with family demographic characteristics such as SES ($r < 0.14$) and may, therefore, be considered relatively randomly assigned. Maternal subjective distress, however, may have roots in genetic predispositions and learning and is, therefore, less likely to be random. Interestingly, the moderating effect of sex on offspring cortisol reactivity did not apply to the objective degree of exposure to the floods. The lack of generalizability across subjective and objective experiences of stress suggests that maternal perception of an event as ''stressful'', and her subsequent levels of distress, may trigger different physiological cascades of events for the developing foetal HPA axis than does the objective degree of hardship from the event.

The Iowa Flood Study is not without its limitations, however. Although we can be quite precise when dating the onset of the stressor in this sample, and about dating the time in each woman's pregnancy when the floods hit, we cannot date the end of the stress experience for each woman; the psychological distress and the logistical difficulties may have lasted well into the postpartum period for women who experienced high levels of flood-related hardship. This timing issue and other related methodological challenges are clearly described in DiPietro (2012). Given that maternal cortisol can be transferred to a baby via breast milk (Patacchioli et al., 1992), for example, some of our results may be due to flood stress extending past the ''prenatal'' period and into infancy. As well, given that experiencing PTSD symptoms in the face of trauma may reflect a genetic vulnerability and predisposition towards

Table 5 Results of hierarchical multiple regression of (a) area under the curve (increase) ($n = 71$) and (b) area under the curve (ground) ($n = 71$).

Predictor variables	β	B	Std. error	R	R^2	ΔR^2	F	ΔF
(a) Area under the curve (increase) ($n = 71$)								
Step 1				.258	.067	.067	4.935*	4.935*
Objective PNMS	.258*	.032*	.014					
Step 2				.297	.088	.021	3.286*	1.595
Objective PNMS	.185	.023	.016					
Subjective PNMS	.164	.018	.014					
Step 3				.401	.161	.073	4.284**	5.816*
Objective PNMS	.144	.018	.016					
Subjective PNMS	.131	.014	.014					
Timing in utero	.277*	.0001*	.0001					
Step 4				.405	.164	.003	3.239*	.247
Objective PNMS	.137	.017	.016					
Subjective PNMS	.139	.015	.014					
Timing in utero	.273*	.0001*	.0001					
Sex (0 = boy; 1 = girl)	−.057	−.013	.025					
Step 5				.484	.234	.070	3.980**	5.967*
Objective PNMS		.019	.015					
Subjective PNMS		−.011	.017					
Timing in utero		.0001*	.0001					
Sex (0 = boy; 1 = girl)		−.088*	.040					
Subjective PNMS × Sex		.059*	.024					
(b) Area under the curve (ground) ($n = 71$)								
Step 1				.053	.003	.003	.191	.191
Objective PNMS	.053	.005	.012					
Step 2				.079	.006	.004	.216	.243
Objective PNMS	.082	.008	.013					
Subjective PNMS	−.067	−.006	.012					
Step 3				.134	.018	.012	.406	.787
Objective PNMS	.099	.010	.014					
Subjective PNMS	−.054	−.005	.012					
Timing in utero	−.110	.0001	.0001					
Step 4				.185	.034	.016	.582	1.107
Objective PNMS	.113	.011	.014					
Subjective PNMS	−.072	−.006	.012					
Timing in utero	−.100	.0001	.0001					
Sex (0 = boy; 1 = girl)	.129	.023	.022					
Step 5				.234	.055	.021	.750	1.410
Objective PNMS	.121	.012	.014					
Subjective PNMS	−.200	−.018	.015					
Timing in utero	−.120	.0001	.0001					
Sex (0 = boy; 1 = girl)	−.054	−.010	.035					
Subjective PNMS × Sex	.273	.026	.022					

Note: PNMS = Prenatal Maternal Stress.
* $p < .05$.
** $p < .01$.

distress, associations between maternal IES-R scores and child HPA axis functioning may be due, in part, to maternal parenting behaviours in the postpartum.

As is the case with all studies that examine biological samples, there are a few limitations associated with cortisol sampling that require mentioning. First and foremost, the absence of a standard definition of a ''normalized'' cortisol stress response within the literature means that research results must be interpreted with caution. What constitutes a ''normal'' or ''dysregulated'' cortisol stress response differs between studies, and is largely attributable to differing research methodologies (Jessop and Turner-Cobb, 2008). To examine the association between PNMS and offspring cortisol reactivity, future studies would benefit from the addition of an age-matched control group; however, generalizability of findings across studies would still be limited due to a lack of methodological consistency. While the present study had a subset of women who did not experience subjective distress

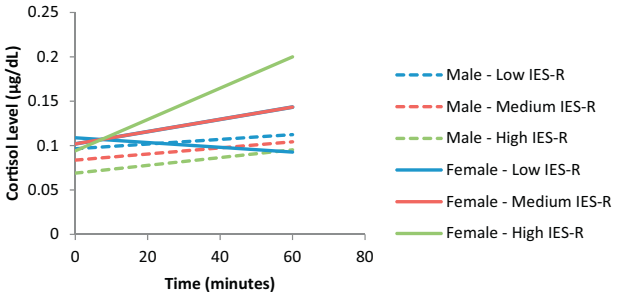

Figure 2 Representation of a three-way interaction between cortisol sampling time, subjective PNMS, and toddler sex, found to be marginally significant in a repeated measures mixed model analysis.

or objective hardship (both IES-R and IF100 scores equal to 0), all participants were recruited from flood-affected areas and were exposed either directly or indirectly to the flood and, thus, were not totally unaffected controls. Secondly, it is well documented that cortisol is released from the adrenal glands into the blood in a pulsatile ultradian manner (Hunter et al., 2011). Unless taking serial plasma cortisol measurements, there is the possibility that the assessment of basal or reactive cortisol levels may capture an artificially high or low hormone profile (Young et al., 2004). This limitation is hard to avoid as salivary sampling continues to be the recommended method for measuring cortisol in young populations due to its non-invasive nature. A final caveat of the present protocol was the absence of a measure of maternal—infant attachment. This was of concern given that studies have found increased postnatal care to reverse the effects of PNMS on the HPA axis in rat pups (Maccari et al., 1995). Moreover in humans, mother—child attachment has been found to moderate the association between cortisol reactivity and behavioural outcomes in toddlers (Nachmias et al., 1996). Thus, secure maternal—infant attachment could potentially correct for any PNMS-associated effects on offspring HPA axis regulation in the present study. Notwithstanding, studies have found prenatal stress or cortisol levels to predict infant stress reactivity independent of maternal—infant attachment or maternal sensitivity, suggesting that our results would likely persist even after controlling for this variable (Grant et al., 2009; O'Connor et al., 2013).

4.1. Conclusions

Overall, the present study is the first of its kind to demonstrate a link between flood-related objective and subjective prenatal maternal stress and cortisol reactivity in response to brief maternal separation in offspring as young as 2½ years of age. Following in the footsteps of Project Ice Storm, this paper provides evidence that the maternal response to the external environment has the potential to programme foetal development during critical stages in utero, and subsequently alter physiological outcomes in toddlerhood (i.e., cortisol reactivity). Given that many natural disasters distribute hardship to a population quasi-randomly, examining disaster-related PNMS allows for the control of external factors that may predispose women to greater distress, thereby increasing internal validity of our results by differentiating between objective exposure and subjective distress. The finding that maternal subjective PNMS was associated with greater cortisol reactivity in female toddlers persisted across two parameters of the cortisol stress response, suggesting that offspring sex strongly influences the hypothesized association between maternal distress in pregnancy and cortisol reactivity. Our results may be indicative of differential susceptibility (Pluess and Belsky, 2011) that renders females more susceptible to prenatal insults than males with respect to HPA axis development; by the same token, however, we see in Fig. 1b that females also benefit more than males from low levels of maternal distress. Our findings, along with the existing limited body of literature, highlight the need for further longitudinal investigation of disaster-related PNMS and cortisol reactivity in offspring, taking into account the moderating effect of sex.

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Conflict of interest

All authors have confirmed that no conflict of interest exists with the submission and publication of this work.

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