Psychoneuroendocrinology 117 (2020) 104697

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Disaster-related prenatal maternal stress predicts HPA reactivity and psychopathology in adolescent offspring: Project Ice Storm

Erin Yong Ping[a], David P. Laplante[b], Guillaume Elgbeili[b], Sherri Lee Jones[b,c], Alain Brunet[b,c], Suzanne King[b,c,*]

[a] Department of Psychology, Concordia University, Montreal, QC, Canada
[b] Douglas Mental Health University Institute, Verdun, QC, Canada
[c] Department of Psychiatry, McGill University, Montreal, QC, Canada

ARTICLE INFO

Keywords:
prenatal stress
HPA axis
psychopathology
adolescents
development
cortisol

ABSTRACT

Background: Prenatal stress has been associated with adverse outcomes in offspring, including elevated risk of psychopathology. Fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis has been posited as a biological mechanism underlying such consequences. The present study aimed to examine whether dysregulation of the offspring HPA axis mediates the relationship between prenatal stress exposure and adolescent psychopathology.

Methods: Five months after the Quebec ice storm of 1998, women who had been pregnant at the time of the storm completed questionnaires about their objective hardship and subjective distress from the disaster. A total of 45 of their children, exposed to the ice storm in utero, participated at 13 years of age. Adolescents completed the Trier Social Stress Test while providing salivary samples to measure circulating cortisol levels. Maternal report of adolescent behaviors was assessed with the Child Behavior Checklist.

Results: Results from the study found that greater objective hardship was associated with elevated offspring cortisol reactivity at 13 years of age. Furthermore, greater subjective distress was associated with greater externalizing behaviors. While lower cortisol reactivity predicted greater externalizing behaviors, it did not mediate the association between maternal objective hardship or subjective distress and offspring externalizing or internalizing behaviors.

Conclusions: Findings suggest that objective hardship in pregnancy has long-term implications for offspring HPA axis functioning, which is also associated with externalizing behaviors. While dysregulation of the offspring HPA axis did not mediate the association between prenatal stress and offspring psychopathological symptoms, further research is warranted to investigate programming of alternative biological systems.

1. Introduction

The lasting consequences of prenatal maternal stress (PNMS) on offspring development are well documented (for review see Graignic-Philippe et al., 2014). Such consequences are hypothesized to be symptomatic of fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis, resulting from changes to the intra-uterine environment. Evidence of elevated maternal glucocorticoid (GC) levels, such as cortisol, and alterations in placental functioning, including down-regulation of the placental enzyme 11β-HSD2, have been documented in times of heightened maternal stress and anxiety (O'Donnell et al., 2012). These changes, resulting in elevated fetal cortisol levels, potentially influence neuronal differentiation and function within the brain during critical periods of development (Glover et al., 2010), including development of the HPA axis. Evidence of PNMS-linked dysregulated HPA axis functioning has been reported at all stages of development, including infancy, childhood, and adolescence (Glover et al., 2010). In general, PNMS-exposed infants typically have lower diurnal cortisol levels at awakening and bedtime (Yehuda et al., 2005) and, conversely, increased reactive cortisol levels in response to stress-provoking paradigms (Brennan et al., 2008; Grant et al., 2009). However, O'Connor et al. (2013) found elevated maternal prenatal cortisol levels to predict higher offspring pre-stressor cortisol levels, as well as blunted changes in cortisol in response to maternal-separation stress.

* Corresponding author at: McGill University & Principal Investigator, Douglas Mental Health University Institute, Perry Pavilion, E-3131, 6875 LaSalle Blvd., Verdun, Quebec, H4H 1R3, Canada.
E-mail address: suzanne.king@mcgill.ca (S. King).

https://doi.org/10.1016/j.psyneuen.2020.104697
Received 30 March 2019; Received in revised form 10 December 2019; Accepted 11 April 2020

Findings of dysregulated diurnal and reactive cortisol levels have been found in children (O'Connor et al., 2005; Yong Ping et al., 2015), adolescents (Huizink et al., 2008; O'Donnell et al., 2013; Van den Bergh et al., 2008), and adults (Entringer et al., 2009).

The inconsistencies between study findings possibly stem from differing methodologies, including the conceptualization of prenatal stress (Tarabulsy et al., 2014). Studies have operationalized PNMS to include psychological reactions to daily hassles, acute stressors (e.g., disasters), and life events, highlighting the complexities within the field. While studies have predominately focused on the prenatal period, postnatal maternal psychopathology has also been associated with elevated and variable morning cortisol levels in offspring compared to controls, demonstrating the continued malleability of the neurobiological stress response system throughout the lifespan (Halligan et al., 2004). This concept has been substantiated by animal cross-fostering studies, whereby PNMS-induced effects in offspring were reversed by increased maternal behavior (Maccari et al., 1995).

Dysregulation of HPA functioning is of great interest, as it has been found to underlie symptoms and disorders of psychopathology, especially those emerging during adolescence and early adulthood (for review see Wingenfeld and Wolf, 2011). Specifically, depressed children and adolescents have been found to have elevated cortisol levels across the diurnal cycle and hyperactive cortisol responses to psychological stressors (for meta-analysis see Lopez-Duran et al., 2009). Given that PNMS influences HPA axis functioning and that altered HPA activity underlies psychopathology, it is pertinent to examine whether HPA axis functioning mediates the relationship between PNMS and psychopathology during adolescence, a period marked by hormonal changes and an increased incidence of psychopathology (Gunnar et al., 2009). For example, Halligan et al. (2007) found that elevated morning cortisol levels at 13 years of age mediated the association between maternal postnatal depression and depressive symptoms at 13 years of age. Similarly, Van den Bergh and colleagues (2008) found that flattened diurnal cortisol profiles in 14-15 year-old females mediated the relationship between maternal anxiety in second trimester and concurrent depressive symptoms. Recently, elevated maternal cortisol levels during pregnancy were found to be associated with greater psychiatric symptoms in offspring at nine years of age, however, this relationship was not influenced by child basal cortisol levels (Isaksson et al., 2015). Collectively, these studies highlight the effects of early-life adversity on mental health functioning in offspring via modifications of fetal biological systems in utero. They also emphasize the need for studies that address the limitations of their predecessors: disentangling genetic transmission and environmental influences on offspring outcomes and assessing the individual and additive effects of maternal objective hardship and subjective distress to a well-defined naturally occurring stressor. Natural disasters allow us to disentangle the maternal stress due to the objective hardship experienced, from a mother's subjective distress. In a study of mothers who were exposed to the Iowa floods in 2008, maternal objective hardship correlated with offspring reactive cortisol levels, and maternal subjective distress (controlling for objective hardship) was associated with reactive cortisol levels in girls but not in boys (Yong Ping et al., 2015). Such findings suggest that objective indices of the maternal stressor, and maternal subjective distress or anxiety in response to that stressor, are independent predictors of HPA responses in the offspring in childhood.

The present study aimed to examine the relationship between PNMS and HPA axis functioning to a stress-provoking paradigm in adolescents. An additional aim of the study was to assess the mediating role of HPA activity on the association between disaster-related PNMS and adolescent internalizing and externalizing symptoms. It was hypothesized that in utero exposure to greater disaster-related PNMS would be associated with greater dysregulation of the offspring's HPA axis, which would be predictive of greater internalizing and externalizing behaviors at 13 years of age, possibly in a sex-specific manner. Sex is of particular interest given that it has been found to influence prevalence rates of depression (Gunnar et al., 2009) and the relationship between PNMS and HPA functioning (Yong Ping et al., 2015). Furthermore, it was hypothesized that timing of in utero stress exposure may affect the findings, as second and third trimester exposure to PNMS have been associated with dysregulated HPA activity in pre-adolescent and adolescent individuals (O'Connor et al., 2005; O'Donnell et al., 2013). As methodological constraints of past studies may have limited the examination of PNMS effects across all trimesters of pregnancy, this will be an additional focus of the present study. Overall, the findings from the present study will shed light on the HPA axis' mechanistic role in the transmission of PNMS to offspring and elucidate its use as a biological marker for psychopathology.

2. Methods and Materials

2.1. Recruitment and Protocol

The present study examined data from Project Ice Storm, a longitudinal, prospective study that recruited a cohort of women from Quebec, Canada, exposed to a severe ice storm while pregnant or up to 3 months preconception. In January of 1998, five consecutive days of freezing rain left millions of residents without power for up to 45 days during the coldest months of the year. Considered one of the worst Canadian natural disasters, the ice storm resulted in an economic loss of $5 to $7 billion.

Women who were pregnant at the time of the ice storm or became pregnant within three months of the storm were contacted and asked to participate. All women who provided written informed consent were asked to complete a recruitment questionnaire packet that assessed their experience to the disaster, psychological symptoms, and demographic information. Subsequently, when their children turned 13 years of age, mothers were re-contacted and asked to participate in the current phase of testing, involving a three-hour laboratory assessment at the Douglas Mental Health University Institute in Montreal, Canada. At the assessment, adolescents were asked to participate in a psychosocial stress-provoking paradigm and provide salivary cortisol samples. In addition, adolescents provided physical measurements (e.g., pulse, blood pressure, weight, and height), and both mother and adolescent completed a variety of questionnaires. All phases of the study were approved by the Ethics Board of the Douglas Mental Health University Institute.

2.2. Participants

Sixty-six of a potential 91 families agreed to participate when the adolescents were 13 years of age, with 61 adolescents completing the stressor paradigm and providing saliva samples. Two mothers did not provide questionnaire data on their children. Because analyses were conducted only on adolescents exposed to the ice storm during the first, second, or third trimester of pregnancy, data from 14 preconception-exposed adolescents were excluded. Thus, the final sample consisted of 45 adolescents; see Table 1 for complete participant characteristics.

2.3. Stress Paradigm

The adolescents' stress response was assessed using the Trier Social Stress Test for children (TSST-C; Buske-Kirschbaum et al., 1997), a widely used paradigm to invoke acute stress reactions in both healthy and clinical adolescent populations (Buske-Kirschbaum et al., 1997; Gordis et al., 2006; Stroud et al., 2009). The protocol commenced with participants watching a film of neutral content for one hour alone in a quiet room. Next, participants were given 10 minutes to prepare the ending of a provided story before reciting it in front of two judges. They were informed that the task would be videotaped for comparison with other performances. Following the story, participants were told that they needed to serially subtract 7 from 1023 rapidly and without error.

Table 1
Participant demographics. Data represent means for continuous variables and frequencies for categorical variables (N = 45).

Variables	
Maternal age at recruitment (years)	30.51 (Range 20.19-41.17; SD = 4.43)
Adolescent age at testing (years)	13.65 (Range 13.42-14.09; SD = 0.13)
Sex	Males (n = 24); Females (n = 21)
Trimester Exposed	
First Trimester	15 (Male = 9; Female = 6)
Second Trimester	16 (Male = 9; Female = 7)
Third Trimester	14 (Male = 6; Female = 8)
Maternal education (years)	15.38 (Range 10-20; SD = 2.41)
Marital status at recruitment	
Married	24 (53.3%)
Common-law	21 (46.7%)
Income	
$12,000-19,999	1 (2.2%)
$20,000-29,999	3 (6.7%)
$30,000-39,999	4 (8.9%)
$40,000-49,999	5 (11.1%)
$50,000 and over	32 (71.1%)
Parity*	
First	15
Second	13
Third or more	7
Mode of delivery	
Vaginal	40
Caesarian section	5

Note. *Data missing for 10 participants

Throughout the protocol, judges were instructed to maintain a serious expression, and to prompt participants whenever they faltered or made a mistake. The tasks were followed by a 60-minute recovery period during which the participant sat alone watching the remainder of the film. Seven salivary cortisol samples were collected: prior to the start of the film (buffer), 5 minutes prior to the TSST-C (pre-stressor), directly following the TSST-C (post-stressor), and 15-, 30-, 45-, and 60-minutes post-stressor. All participants were then debriefed.

2.4. Predictor Variables

2.4.1. Objective hardship

Maternal objective hardship was estimated based on responses to a questionnaire tailored to target mothers' experiences directly associated with the ice storm that tapped into four categories of exposure: loss, threat, change, and scope (Bromet and Dew, 1995). Scores for each category ranged from 0 (no exposure) to 8 (high exposure). A total score (Storm32) was computed by summing each category. Maternal scores in the present sample ranged from 5 to 24, out of a possible 32 points.

2.4.2. Subjective distress

Maternal subjective response to the ice storm was assessed using a validated French adaptation (Brunet et al., 2003) of the 22-item Impact of Event Scale – Revised (IES-R; Weiss and Marmar, 1997), which measured three categories of post-traumatic stress symptoms: intrusive thoughts, avoidance, and hyperarousal. Mothers rated the severity of each symptom during the preceding seven days using a 5-point Likert scale ranging from 0 "Not at all" to 4 "Extremely". Items were summed to provide a total score, ranging from 0 to 40 points, out of a total 88 points. IES-R scores of 22 (Rash et al., 2008) or 33 (Creamer et al., 2003) have been suggested for screening for possible PTSD. In the present sample, IES-R scores (M = 9.84, SD = 10.48) of 22 or 33 and higher were found in eight and one participant, respectively, indicating that a fair number of our participants were significantly stressed by the floods. Due to positive skewness, a natural log transformation was conducted to normalize the data.

2.4.3. Timing of Exposure

The time at which the fetus was exposed to the ice storm in utero was defined as the number of days between the estimated date of conception and January 9, 1998, the date at which the ice storm peaked. The greater the number of days, the later in pregnancy the fetus was exposed to the disaster. Women's date of conception was estimated by subtracting 280 days (40 weeks) from women's due date, which was calculated using babies' gestational age and date of delivery.

2.5. Outcome Variables

2.5.1. Cortisol

Passive-drool saliva samples were collected from participants to assay for free cortisol levels via competitive enzyme-linked immunoassay (EIA) using Salimetrics kits (catalogue number 1-3002) at the Douglas Mental Health University Institute. The inter- and intra-assay coefficients of variation were 6.6% and 6.3%, respectively. In total, six cortisol values belonging to four participants were missing due to invalid assay results or failure to provide a sufficient amount of saliva: buffer (n = 1), pre-stressor (n = 1), 45-mins post-stressor (n = 1), and 60-mins post-stressor (n = 3). Missing cortisol values were imputed with regression analyses using participants' available cortisol levels as predictors. Area Under the Curve with respect to increase (AUCi) and ground (AUCg) were computed using the cortisol values collected from pre-stressor to 60-mins post-stressor and represent change in cortisol secretion over time and total cortisol production, respectively (Pruessner et al., 2003). Given that AUCi and AUCg likely represent distinct physiological processes, they were both included in this study as has been done by others (Khoury et al., 2015). AUC values were standardized by dividing total cortisol output by the total amount of time elapsed between the first and last saliva collection. Finally, pre-stressor cortisol levels were also examined as an indicator of baseline HPA axis functioning.

2.5.2. Adolescent Internalizing and Externalizing Behaviors

Maternal-reported adolescent internalizing and externalizing behaviors were obtained using the Child Behavior Checklist 6-18 year version (CBCL; Achenbach, 2001). The CBCL consisted of 112-items that described various problem behaviors. Mothers were asked to rate each statement on a 3-point Likert scale based on how often their child engaged in the behavior over the past 12 months: 0 being "not applicable", 1 "more or less or sometimes true", and 2 "always or sometimes true". Using adolescents' age and sex, raw parent-report scores were converted to standardized scores. The Internalizing Problem scale was computed by combining scores on the anxiety/depression, social withdrawal, and somatic complaints subscales. The Externalizing Problem scale was computed by combining scores on the rule-breaking behavior and aggressive behavior subscales.

2.6. Covariates

2.6.1. Maternal Characteristics

The numbers of women who reported daily smoking and/or weekly alcohol consumption are presented in Table 2. Information on maternal and paternal level of education and employment were also collected to compute socioeconomic status based on the Hollingshead Index criteria (Hollingshead, 1973). The women reported on other major life events that they experienced during their pregnancies and up to six-months postpartum using the Life Experiences Survey (LES; Sarason et al., 1978). The modified LES assessed the presence and impact of various stressful life events, including the death of a spouse or a promotion at work.

2.6.2. Adolescent Characteristics

Pubertal development was assessed using the Puberty Development Scale (PDS; Petersen et al., 1988) and the Tanner Sexual Maturity Scale

Table 2
Predictor, outcome, and covariate variable descriptive statistics.

Variables	Mean	Standard Deviation	Range
Stress Measures			
Objective hardship	11.18	4.43	5.00-24.00
Subjective distress	1.79	1.21	0-3.71
Timing of exposure (days)	142.51	82.01	1-274
Maternal Measures			
Smoking (count)			
0	41		
1-10	1		
11-20	3		
Alcohol consumption (count)			
0	35		
]0,1]	8		
]1-2]	2		
Socioeconomic status	26.49	11.17	11-65
Life experiences survey	5.87	3.67	2-17
Adolescent Measures			
Buffer cortisol (μg/dL)	0.11	0.06	0.02-0.29
Pre-stressor cortisol (μg/dL)	0.08	0.05	0.02-0.22
Post-stressor cortisol (μg/dL)	0.13	0.09	0.03-0.42
15-mins post-stressor (μg/dL)	0.17	0.11	0.03-0.54
30-mins post-stressor (μg/dL)	0.12	0.06	0.02-0.32
45-mins post-stressor (μg/dL)	0.10	0.05	0.03-0.24
60-mins post-stressor (μg/dL)	0.08	0.05	0.01-0.20
AUC ground (μg/dL)	0.12	0.06	0.03-0.33
AUC increase (μg/dL)	0.04	0.05	−0.10-0.15
Adolescent internalizing	52.20	11.16	33-76
Adolescent externalizing	45.76	9.07	34-66
Puberty development scale*	2.73	0.53	1.80-3.50
Tanner sexual maturity scale*	3.56	0.67	3-5
Body Mass Index (13 years)	21.78	5.54	15.96-41.51
Birth weight	3327.06	573.49	1655-4320
Gestational age at birth	39.29	2.05	32.29-41.29

Note. N = 45. AUC = Area Under the Curve. Socioeconomic status (SES) – higher score indicates higher SES. Timing of exposure in utero - higher days indicates exposure later in pregnancy. *Data missing for 2 participants.

(SMS; Tanner, 1981). Body Mass Index was computed based on weight and height measurements. Birth outcome data, including birth weight, gestational age at birth, and mode of delivery were obtained from participant's hospital records. Finally, the time at which the pre-stressor cortisol was sampled was recorded to control for the time of day of testing.

2.7. Statistical Analyses

Descriptive statistics were computed for all variables. To determine whether the TSST-C successfully elevated adolescent cortisol levels, paired samples t-tests were conducted between pre-stressor and all other values. Bivariate correlations were then conducted to assess the relationship between all maternal and adolescent predictor, outcome, and control variables.

Next, separate hierarchical multiple linear regression models were conducted for AUCi, AUCg, and pre-stressor cortisol in which objective hardship, subjective distress, timing of exposure, and sex were entered in Steps 1 through 4, respectively. Covariates that significantly correlated at $p < .05$ with the outcome variable were allowed to enter in the next step(s), in a stepwise manner. Finally, objective hardship × timing of exposure, objective hardship × child sex, subjective distress × timing of exposure, or subjective distress × child sex interaction terms were entered into the model (separate model for each interaction term). Non-significant covariates and interactions were removed from the final model due to constraints of power.

Next, hierarchical multiple linear regressions were conducted separately for adolescent internalizing and externalizing behaviors. Similar to above, objective hardship and subjective distress were entered into Step 1 and Step 2, respectively. AUCi, AUCg, or pre-stressor cortisol were then entered in Step 3. Finally, covariates that significantly correlated with the outcome variable were allowed to enter in next step(s), in a stepwise manner.

To examine whether reactive (AUCi or AUCg) or baseline (pre-stressor) cortisol levels mediated the relationship between objective hardship or subjective distress and behavioral outcomes (internalizing or externalizing behaviors) in the adolescents, the percentile-based bootstrapping procedure was employed using 10000 resampling to test the 12 different mediation analyses, controlling for covariates that were included in the regression analyses. They were considered significant if 0 fell outside of the 95% confidence interval. The moderating effects of offspring sex and timing of exposure on the mediation were tested only if the interactions effects of PNMS by sex (or timing) on cortisol levels were significant ($p < 0.05$) in hierarchical regression analyses.

All analyses were conducted with SPSS version 20.0. The PROCESS macro (Hayes, 2013) was used to probe significant interactions and to run the bootstrapping procedure for the mediation analyses.

3. Results

3.1. Descriptives

Descriptive statistics for all variables are presented in Table 2. On average, pre-stressor cortisol levels were significantly lower than post-stressor levels at all time-points ($p < .001$) except at 60-mins post-stressor. Cortisol levels increased from pre-stressor to 15-mins post-stressor, and then steadily declined until 60-mins post-stressor.

3.2. Correlations

Correlation coefficients between predictor, outcome, and potential covariates are reported in Table 3. Higher levels of objective hardship tended to be associated with higher AUCi (i.e., a greater cortisol increase in response to the TSST-C; $r = .269$, $p = .074$) Mode of delivery was associated with AUCi ($r = -.314$, $p = .036$), such that adolescents born via Caesarean-section (C-section) secreted less cortisol with respect to baseline during the TSST-C than those born vaginally. Pre-stressor cortisol levels were correlated with sex ($r = .298$, $p = .047$), such that females demonstrated higher cortisol levels than males prior to the introduction of the TSST-C.

With respect to adolescent behaviors, higher externalizing scores were associated with lower AUCi values ($r = -.302$, $p = .044$) and moderately higher subjective distress ($r = .279$, $p = .063$). Lower gestational age at birth ($r = -.421$, $p = .004$) and C-section delivery ($r = .491$, $p = .001$) were also associated with higher externalizing scores.

3.3. Hierarchical Regression Models

3.3.1. Area Under the Curve increase

Results of the hierarchical multiple regression are presented in Table 4. Objective hardship was moderately related to AUCi ($p = .074$) and accounted for 7.2% of the variance in Step 1. Subjective distress, timing of exposure, and offspring sex were not significantly associated with AUCi, accounting for an additional 6.0% of the total variance. Mode of delivery accounted for an additional 14.6% of the variance ($p = .008$). The inclusion of mode of delivery resulted in the effect of objective PNMS becoming significant ($p = .010$). Neither sex nor timing interactions with either PNMS variable were significant (see Supplementary Table 1 for all interaction term regression coefficients). The final model accounted for 27.8% of the variance of AUCi.

3.3.2. Area Under the Curve ground

Results of the hierarchical multiple regression are presented in Table 4. Objective hardship and subjective distress did not significantly predict AUCg, accounting for 4.1% of the variance. Similarly, timing of exposure and offspring sex were unrelated to the outcome, accounting for an additional 10.1% of the variance. The addition of sex did result in

Table 3
Pearson correlation coefficients between maternal and adolescent predictor, outcome, and covariate variables.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Internalizing																			
2. Externalizing	.54**																		
3. Objective	.16	-.12																	
4. Subjective	.23	.28†	.23																
5. AUCincrease	-.03	-.30*	.27†	.13															
6. AUCground	.11	-.24	.19	-.03	.68**														
7. Pre-stressor	.18	.03	-.06	-.19	-.24	.55**													
8. Timing	-.00	.09	-.19	.15	.19	.16	-.01												
9. SES	-.13	.17	-.10	.14	-.08	.03	.14	.14											
10. LES	.28†	.10	.30*	.35*	.22	.17	-.03	.25	-.12										
11. Smoking	-.12	-.04	-.11	-.05	-.24	-.07	.18	-.06	.12	-.04									
12. Alcohol	-.20	.15	-.09	-.06	-.03	-.22	-.26†	-.10	.04	-.26	.16								
13. Delivery	.20	.49**	.18	-.01	-.31*	-.07	.26†	-.00	.27†	.05	-.10	.18							
14. Sex	.21	.14	-.24	-.09	-.02	.21	.30*	.15	.07	-.14	-.22	-.02	.09						
15. Gestation	-.15	-.42**	.08	.01	.27†	.13	-.13	.09	.04	.19	-.16	-.42**	-.40**	-.06					
16. Weight	-.14	-.27†	.03	.11	.10	.04	-.06	.12	.16	.09	-.11	-.28†	-.27†	-.01	.77**				
17. PDS	.10	.14	.00	-.01	-.09	.00	.11	.17	.00	-.04	-.16	.06	-.07	.49**	-.02	.17			
18. Tanner	.11	.13	.14	.04	-.04	-.12	-.11	-.01	-.01	.09	-.16	.19	.03	-.01	.03	.23	.53**		
19. BMI	-.16	.17	.32*	.30*	-.06	-.11	-.08	-.23	-.04	.33*	-.07	-.02	.06	-.25†	.10	.09	.07	.22	
20. Time of sampling	-.08	.03	.12	.08	.21	.15	-.05	.28†	.17	.14	.01	.23	.12	.12	-.03	-.18	-.10	-.23	-.07

Note. AUC = Area Under the Curve. SES = socioeconomic status. LES = Life Events Survey. Sex: 1 = boy; 2 = girl. Delivery: 1 = Caesarian section; 0 = vaginal. PDS = Puberty development scale. BMI = body mass index. † $p < .1$; * $p < .05$; ** $p < .01$.

the effect of objective PNMS becoming marginally significant ($p = .058$). Neither offspring sex nor timing interactions with either PNMS variable were significant. The final model accounted for 14.2% of the variance of AUCg.

3.3.3. Pre-Stressor Cortisol

Results of the hierarchical multiple regression are presented in Table 4. Objective hardship and subjective distress did not significantly predict pre-stressor cortisol levels, accounting for 3.7% of variance. While timing of exposure was not a significant predictor, offspring sex was marginally significant ($p = .062$), accounting for an additional 8.1% of the variance. With respect to interactions, timing of exposure significantly interacted with objective hardship to predict pre-stressor cortisol levels, accounting for 9.5% ($p = .036$; see Fig. 1). Specifically, higher objective distress predicted elevated pre-stressor cortisol levels for offspring exposed to the ice storm during the third trimester compared to the first trimester of pregnancy. Probing the interaction, the effect of objective hardship on pre-stressor cortisol levels became marginally significant when exposure occurred after approximately the 209th day of pregnancy, that is, during the seventh month of gestation. Similarly, sex moderately interacted with objective hardship and accounted for 7.1% of the variance ($p = .071$; see Fig. 2). Specifically, at high levels of objective hardship, females had significantly elevated pre-stressor cortisol levels compared to males. Conversely, neither offspring sex nor timing interacted with subjective distress to predict pre-stressor cortisol levels. Final models with the timing of exposure and sex interactions accounted for 21.3% and 19.0% of the variance of pre-stressor cortisol, respectively.

3.3.4. Adolescent Externalizing Behaviors

Results of the hierarchical multiple regressions are presented in Table 5. Controlling for objective hardship, subjective distress predicted externalizing behaviors, and accounted for 9.9% of the variance ($p = .036$). AUCi explained an additional 9.3% of the variance ($p = .034$). Gestational age at birth explained an additional 11.8% of the variance ($p = .012$). Finally, mode of delivery accounted for an additional 20.8% of the variance ($p = .001$). Given that the addition of mode of delivery resulted in gestational age becoming non-significant, gestational age was trimmed from the final model. After controlling for mode of delivery, subjective distress remained significant ($p = .006$) and objective hardship became marginally significant ($p = .064$). Overall, the final model including objective hardship, subjective distress, and mode of delivery accounted for 41.3% of the variance of adolescent externalizing behaviors.

The hierarchical multiple regression was repeated using AUCg as a predictor variable (Table 5). As before, controlling for objective hardship, subjective distress predicted externalizing behaviors, and accounted for 9.9% of the variance ($p = .036$). AUCg did not significantly account for any additional variance. As before, mode of delivery accounted for an additional 26.9% of the variance. The overall model, including mode of delivery, explained 41.9% of the variance.

The same analyses were repeated using pre-stressor cortisol levels as a predictor variable (Table 5). Pre-stressor cortisol level was found to be a non-significant predictor accounting for 0.7% of the variance. The overall model explained 40.6% of the variance.

3.3.5. Adolescent Internalizing Behaviors

None of the PNMS predictor variables, AUCi, AUCg, or pre-stressor cortisol, or covariates were significantly related to the offspring's internalizing scores. The final models accounted for 7.5%, 7.4%, and 11.9% of the variance and are presented in Table 5.

3.4. Mediation Analyses

Mediation analyses found no significant effect of objective or subjective PNMS on adolescent internalizing or externalizing behavior through either AUCi, AUCg, or pre-stressor cortisol levels (see supplemental Table 2). Only those interactions that were significant in the hierarchical regression analyses were tested through moderated mediation analyses (see section 3.3.3). Neither sex nor timing significantly moderated the mediation from objective PNMS to adolescent internalizing or externalizing behaviors through pre-stressor cortisol levels.

4. Discussion

The purpose of the present study was, first, to determine whether in utero exposure to prenatal maternal stress influences HPA axis functioning in response to a psychosocial stressor in adolescents. The second aim was to test whether the magnitude of HPA responsivity mediated the relationship between prenatal stress and adolescent psychopathology, assessed via maternal-reported internalizing and externalizing behaviors. To our knowledge, this is the first study to

Table 4
Results of hierarchical multiple regressions of Area Under the Curve increase, Area Under the Curve ground, and Pre-Stressor Cortisol.

Predictor Variables	β	B	SE	R	R^2	ΔR^2	F	ΔF
Area Under the Curve increase								
Step 1				0.269	0.072	0.072	3.352†	3.352†
Objective hardship	0.269†	0.003†	0.002					
Step 2				0.278	0.077	0.005	1.763	0.233
Objective hardship	0.252	0.003	0.002					
Subjective distress	0.073	0.003	0.006					
Step 3				0.364	0.132	0.055	2.083	2.591
Objective hardship	0.311*	0.004*	0.002					
Subjective distress	0.024	0.001	0.006					
Timing	0.244	0.000	0.000					
Step 4				0.364	0.133	0.000	1.529	0.018
Objective hardship	0.315†	0.004†	0.002					
Subjective distress	0.025	0.001	0.007					
Timing	0.241	0.000	0.000					
Sex	0.021	0.002	0.015					
Step 5				0.528	0.278	0.146	3.010*	7.880**
Objective hardship	0.406*	0.005*	0.002					
Subjective distress	0.004	0.000	0.006					
Timing	0.253†	0.000†	0.000					
Sex	0.076	0.008	0.014					
Mode of delivery	−0.393**	−0.063**	0.022					
Area Under the Curve ground								
Step 1				0.187	0.035	0.035	1.553	1.553
Objective hardship	0.187	0.002	0.002					
Step 2				0.202	0.041	0.006	0.891	0.256
Objective hardship	0.205	0.003	0.002					
Subjective distress	−0.079	−0.004	0.008					
Step 3				0.295	0.087	0.047	1.307	2.094
Objective hardship	0.259	0.003	0.002					
Subjective distress	−0.124	−0.006	0.008					
Timing	0.225	0.000	0.000					
Step 4				0.376	0.142	0.054	1.651	2.536
Objective hardship	0.308†	0.004†	0.002					
Subjective distress	−0.109	−0.005	0.008					
Timing	0.196	0.000	0.000					
Sex	0.242	0.028	0.018					
Pre-Stressor Cortisol								
Step 1				0.059	0.004	0.004	0.152	0.152
Objective hardship	−0.059	−0.001	0.002					
Step 2				0.191	0.037	0.033	0.798	1.443
Objective hardship	−0.017	0.000	0.002					
Subjective distress	−0.187	−0.007	0.006					
Step 3				0.192	0.037	0.000	0.525	0.015
Objective hardship	−0.012	0.000	0.002					
Subjective distress	−0.191	−0.007	0.006					
Timing	0.019	1.063E-5	0.000					
Step 4				0.344	0.118	0.081	1.339	3.681
Objective hardship	0.048	0.000	0.002					
Subjective distress	−0.172	−0.006	0.006					
Timing	−0.015	−8.391E-6	0.000					
Sex	0.296†	0.026†	0.014					
Step 5.1				0.461	0.213	0.095	2.109†	4.695
Objective hardship		−0.005	0.003					
Subjective distress		−0.006	0.006					
Timing		−0.001*	0.000					
Sex		0.025†	0.013					
Objective X Time		4.112E-5*	0.000					
Step 5.2				0.435	0.190	0.071	1.825	3.440
Objective hardship		−0.008	0.005					
Subjective distress		−0.005	0.006					
Timing		−2.286E-5	0.000					
Sex		−0.036	−0.036					
Objective X Sex		0.006†	0.003					

Note. Sex: 1 = boy; 2 = girl. Mode of delivery: 1 = Caesarian section; 0 = vaginal. † $p < .1$; * $p < .05$; ** $p < .01$.

examine whether disaster-related PNMS functions to elevate risk for psychopathology in offspring via alterations to the HPA axis, as has been previously suggested (Beijers et al., 2014; O'Donnell et al., 2012) in non-disaster exposed cohorts. The Quebec ice storm was a natural disaster that functioned as a discrete, "independent stressor", primarily affecting individuals located in a specific geographical location, irrespective of psychosocial risk factors and sociodemographic variables. Thus, this natural experimental design allowed for the examination of prenatal stress and its associated effects on offspring outcomes, while simultaneously controlling for various confounding variables (Barker et al., 2011).

On average, adolescents in our study experienced an increase in salivary cortisol levels in response to the TSST-C, with levels reaching maximum values 15 minutes after the end of the stressor. Our findings

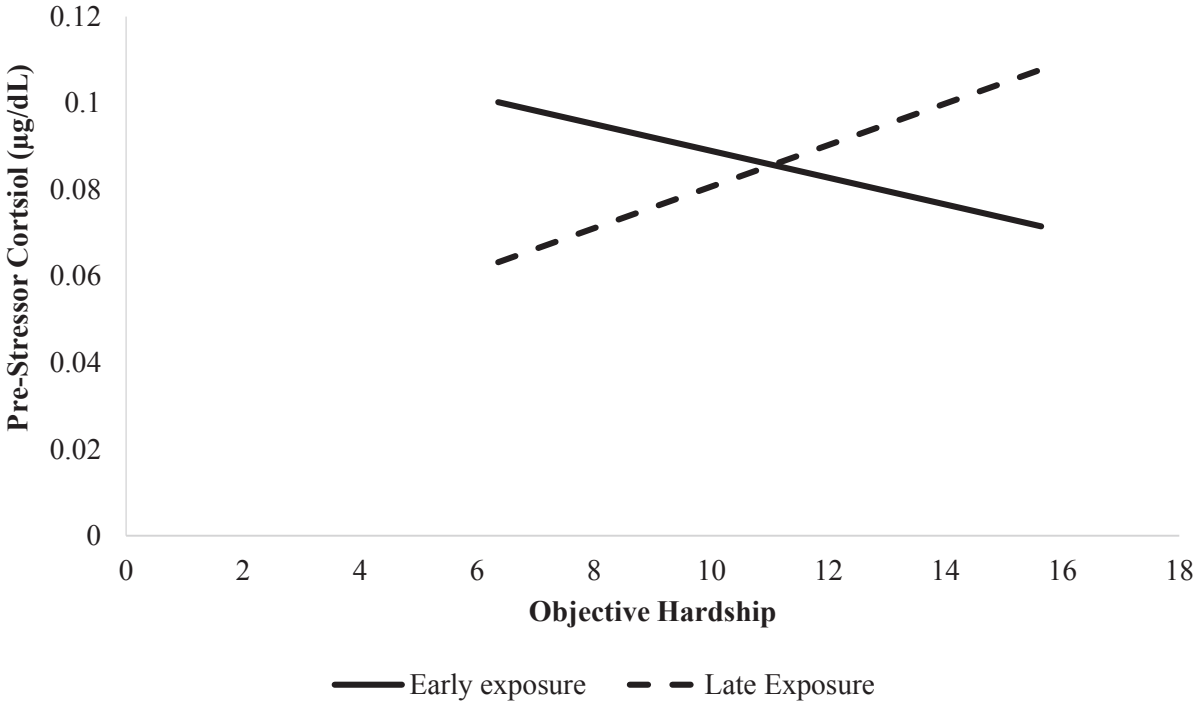

Fig. 1. Adolescent pre-stressor cortisol (µg/dL) at low (16th percentile) and high (84th percentile) objective hardship levels for individuals exposed to the ice storm during the first (48th day) and third (241st day) trimesters of pregnancy. A significant interaction found higher objective hardship to moderately predict ($p = 0.064$) elevated pre-stressor cortisol levels for those individuals exposed during the third trimester of pregnancy.

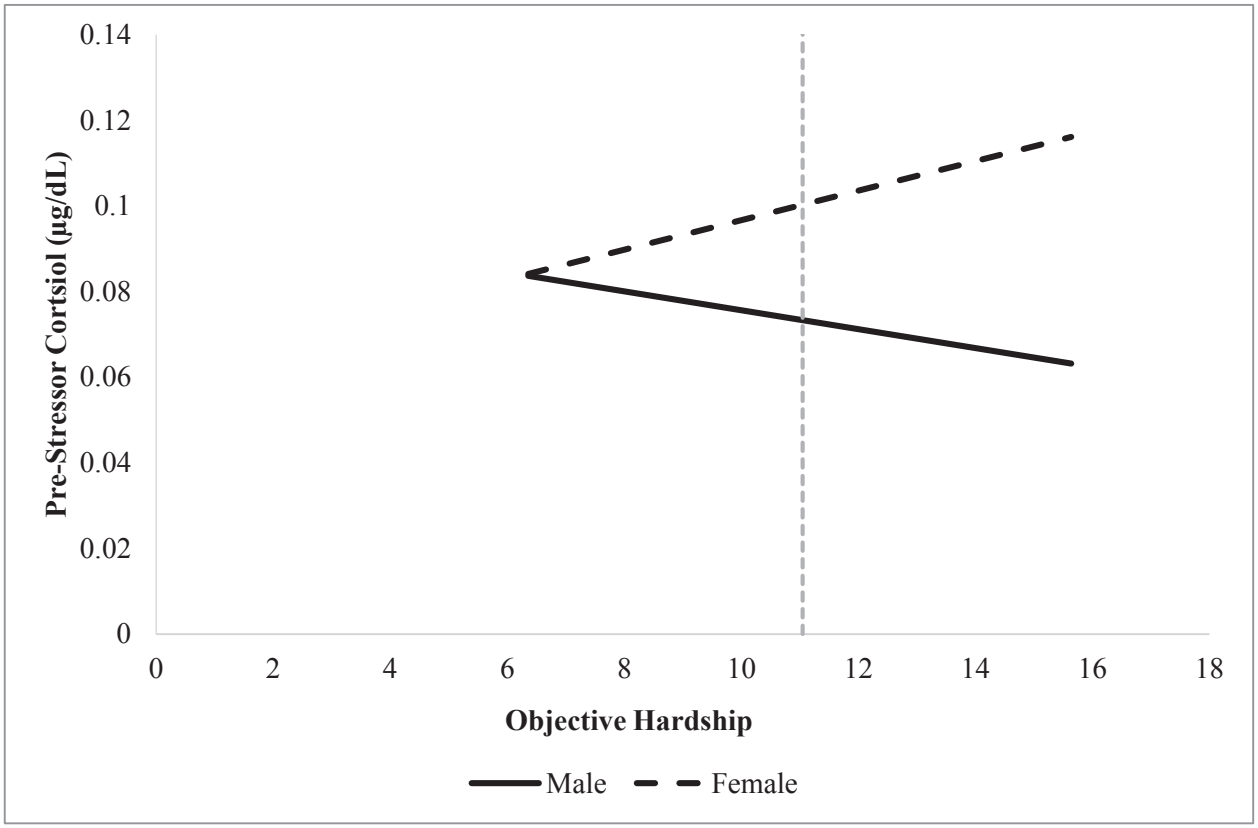

Fig. 2. Adolescent pre-stressor cortisol levels (µg/dL) at varying objective hardship levels for males and females. Sex moderately influenced the association between objective hardship and pre-stressor cortisol levels such that females exposed to higher levels of hardship presented with generally higher pre-stressor cortisol levels than males. The region of significance was identified by probing the interaction (dotted vertical line; objective hardship = 11.05).

Table 5
Results of hierarchical multiple regression of adolescent externalizing and internalizing problem scores.

Predictor Variables	β	B	SE	R	R^2	ΔR^2	F	ΔF
Externalizing Problem Scores								
AUCi								
Step 1				0.117	0.014	0.014	0.593	0.593
Objective hardship	−0.117	−0.239	0.310					
Step 2				0.335	0.112	0.099	2.659†	4.676*
Objective hardship	−0.190	−0.390	0.306					
Subjective distress	0.323*	2.430*	1.124					
Step 3				0.453	0.206	0.093	3.538*	4.811*
Objective hardship	−0.110	−0.226	0.302					
Subjective distress	0.346*	2.606*	1.079					
AUCi	−0.318*	−56.565*	25.788					
Step 4				0.643	0.413	0.208	7.045**	14.160**
Objective hardship	−0.256†	−0.524†	0.275					
Subjective distress	0.359**	2.703**	0.939					
AUCi	−0.123	−21.926	24.252					
Mode of Delivery	0.501**	14.311**	3.803					
AUCg								
Step 1				0.117	0.014	0.014	0.593	0.593
Objective hardship	−0.117	−0.239	0.310					
Step 2				0.335	0.112	0.099	2.659†	4.676*
Objective hardship	−0.190	−0.390	0.306					
Subjective distress	0.323*	2.430*	1.124					
Step 3				0.388	0.151	0.038	2.422†	1.841
Objective hardship	−0.150	−0.307	0.309					
Subjective distress	0.307*	2.312*	1.116					
AUCg	−0.199	−30.575	22.531					
Step 4				0.648	0.419	0.269	7.225**	18.527**
Objective hardship	−0.264*	−0.541*	0.264					
Subjective distress	0.341**	2.565**	0.936					
AUCg	−0.138	−21.213	18.983					
Mode of Delivery	0.531**	15.165**	3.523					
Pre-Stressor Cortisol								
Step 1				0.117	0.014	0.014	0.593	0.593
Objective hardship	−0.117	−0.239	0.310					
Step 2				0.335	0.112	0.099	2.659†	4.676*
Objective hardship	−0.190	−0.390	0.306					
Subjective distress	0.323*	2.430*	1.124					
Step 3				0.345	0.119	0.007	1.849	0.316
Objective hardship	−0.189	−0.387	0.308					
Subjective distress	0.339*	2.548*	1.152					
Pre-stress cortisol	0.084	16.962	30.173					
Step 4				0.637	0.406	0.286	6.825**	19.279**
Objective hardship	−0.300*	−0.615*	0.262					
Subjective distress	0.341*	2.563*	0.958					
Pre-stress cortisol	−0.070	−14.068	26.070					
Mode of Delivery	0.566**	16.155**	3.679					
Internalizing Problem Scores								
AUCi								
Step 1				0.158	0.025	0.025	1.107	1.107
Objective hardship	0.158	0.399	0.379					
Step 2				0.256	0.066	0.040	1.473	1.817
Objective hardship	0.111	0.280	0.386					
Subjective distress	0.207	1.912	1.419					
Step 3				0.273	0.074	0.009	1.098	0.393
Objective hardship	0.136	0.342	0.402					
Subjective distress	0.214	1.979	1.433					
AUCi	−0.098	−21.466	34.245					
AUCg								
Step 1				0.158	0.025	0.025	1.107	1.107
Objective hardship	0.158	0.399	0.379					
Step 2				0.256	0.066	0.040	1.473	1.817
Objective hardship	0.111	0.280	0.386					
Subjective distress	0.207	1.912	1.419					
Step 3				0.273	0.075	0.009	1.102	0.404
Objective hardship	0.091	0.230	0.397					
Subjective distress	0.214	1.983	1.433					
AUCg	0.097	18.380	28.931					
Pre-Stressor Cortisol								
Step 1				0.158	0.025	0.025	1.107	1.107
Objective hardship	0.158	0.399	0.379					
Step 2				0.256	0.066	0.040	1.473	1.817
Objective hardship	0.111	0.280	0.386					
Subjective distress	0.207	1.912	1.419					
Step 3				0.344	0.119	0.053	1.837	2.464

(*continued on next page*)

Table 5 (*continued*)

Predictor Variables	β	B	SE	R	R^2	ΔR^2	F	ΔF
Objective hardship	0.115	0.290	0.380					
Subjective distress	0.250	2.318	1.418					
Pre-stress cortisol	0.235	58.292	37.135					

Note. AUCi = Area under the curve increase; AUCg = Area under the curve ground. Mode of delivery: 1 = Caesarian section; 0 = vaginal. † $p < .1$; * $p < .05$; ** $p < .01$.

contribute to the existing body of literature that supports the efficacy of the TSST-C to activate an HPA axis response in diverse populations (Kudielka et al., 2007).

The present study found that maternal objective hardship was associated with greater cortisol responsivity in adolescents. In other words, women who experienced greater exposure to the ice storm in terms of threat, loss, scope (duration), and change had children who, as adolescents, released higher levels of cortisol while stressed than adolescents of mothers who experienced lower levels of hardship, a finding supported elsewhere (Brennan et al., 2008; Davis et al., 2011; Entringer et al., 2009; Grant et al., 2009; Gutteling et al., 2004, 2005; Huizink et al., 2008; O'Connor et al., 2005; Tollenaar et al., 2011). Our results, coupled with previous findings, suggest that the link between objective measures of PNMS and cortisol is fairly robust: increased maternal stress is associated with increased offspring reactive cortisol levels.

This result did not extend to maternal subjective distress, suggesting that objective changes to one's daily life that are associated with exposure to a disaster may result in greater changes to the in utero environment than those associated with disaster-related distress and, in turn, influence development of the fetus. The different results associated with objective and subjective aspects of prenatal maternal stress add to a host of other previous Project Ice Storm results that highlight the importance of teasing apart their effects. For example, while objective hardship is highly associated with DNA methylation in these same children at age 13, subjective distress is not (Cao-Lei et al., 2014); we found similar associations with these children's cytokine levels (Veru et al., 2015), insulin secretion (Dancause et al., 2013), and BMI (Dancause et al., 2012). It is difficult to speculate as to why these two variables have different associations with child outcomes. Objective exposure could be a proxy variable for some related, unmeasured exposure variable from the time of the ice storm that we did not assess and that might have fetal programming effects, such as exposure to the cold. As well, it should be recognized that the subjective distress measure is from a scale of PTSD symptoms which was administered 5-6 months after the disaster. Although it is normal for a person to have PTSD symptoms in the immediate aftermath of a trauma, only a specific profile of person still experiences those symptoms 5-6 months later; these mothers may well have a history of blunted cortisol themselves. Thus, one must consider the exact meaning of the IES-R scores as those of extended, persistent symptoms rather than reflective of their response at the time of the event. In summary, we cannot say for certain why objective exposure and persistent PTSD symptoms often have different associations with child outcomes.

Next, the relationship between adolescent HPA axis responsivity to a psychosocial stressor and severity of behavioral problems was examined. Results showed that adolescents who released lower levels of cortisol while stressed had greater externalizing behaviors. Despite a positive association between objective PNMS and AUCi, and a negative association between AUCi and externalizing behaviors, functioning of the HPA axis was not found to mediate the association between objective PNMS and symptoms of behavioral problems in the adolescents, as originally hypothesized.

Considering both parts of the non-significant mediation model, it is worth noting that elevated activity of the HPA axis may have functioned to protect offspring exposed to elevated maternal objective hardship, subsequently contributing to lower externalizing behaviors in adolescence. It has been hypothesized that underactivity of the stress response system may lead to physiological hypo-arousal, predisposing individuals to actively seek out stimulation and increase risk-taking behaviors, in turn increasing their risk for externalizing disorders, such as attention-deficit hyperactivity, conduct, and oppositional defiant disorders (van Goozen et al., 2007). As such, in the face of an adverse early environment, as was the case in the present study, elevated offspring HPA axis functioning may have operated to counter the manifestation of later behavioral problems in adolescence.

The present study also demonstrated that elevated maternal subjective distress levels were directly associated with greater externalizing behaviors in adolescents, but only once objective hardship levels were controlled for. These results are in accordance with past studies that have found an association between elevated maternal anxiety and childhood emotional problems and externalizing behaviors, including hyperactivity and conduct problems (Isaksson et al., 2015; O'Connor et al., 2003; Van den Bergh and Marcoen, 2004). Maternal anxiety has also been associated with elevated internalizing symptoms in 7-8 year old children (Barker et al., 2011), however this was not the case in the present study. In contrast, internalizing behaviors as measured by maternal report on the CBCL, were not predicted by maternal objective hardship or subjective distress in our current analyses at 13 ½ years of age. This was interesting given that we found consistent associations with objective hardship and subjective distress at earlier ages in this cohort (King et al., 2012), but suggests that the direct associations diminish with increasing age. The value of our natural disaster study is in its ability to disentangle the effects of what happened to the women objectively from their subjective distress. Our findings suggest that it is the maternal subjective distress associated with the natural disaster that is a more important factor than the objective hardship experienced, in predicting childhood externalizing behaviors. Thus, future studies should examine whether maternal psychological support systems can offset the effects of maternal subjective distress levels in response to a natural disaster during pregnancy on child externalizing behaviors.

We found that the association between PNMS and adolescent HPA axis responsivity was not moderated by either sex or timing of exposure, in contrast to other studies (Glover and Hill, 2012; Yong Ping et al., 2015). Nevertheless, a moderately significant main effect of timing of exposure was found on adolescent cortisol reactivity, such that exposure to the ice storm later in pregnancy was associated with greater cortisol secretion in response to the TSST-C, suggesting that mid- to late-pregnancy may represent a sensitive period for fetal development of the stress response system (Van den Bergh et al., 2008; Yehuda et al., 2005).

Conversely, with respect to baseline HPA axis functioning, timing of exposure was found to significantly influence the association between maternal objective hardship and pre-stressor cortisol levels. Specifically, pre-stressor cortisol levels increased with increasing maternal objective hardship in offspring exposed to the ice storm later in pregnancy. These results suggest that late pregnancy may represent a period of vulnerability for HPA axis functioning at both baseline and in response to a psychosocial stressor. Given that pregnant women demonstrate a 3-fold increase in circulating cortisol by their third trimester (Jung et al., 2011), it is possible that PNMS-induced cortisol secretion that occurs later in pregnancy overwhelms an already saturated

in utero environment, leading to long-term consequences on the development of the fetal HPA axis. In addition, we also found a moderately significant main effect of sex, such that females tended to have higher pre-stressor cortisol levels than males. Furthermore, sex moderately interacted with objective hardship to predict baseline cortisol, such that females whose mothers reported higher levels of objective distress were found to express higher pre-stressor cortisol levels compared to males. Similar results of elevated pre-stressor cortisol levels in females have been reported elsewhere (O'Connor et al., 2013).

While results from the study did not provide direct support for the fetal programming hypothesis involving the HPA axis, they do suggest that maternal objective hardship and subjective distress may impact adolescent behavioral functioning through different pathways. Specifically, the pathway from maternal subjective distress to adolescent externalizing behaviors may be genetic, with higher levels of distress in the mother predicting greater externalizing behaviors in the child. Conversely, it is possible that the pathway from maternal objective hardship (which does not have a heritable component) to adolescent externalizing behaviors may be indirect (e.g., via extended cold exposure), as both HPA axis hyper- and hypo-reactivity were associated with elevated objective hardship and externalizing behaviors, respectively. Continued exploration of these two independent pathways of maternal subjective and objective stress on developmental outcomes is needed.

The results from the present study did not support the fetal programming hypothesis, whereby PNMS influences development of the fetal HPA axis, in turn predisposing adolescents to elevated psychopathology. A lack of support for the fetal programming hypothesis is in contrast to findings by Van den Bergh et al. (2008), in which dysregulation of the HPA axis was found to mediate the relationship between maternal anxiety early in pregnancy and depressive symptoms in post-pubertal female adolescents. It is important to note that adolescence is associated with significant changes to the HPA axis, including increasing basal and reactive cortisol levels (Gunnar et al., 2009). Such developmentally-induced biological changes may have made it difficult to capture the long-lasting effects of PNMS on HPA axis functioning in our study. While the present study did not support the HPA axis as the mechanism mediating the association between prenatal stress and greater offspring psychopathological symptoms, the relationship between maternal subjective distress and adolescent externalizing behaviors posits the occurrence of in utero programming, with the specific physiological mechanism remaining unknown.

In the present study, delivery by C-section was a significant predictor of dampened HPA axis responsivity and greater externalizing behaviors in the adolescents. With respect to the latter finding, our results replicate earlier reports of elevated emotional and behavioral problems in infants born via C-section (Sirvinskiene et al., 2016). In addition, mode of delivery was an important covariate which, when controlled for, resulted in timing of exposure significantly predicting HPA axis responsivity. Past research has found an effect of delivery complications (e.g., use of forceps, bleeding during delivery, abnormal fetal heart rate at delivery) on infant cortisol levels (Brennan et al., 2008). Moreover, elective C-section has been associated with less pronounced stress responses in infants compared to those born via assisted deliveries (Taylor et al., 2000). Overall, maternal stress associated with delivery complications may contribute to dysregulation of the fetal HPA axis (Glover et al., 2010). The 'hygiene hypothesis' has been put forth to explain these findings, positing that overly clean environments contribute to greater psychological and physical pathology (Neu and Rushing, 2011). In instances of C-sections, the lack of contact between the fetus and maternal vaginal flora may negatively impact functioning of the offspring immune system, rendering them susceptible to later pathology. Restrictions of sample size prohibited us from examining these associations in greater depth, but our findings suggest that further research is warranted.

There were a number of limitations to the present study. First, due to the longitudinal design of the study, participant attrition influenced the final sample size of this project. Our relatively small sample size resulted in reduced statistical power, which may have affected the outcome of our analyses and resulted in marginal or non-significant findings. Nevertheless, our sample size followed recent recommendations of having at least two subjects per variable in order to make adequate estimations in our regression analyses (Austin and Steyerberg, 2015). Due to the sample size, it is important that results be interpreted with caution. Second, participants were recruited from a non-clinical, community sample that consisted primarily of Caucasian participants from two-parent households. Furthermore, our Ice Storm sample is not representative of the general population of the Montérégie sector of Quebec: in essence, the mothers in our sample are generally more affluent and educated than the typical family of this region. As such, the demographic profile of the present sample likely contributed to the relatively low levels of adolescent behavioral problems reported in this paper. The effect of PNMS on adolescent behaviors in the present sample suggests that further investigation utilizing high-risk samples, at an already elevated risk for behavioral problems and with limited access to resources, is warranted. Third, adolescent behaviors may have been affected by reporter bias as they were measured via maternal report. Specifically, mothers with elevated anxiety or depression may have been more likely to rate their child's behavior negatively. Despite this limitation, past studies have found maternal-report of adolescent problems to be predictive of later clinical psychopathology (Hofstra et al., 2000). Fourth, the present study lacked a control group of offspring born of similar demographics and not exposed to a natural disaster in utero, such as offspring born prior to the ice storm. Such a limitation stemmed from methodological constraints of the study at its conception and should be incorporated into future disaster-related studies. Fifth, while pre-stressor cortisol levels were used as an index of baseline HPA axis functioning, results must be interpreted with caution, as the laboratory assessment may have inherently activated a stress response in participants at baseline due to the novel surroundings. Finally, both cortisol responsivity to the TSST-C and symptoms of psychopathology were assessed at the same age, making it difficult to determine the direction of causality between these two variables.

Regardless of these limitations, the present study possessed a number of strengths including its longitudinal design that allowed for prospective hypothesis testing of PNMS on later child development. While a significant mediation involving the HPA axis was not supported, the association between subjective PNMS and offspring externalizing behaviors supports the intergenerational transmission of prenatal stress to offspring (Bowers and Yehuda, 2016). Further research is now needed to examine additional physiological mechanisms that may underlie this transmission, including maternal immune function, catecholamines, and vaginal microbiota, as well as the complex interactions among biological systems (Beijers et al., 2014; Markham and Koenig, 2011; Quas et al., 2014). Based on the findings of the present study, it is also recommended that future studies examine the moderating effects of mode of delivery on offspring outcomes, potentially via disrupted transmission of maternal microbiome during C-section delivery.

5. Conclusions

Overall, the present study uncovered meaningful associations between PNMS and offspring physiological and psychological outcomes, including HPA axis responsivity and externalizing behaviors at 13 years of age, respectively. Despite the study's inability to support the fetal programming hypothesis through dysregulation of the HPA axis, findings from the study did support the in utero environment as being susceptible to prenatal insults, which can result in long-term developmental consequences for offspring. At this time, further research is needed to investigate additional physiological mechanisms that may predispose offspring of prenatally stressed mothers to long-term

consequences, including elevated psychopathology.

Competing Interests

The authors declare that they have no competing interests.

Funding Source

This project was funded by the Canadian Institutes of Health Research (CIHR) grants (MOP111177 and MOP79424), as well as grants from the McGill University Stairs Memorial Fund. Sherri Lee Jones was supported by a Postdoctoral Fellowship awarded by the Fonds de recherche Québec – Santé. There are no financial conflicts of interest with the publication of this paper with either the authors or the financial institutions.

Declaration of Interest Form

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Erin Yong Ping: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **David P. Laplante:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition. **Guillaume Elgbeili:** Validation, Methodology, Formal analysis, Writing - review & editing. **Sherri Lee Jones:** Writing - review & editing. **Alain Brunet:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Suzanne King:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition.

Acknowledgements

We would like to acknowledge all participants who took part in this study, as well as all research personnel who made this project possible, including Isabelle Bouchard and Marie-Eve Roy.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104697.

References

Achenbach, T., 2001. Child Behavior Checklist. Achenbach System of Emprically Based Assessments (ASEBA), Burlington, VT.
Austin, P.C., Steyerberg, E.W., 2015. The number of subjects per variable required in linear regression analyses. J Clin Epidemiol 68, 627–636.
Barker, E.D., Jaffee, S.R., Uher, R., Maughan, B., 2011. The contribution of prenatal and postnatal maternal anxiety and depression to child maladjustment. Depression and Anxiety 28, 696–702.
Beijers, R., Buitelaar, J.K., de Weerth, C., 2014. Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. European child & adolescent psychiatry 23, 943–956.
Bowers, M.E., Yehuda, R., 2016. Intergenerational Transmission of Stress in Humans. Neuropsychopharmacology 41, 232–244.
Brennan, P.A., Pargas, R., Walker, E.F., Green, P., Newport, D.J., Stowe, Z., 2008. Maternal depression and infant cortisol: influences of timing, comorbidity and treatment. Journal of Child Psychology and Psychiatry 49, 1099–1107.
Bromet, E., Dew, M.A., 1995. Review of psychiatric epidemiologic research on disasters. Epidemiologic Reviews 17, 113–119.
Brunet, A., St-Hilaire, A., Jehel, L., King, S., 2003. Validation of a French version of the Impact of Event Scale - Revised. Canadian Journal of Psychiatry 48, 55–60.
Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., Hellhammer, D., 1997. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. Psychosomatic medicine 59, 419–426.
Cao-Lei, L., Massart, R., Suderman, M.J., Machnes, Z., Elgbeili, G., Laplante, D.P., Szyf, M., King, S., 2014. DNA Methylation Signatures Triggered by Prenatal Maternal Stress Exposure to a Natural Disaster: Project Ice Storm. PLoS ONE 9, e107653.
Creamer, M., Bell, R., Failla, S., 2003. Psychometric properties of the Impact of Event Scale - Revised. Behav Res Ther 41, 1489–1496.
Dancause, K.N., Laplante, D.P., Fraser, S., Brunet, A., Ciampi, A., Schmitz, N., King, S., 2012. Prenatal exposure to a natural disaster increases risk for obesity in 5½-year-old children. Pediatr. Res. 71, 126–131.
Dancause, K.N., Veru, F., Andersen, R.E., Laplante, D.P., King, S., 2013. Prenatal stress due to a natural disaster predicts insulin secretion in adolescence. Early Hum. Dev. 89, 773–776.
Davis, E.P., Glynn, L.M., Waffarn, F., Sandman, C.A., 2011. Prenatal maternal stress programs infant stress regulation. Journal of child psychology and psychiatry, and allied disciplines 52, 119–129.
Entringer, S., Kumsta, R., Hellhammer, D.H., Wadhwa, P.D., Wust, S., 2009. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. Hormones and behavior 55, 292–298.
Glover, V., Hill, J., 2012. Sex differences in the programming effects of prenatal stress on psychopathology and stress responses: An evolutionary perspective. Physiology & Behavior 106, 736–740.
Glover, V., O'Connor, T.G., O'Donnell, K., 2010. Prenatal stress and the programming of the HPA axis. Neuroscience & Biobehavioral Reviews 35, 11–22.
Gordis, E.B., Granger, D.A., Susman, E.J., Trickett, P.K., 2006. Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: relation to aggressive behavior in adolescents. Psychoneuroendocrinology 31, 976–987.
Graignic-Philippe, R., Dayan, J., Chokron, S., Jacquet, A.Y., Tordjman, S., 2014. Effects of prenatal stress on fetal and child development: a critical literature review. Neurosci Biobehav Rev 43, 137–162.
Grant, K.-A., McMahon, C., Austin, M.-P., Reilly, N., Leader, L., Ali, S., 2009. Maternal prenatal anxiety, postnatal caregiving and infants' cortisol responses to the still-face procedure. Developmental Psychobiology 51, 625–637.
Gunnar, M.R., Wewerka, S., Frenn, K., Long, J.D., Griggs, C., 2009. Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty. Development and psychopathology 21, 69–85.
Gutteling, B.M., de Weerth, C., Buitelaar, J.K., 2004. Maternal prenatal stress and 4-6 year old children's salivary cortisol concentrations pre- and post-vaccination. Stress 7, 257–260.
Gutteling, B.M., de Weerth, C., Buitelaar, J.K., 2005. Prenatal stress and children's cortisol reaction to the first day of school. Psychoneuroendocrinology 30, 541–549.
Halligan, S.L., Herbert, J., Goodyer, I., Murray, L., 2007. Disturbances in morning cortisol secretion in association with maternal postnatal depression predict subsequent depressive symptomatology in adolescents. Biol Psychiatry 62, 40–46.
Halligan, S.L., Herbert, J., Goodyer, I.M., Murray, L., 2004. Exposure to postnatal depression predicts elevated cortisol in adolescent offspring. Biol Psychiatry 55, 376–381.
Hayes, A.F., 2013. Introduction to mediation, moderation, and conditional process analysis. The Guilford Press, New York.
Hofstra, M.B., Van der Ende, J., Verhulst, F.C., 2000. Continuity and change of psychopathology from childhood into adulthood: a 14-year follow-up study. J Am Acad Child Adolesc Psychiatry 39, 850–858.
Hollingshead, A.B., 1973. Four-factor index of social status. Yale University Press, New Haven.
Huizink, A.C., Bartels, M., Rose, R.J., Pulkkinen, L., Eriksson, C.J.P., Kaprio, J., 2008. Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring. Journal of Epidemiology and Community Health 62, e5.
Isaksson, J., Lindblad, F., Valladares, E., Hogberg, U., 2015. High maternal cortisol levels during pregnancy are associated with more psychiatric symptoms in offspring at age of nine - A prospective study from Nicaragua. J Psychiatr Res 71, 97–102.
Jung, C., Ho, J.T., Torpy, D.J., Rogers, A., Doogue, M., Lewis, J.G., Czajko, R.J., Inder, W.J., 2011. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. J Clin Endocrinol Metab 96, 1533–1540.
Khoury, J.E., Gonzalez, A., Levitan, R.D., Pruessner, J.C., Chopra, K., Basile, V.S., Masellis, M., Goodwill, A., Atkinson, L., 2015. Summary cortisol reactivity indicators: Interrelations and meaning. Neurobiol Stress 2, 34–43.
King, S., Dancause, K., Turcotte-Tremblay, A.M., Veru, F., Laplante, D.P., 2012. Using natural disasters to study the effects of prenatal maternal stress on child health and development. Birth Defects Res C Embryo Today 96, 273–288.
Kudielka, B.M., Hellhammer, D.H., Kirschbaum, C., Harmon-Jones, E., Winkielman, P., 2007. Ten years of research with the Trier Social Stress Test—revisited. In: Harmon-Jones, E., Winkielman, P. (Eds.), Social neuroscience: Integrating biological and psychological explanations of social behavior. Guilford Press, New York, pp. 56–83.
Lopez-Duran, N.L., Kovacs, M., George, C.J., 2009. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology 34, 1272–1283.
Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. Journal of Neuroscience 15, 110–116.
Markham, J., Koenig, J., 2011. Prenatal stress: Role in psychotic and depressive diseases. Psychopharmacology 214, 89–106.
Neu, J., Rushing, J., 2011. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol 38, 321–331.
O'Connor, T.G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., Glover, V., 2005. Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. Biological Psychiatry 58, 211–217.
O'Connor, T.G., Bergman, K., Sarkar, P., Glover, V., 2013. Prenatal cortisol exposure predicts infant cortisol response to acute stress. Dev Psychobiol 55, 145–155.
O'Connor, T.G., Heron, J., Golding, J., Glover, V., 2003. Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis.

Journal of Child Psychology and Psychiatry 44, 1025–1036.
O'Donnell, K.J., Bugge Jensen, A., Freeman, L., Khalife, N., O'Connor, T.G., Glover, V., 2012. Maternal prenatal anxiety and downregulation of placental 11β-HSD2. Psychoneuroendocrinology 37, 818–826.
O'Donnell, K.J., Glover, V., Jenkins, J., Browne, D., Ben-Shlomo, Y., Golding, J., O'Connor, T.G., 2013. Prenatal maternal mood is associated with altered diurnal cortisol in adolescence. Psychoneuroendocrinology 38, 1630–1638.
Petersen, A.C., Crockett, L., Richards, M., Boxer, A., 1988. A self-report measure of pubertal status: Reliability, validity, and initial norms. Journal of Youth and Adolescence 17, 117–133.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Quas, J.A., Yim, I.S., Oberlander, T.F., Nordstokke, D., Essex, M.J., Armstrong, J.M., Bush, N., Obradovic, J., Boyce, W.T., 2014. The symphonic structure of childhood stress reactivity: patterns of sympathetic, parasympathetic, and adrenocortical responses to psychological challenge. Development and psychopathology 26, 963–982.
Rash, C.J., Coffey, S.F., Baschnagel, J.S., Drobes, D.J., Saladin, M.E., 2008. Psychometric properties of the IES-R in traumatized substance dependent individuals with and without PTSD. Addict Behav 33, 1039–1047.
Sarason, I.G., Johnson, J.H., Siegel, J.M., 1978. Assessing the impact of life changes: Development of the Life Experience Survey. Journal of Consulting & Clinical Psychology 46, 932–946.
Sirvinskiene, G., Zemaitiene, N., Jusiene, R., Markuniene, E., 2016. Predictors of Emotional and Behavioral Problems in 1-Year-Old Children: A Longitudinal Perspective. Infant Ment Health J 37, 401–410.
Stroud, L.R., Foster, E., Papandonatos, G.D., Handwerger, K., Granger, D.A., Kivlighan, K.T., Niaura, R., 2009. Stress response and the adolescent transition: performance versus peer rejection stressors. Development and psychopathology 21, 47–68.
Tanner, J.M., 1981. Growth and maturation during adolescence. Nutrition Reviews 39, 43–55.
Tarabulsy, G.M., Pearson, J., Vaillancourt-Morel, M.P., Bussieres, E.L., Madigan, S., Lemelin, J.P., Duchesneau, A.A., Hatier, D.E., Royer, F., 2014. Meta-analytic findings of the relation between maternal prenatal stress and anxiety and child cognitive outcome. Journal of developmental and behavioral pediatrics : JDBP 35, 38–43.
Taylor, A., Fisk, N.M., Glover, V., 2000. Mode of delivery and subsequent stress response. Lancet 355, 120.
Tollenaar, M.S., Beijers, R., Jansen, J., Riksen-Walraven, J.M., de Weerth, C., 2011. Maternal prenatal stress and cortisol reactivity to stressors in human infants. Stress 14, 53–65.
Van den Bergh, B.R., Van Calster, B., Smits, T., Van Huffel, S., Lagae, L., 2008. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. Neuropsychopharmacology 33, 536–545.
Van den Bergh, B.R.H., Marcoen, A., 2004. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year olds. Child Development 75, 1085–1097.
van Goozen, S.H., Fairchild, G., Snoek, H., Harold, G.T., 2007. The evidence for a neurobiological model of childhood antisocial behavior. Psychol Bull 133, 149–182.
Veru, F., Dancause, K., Laplante, D.P., King, S., Luheshi, G., 2015. Prenatal maternal stress predicts reductions in CD4+ lymphocytes, increases in innate-derived cytokines, and a Th2 shift in adolescents: Project Ice Storm. Physiol. Behav. 144, 137–145.
Weiss, D.S., Marmar, C.R., 1997. The Impact of Event Scale - Revised. In: Wilson, J.P., Keane, T.M. (Eds.), Assessing psychological trauma and PTSD: A practitioner's handbook. Guilford, New York, pp. 399–411.
Wingenfeld, K., Wolf, O.T., 2011. HPA axis alterations in mental disorders: impact on memory and its relevance for therapeutic interventions. CNS neuroscience & therapeutics 17, 714–722.
Yehuda, R., Engel, S.M., Brand, S.R., Seckl, J., Marcus, S.M., Berkowitz, G.S., 2005. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. J Clin Endocrinol Metab 90, 4115–4118.
Yong Ping, E., Laplante, D.P., Elgbeili, G., Hillerer, K.M., Brunet, A., O'Hara, M.W., King, S., 2015. Prenatal maternal stress and toddler stress reactivity at 2 1/2 years of age: the Iowa flood study. Psychoneuroendocrinology 56, 62–78.