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Natural disaster-related prenatal maternal stress is associated with alterations in placental glucocorticoid system: The QF2011 Queensland Flood Study

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ABSTRACT

We investigated the effects of a natural disaster (a sudden flood) as a source of prenatal maternal stress (PNMS) on the placental glucocorticoid system and glucose transporters. Whether the gestational age at the time of the flood moderated these effects was also evaluated. Placental samples were collected from participants in the 2011 Queensland Flood Study (QF2011) who were pregnant in the first or second trimester at the onset of the flood. Detailed questionnaire results for objective hardship and composite subjective distress were obtained to assess stress levels. Subjective distress was significantly associated with a reduction in placental *NR3C1-β* mRNA levels for males only ($\beta = -0.491$, $p = 0.005$). In female placentas, objective hardship was marginally linked with lower *SLC2A1* mRNA levels while subjective distress was a marginally significant predictor of higher placental *SLC2A4* mRNA levels. Gestational age at the time of the flood was a significant moderator of the effect of subjective distress on placental mRNA levels for *NR3C1-α* ($p = 0.046$) and *HSD11B1* ($p = 0.049$) in male placentas: if the flood occurred in mid-pregnancy, lower subjective distress predicted higher *HSD11B1* while higher subjective distress predicted lower *NR3C1-α* placental mRNA level. While results did not show any PNMS effects on placental *HSD11B2* mRNA and protein levels, and activity, we showed a reduction in placental *NR3C1-β* mRNA level in male placentas. Our results show evidence of distinct placental glucocorticoid and glucose systems adaptations to PNMS as a function of fetal sex and gestational timing of exposure, with high subjective PNMS in mid-pregnancy associated with lower levels of expression of glucocorticoid-promoting gene in males, leaving the fetus less protected against maternal stress. The exact mechanism by which natural disaster-related PNMS acts on the placenta and the impact on fetal programming requires further investigation.

1. Introduction

There is growing evidence that prenatal maternal stress (PNMS) due to a natural disaster is linked to adverse fetal development and alterations in child outcomes (Dancause et al., 2015; King et al., 2005; Laplante et al., 2004; Simcock et al., 2017; Simcock et al., 2016). Several studies have linked maternal depression, anxiety and stress in pregnancy with adverse fetal outcomes (Brunton and Russell, 2011; Buss et al., 2010; Davis et al., 2011; O'Connor et al., 2005; Ponder et al., 2011). Studies also suggest that such programming effects are an evolutionary adaptation, preparing the child to unfavorable living conditions experienced by the mother (Glover and Hill, 2012).

The mechanisms underlying this phenomenon are still largely unknown but there is growing evidence linking the mother's hypothalamic–pituitary–adrenal (HPA) axis, and its end product, glucocorticoids (cortisol in humans), to fetal programming (Seckl and Holmes, 2007). In pregnancy, there is a modification in the maternal HPA axis as the placenta produces its own corticotropin releasing hormone (CRH) in response to cortisol, which modulates the maternal HPA axis in a positive feedback loop to increase blood cortisol levels (reviewed in St-

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Pierre et al., 2016b). To counterbalance the effect of higher circulating cortisol levels, the placenta expresses type 2, 11 beta-hydroxysteroid dehydrogenase enzyme (11β-HSD2, *HSD11B2* gene) that converts cortisol into inactive cortisone (Draper and Stewart, 2005). The placenta also expresses other proteins involved in the glucocorticoid system such as glucocorticoid receptors (GR, *NR3C1*) α and β as well as the cortisol-producing enzyme 11β-HSD1 (*HSD11B1*), although at lower levels than 11β-HSD2 (Saif et al., 2015; Tomlinson et al., 2004). After cortisol binding, the GR-α receptor can work as a transcription factor when dimerized, or can be linked to the nuclear bound inhibitory form GR-β (Oakley and Cidlowski, 2013). Studies of stress, anxiety and/or depression in pregnancy in humans, and studies of experimental stressors in rodents, have associated these maternal conditions with an altered placental glucocorticoid system and linked these to developmental programming in the offspring (Mairesse et al., 2007; Mina et al., 2015; O'Donnell et al., 2011; Peña et al., 2012; Räikkönen et al., 2015; Seth et al., 2015).

The placenta mediates the transfer of obligate nutrients, notably glucose, to meet fetal demands. Glucose is passed from maternal to fetal circulation via the placenta by glucose transporters (GLUTs). The main placental glucose transporter isoform is GLUT1 (*SLC2A1* gene; Brown et al., 2011); it is primarily expressed on the apical microvillus membrane of syncytiotrophoblast adjacent to maternal circulation, and to a lesser extent on the basolateral membrane adjacent to the fetal endothelial cells (Jansson et al., 1993). It is also found in the syncytiotrophoblast precursor cells, the villous cytotrophoblasts (Baumann et al., 2002). GLUT3 (*SLC2A3*) and GLUT4 (*SLC2A4*) are also expressed in the human placenta. GLUT3 is found in the trophoblast layer of term placenta but is predominantly expressed in the first trimester and it is thought to be an important component of the glucose transport system (Brown et al., 2011). GLUT4 is primarily expressed in early gestation syncytiotrophoblast and is insulin-regulated, unlike GLUT1 (Ericsson et al., 2005). In rats, it has been shown that maternal restraint stress induces a reduction in placental GLUT1 and an increase in GLUT3 and GLUT4 protein levels at term (Mairesse et al., 2007). In humans, it has been shown that glucocorticoids down-regulate GLUT1 and GLUT3 in primary cultured villous trophoblastic cells *in vitro* (Hahn et al., 1999).

Fetal sex is an important factor to account for in studies regarding PNMS and its effect on placental function, fetal development and programming. Studies have shown that the stress response in the placenta can differ depending on fetal sex (reviewed in Clifton, 2010). This different response in regards to PNMS can be attributed to different placental glucocorticoid receptor isoforms expressed (Saif et al., 2015). Likewise, data suggests that the timing of the stress exposure during pregnancy may also affect fetal programming. For example, Project Ice Storm, a study of PNMS from a natural disaster, found that: (i) early gestational exposure predicts more severe autistic-like symptoms (Walder et al., 2014) and lower IQ (Laplante et al., 2004) in toddlers; and (ii) mid-gestational exposure predicts greater fluctuating asymmetry (King et al., 2009), while late exposure predicts poorer motor abilities (Cao et al., 2014). Although these different aspects of PNMS (objective hardship and subjective distress) have been linked to different effects on a variety of child outcomes, to date, it is unknown how these different aspects of the natural disaster-related PNMS impact the glucocorticoid and glucose transporter systems in the human placenta, and how these relationships may be moderated by timing of exposure. In the current study, we took advantage of a natural disaster in Queensland, Australia, to study the effects of PNMS on placental functioning. On January 10, 2011 the Brisbane River overflowed its banks, and heavy rains flooded 70% of the state of Queensland. Nearly 15,000 homes were completely inundated, with another 18,000 partially flooded. There were 23 flood-related deaths, and the economic costs were more than AUS$2-billion, making it one of the worst natural disasters in Queensland history.

The aim of this study was to determine the effect of *in utero* exposure to two aspects of natural disaster-related PNMS (i.e., objective hardship and subjective distress) on the placental glucocorticoid system and glucose transporters. We hypothesized that higher levels of PNMS would be associated with a decreased expression level of genes associated with reducing glucocorticoid effects (*HSD11B2, NR3C1-β*), and an increase in the expression level genes associated with promoting glucocorticoid effects (*HSD11B1, NR3C1-α, CRH*) in the placenta. We also hypothesized that higher levels of PNMS would be associated with a reduction in placental *SLC2A1* levels and an increase in *SLC2A3* and *SLC2A4* levels. We suspected that the timing of the stress exposure in pregnancy would moderate any PNMS effect. To test our hypotheses, we used a cohort of women who were pregnant during the Queensland flood in January 2011: The QF2011 Queensland Flood Study. Details on the cohort and methodology have already been described in detail elsewhere (King et al., 2015).

2. Materials and methods

2.1. Participants

Participants were women who were in any month of pregnancy during the flood on January 10, 2011 (n = 230). Recruitment began with ethics approval on April 1, 2011 and continued until mid-January 2012. Collection of the placentas began in April 2011; as such, our sample includes women who were exposed to the flood at some point in the first 172 days (first 24 weeks, or 6.6 months) of pregnancy, and does not include the 124 women who had been in their third trimester at the time of the flood and who gave birth between January 10 and April 1, 2011. Further details on eligibility and recruitment are described in (King et al., 2015). For this study, the 10 women who gave birth by elective C-section were excluded to minimize the effect of delivery method on gene expression (Burton et al., 2014): the ten elective C-sections in our sample are too few for testing differential effects of labor. The final sample size used for our analyses was 96 women who completed the stress questionnaires and from whom our team was able to collect placental samples (51 males and 45 females, Fig. S1). The QF2011 study was approved by the Mater Hospital Human Research Ethics Committee on April 1, 2011 and The University of Queensland. Participants provided informed written consent.

2.2. Placental sampling

Placentas were obtained within 30 min of expulsion, and sampling occurred within one hour. Placentas from women who gave birth by elective cesarean section were excluded. Placentas were rinsed to remove excess blood and eight biopsies were taken from the trophoblast layer using a stereological grid as previously described (Mayhew, 2006). Samples were flash frozen immediately and kept at −80 °C until further analysis. Pools of five placental samples were ground into powder using a mortar and pestle on dry ice, taking precautions to keep tissue samples frozen.

2.3. RNA isolation and cDNA synthesis

Frozen placental tissue samples (15–20 mg) were mixed with the appropriate amount of RLT buffer from the Allprep DNA/RNA/Protein mini kit (Qiagen, Toronto, ON) and placed in Qiashredder spin columns (Qiagen) to further disrupt the tissue before isolating RNA according to the manufacturer's instructions. RNA quantity and purity were assessed using a ND-1000 nanodrop (Thermo Scientific, Waltham, MA), and RNA integrity was assessed using an Experion electrophoresis system (Bio-Rad, Mississauga, ON). First strand cDNA was generated from 500 ng purified RNA using iScript reverse transcription supermix for RT-qPCR (Bio-Rad) for highly expressed genes (*CRH, HSD11B2, HSD11B1, NR3C1, NR3C1-α* and *SLC2A1*). For those genes expressed at lower levels (*NR3C1-β, SLC2A3 and SLC2A4*) the iScript advanced cDNA synthesis kit was used with 2 µg of purified RNA and an

additional pre-amplification step using SsoAdvanced PreAmp Supermix (Bio-Rad). Primer sequences are presented in Table S1.

2.4. RT-qPCR

cDNA samples were diluted 1:32 for highly expressed genes, and 1:20 for genes expressed at lower levels. A 2-step PCR was performed using a CFX96 (Bio-Rad) with either SsoFast or SsoAdvanced PCR mastermix (Bio-Rad) for high and low mRNA expression, respectively. Assays were performed in triplicates with *HPRT1* and *TOP-1* as reference genes selected using Qbase plus software (BioGazelle, Zwijnaarde, Belgium) (Lanoix et al., 2012; Vandesompele et al., 2002). Reference genes were tested for their expression level according to the child's sex and showed no significant differences in levels between males and females (St-Pierre et al., 2017).

2.5. Protein isolation and Western blot

Proteins for Western blot analysis were obtained using a radio-immunoprecipitation assay (RIPA) buffer containing Halt protease and Halt phosphatase inhibitor cocktails (Thermo scientific, Waltham, MA) buffer with 15–20 mg of frozen placental tissue powder. Protein concentration was evaluated by bicinchoninic acid assay (BCA) assay following the manufacturer's instructions (Pierce Biotechnology, Rockford, IL). Proteins (40 µg for 11b-HSD2 and 20 µg for GLUT1) were separated in 4–15% mini protean TGX gels (Bio-Rad) and transferred on Polyvinylidene fluoride (PVDF) membrane. Five percent skimmed milk in 0.5% PBS-Tween was used as membrane blocking agent. The antibodies used were ab80317 for 11β-HSD2 and ab652 for GLUT1 from Abcam (Cambridge, UK). Chemiluminescence was detected on a Chemidoc MP imaging system (Bio-Rad) with Clarity Western ECL blotting substrate (Bio-Rad). Protein expression level was evaluated by densitometry analysis of images and normalized to total protein coloration by MemCode reversible protein stain (Pierce). Results were obtained with the ImageLab 4.1 software.

2.6. 11β-HSD2 activity assay

11β-HSD2 activity was estimated by radio-enzymatic conversion adapted from a recent study (St-Pierre et al., 2016a). Briefly, proteins were isolated as described in Section 2.5 for Western blot analysis and incubated at 37 °C for 30 min with ^{3}H-Cortisol (Perkin-Elmer, Akron, OH) and unlabeled cortisol. Reaction was stopped by adding one volume of diethyl ether. Steroids were extracted by freezing the aqueous phase and removing the solvent phase. Solvent was evaporated and the steroids were suspended in dichloromethane. A small fraction of the suspension was placed on a thin layer chromatography plate (silica gel HLF 250 µm, Analtech, Newark, Delaware). Steroids were separated using a solution of dichloromethane:methanol (95:5, v:v) and bands were identified under UV light with unlabeled cortisol and cortisone for reference. The identified bands were removed from the plates by scraping carefully and placed in scintillation vials for measurement in a Tri-Carb 2100TR (Perkin Elmer). Experiments were performed in duplicate and background values subtracted (cortisone conversion without placental proteins).

2.7. Maternal stress assessment

Maternal objective hardship was assessed using the Queensland Flood Objective Stress Scale (QFOSS) questionnaire at recruitment and 12-month post-flood. This questionnaire was designed to assess the distinctive experience of the 2011 Queensland flood based on previous disaster-related PNMS studies (Laplante et al., 2007; Yong Ping et al., 2015). The questionnaire items tapped into four categories of exposure: Threat, Loss, Scope and Change. Each of the four categories was scaled from 0 to 50 (from no impact to extreme impact) for a total possible score of 200, with higher scores indicating a higher level of objective hardship. Comprehensive details on the QFOSS questionnaire are available from previous publications (King et al., 2015; Simcock et al., 2016).

To assess subjective distress, women completed three questionnaires at recruitment. The Impact of Event Scale-Revised (IES-R) (Weiss and Marmar, 1997) which assesses current PTSD-type symptoms as well as the Peritraumatic Distress Inventory (PDI-Q) (Brunet et al., 2001) and the Peritraumatic Dissociation Experience Questionnaire (PDEQ) (Marmar et al., 1997) that are retrospective reports of distress and dissociation at the time of the flood. To reduce the number of analyses, the three subjective distress measures were combined according to a Principle Components Analysis into the COmposite Score for MOther's Subjective Stress (COSMOSS). Scores on COSMOSS are centered around a mean of 0 with negative scores indicating below average subjective stress and positive scores indicating above average distress. Further details have already been described (King et al., 2015; Simcock et al., 2016).

The gestational age at the time of flood exposure (timing of exposure) was calculated as the number of days of pregnancy at the peak of the Queensland flood on January 10, 2011.

2.8. Covariates

The women's level of depression was assessed when they were first assigned their midwife (Mean = 14.6 weeks of gestation; range 6–36 weeks of gestation) using the Edinburgh Depression Scale (EDS) (Cox et al., 1996). Socioeconomic status was estimated using the Australian socio-economic indexes for area (SEIFA) scores (Pink, 2011) at recruitment into the study. Finally, the women's current anxiety levels were assessed at recruitment using the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010).

2.9. Statistical analyses

We tested sex differences using Student's *t*-tests for data from questionnaires, maternal biological factors and child outcome measures. Pearson's product moment correlations were used to test for associations among mRNA levels of each gene, and also for associations between predictors and covariates and placental mRNA levels. The Shapiro-Wilk test indicated that all the mRNA levels and QFOSS questionnaire results were not normally distributed (Table S2). Thus, these data were log-transformed for them to be closer to a normal distributions.

In order to determine the importance of the effect of PNMS on placental mRNA, protein and activity levels over and beyond the effects of covariates (e.g. SEIFA score or maternal mood), hierarchical multiple regression was used for each outcome. In the first step, SEIFA score and mood outcomes were introduced into the model. For the second step, the timing of exposure to the flood in pregnancy was added. It was included in all of our analyses as timing of exposure to the flood during pregnancy was of particular interest and part of our initial hypothesis. Objective hardship (QFOSS) was added at the third step. Composite subjective distress (COSMOSS) was entered in the fourth step; thus allowing us to assess the effects of subjective distress while controlling for objective hardship levels. Finally, objective hardship × timing of exposure or subjective distress × timing of exposure interactions terms was entered separately into the model at the final step. Composite subjective distress was not included if the model tested the interaction between objective hardship and timing of exposure. When composite subjective stress had a significant effect on placental outcomes, the effects for its three components were tested separately in exploratory analyses. Finally, SEIFA score and mood outcomes were subsequently removed from the model if they did not contribute sufficiently to the model ($p \geq 0.10$) (backwards approach). To determine the regions of significance, i.e. the levels of timing at which the effect of PNMS on

Table 1
Characteristics of the cohort.

	Male Mean (n, SD)	Female Mean (n, SD)	Sig.
Predictor variables			
QFOSS	16.45 (51, 15.51)	18.20 (45, 14.72)	0.574
COSMOSS	−0.08 (51, 1.02)	−0.25 (45, 0.59)	0.324
IES-R	6.30 (51, 10.86)	4.25 (45, 7.07)	0.282
PDI	11.50 (51, 9.03)	9.72 (45, 6.38)	0.273
PDEQ	4.85 (51, 7.18)	4.41 (45, 4.10)	0.722
Covariates			
STAI	38.26 (51, 8.45)	34.40 (45, 9.37)	**0.036***
EDS	5.07 (44, 3.55)	4.87 (39, 4.41)	0.823
Days of pregnancy at the flood	78.34 (51, 46.58)	86.79 (45, 50.25)	0.395
SEIFA socioeconomic status (SES)	1043.43 (51, 67.95)	1057.47 (45, 50.65)	0.259
Upper SES (n, %)	29, 56.9	29, 64.4	
Upper-Middle SES (n, %)	13, 25.5	11, 24.4	
Middle SES (n, %)	3, 5.9	4, 8.9	
Lower-Middle SES (n, %)	1, 2.0	0, 0	
Lower SES (n, %)	5, 9.8	1, 2.2	
Pregnancy characteristics			
Gestation length (weeks)	39.37 (51, 1.25)	39.53 (45, 1.12)	0.510
Birth weight (Kg)	3.62 (51, 0.43)	3.58 (45, 0.38)	0.635
Birth weight for gestational age	0.26 (51, 0.78)	0.40 (45, 0.67)	0.335
Placental weight (kg)	0.65 (50, 0.12)	0.65 (43, 0.13)	0.889
Placental index	0.18 (50, 0.03)	0.18 (43, 0.03)	0.973
Mothers' characteristics			
Previous pregnancies	0.67 (51, 0.88)	0.84 (43, 1.07)	0.400
BMI	24.57 (51, 4.46)	24.50 (44, 5.77)	0.943
Age at birth	30.84 (51, 5.11)	31.15 (45, 5.69)	0.779

Student's *t*-test, * $p < 0.05$. QFOSS: Queensland Flood Objective Stress Scale; COSMOSS: COmposite Score of the MOther's Subjective Stress; IES-R: Impact of Event Scale – Revised; PDI: Peritraumatic Distress Inventory; PDEQ: Peritraumatic Dissociative Experiences Questionnaire; STAI: State-Trait Anxiety Inventory; EDS: Edinburgh Depression Scale; SEIFA: Socio-Economic Indexes for Areas; BMI: Body mass index; Placental index: placental weight divided by birth weight.

biomarker levels is significant, and to facilitate the graphical representation of significant interactions, the PROCESS macro v2.11 was used (Hayes, 2013). Analyses were performed using SPSS v.21 (IBM).

3. Results

3.1. Descriptive statistics

Table 1 presents the descriptive statistics of the participants as a function of placental sex. Student's *t*-test showed that anxiety levels were higher for women carrying male compared to female fetuses ($t(94) = 2.123$, $p = 0.036$). There were no other statistically significant differences. We also compared these characteristics with the rest of the QF2011 cohort that was exposed to the flood during the first and second trimester of pregnancy (for whom the placentas were not collected) and the mothers reported significantly higher objective hardship for the participants that the placenta was not collected ($t(90) = 2.439$, $p = 0.017$) for women carrying a male fetus. Furthermore, there was a slightly shorter average gestation length for women for whom we did not collect the placenta ($t(92) = -1.985$, $p = 0.050$) carrying a female fetus. No other statistically significant differences were observed for the descriptive statistics between the participants for whom the placenta was available for analysis and those that was not. Furthermore, out of 96 women participating in the study, 94 were Caucasian. No significant differences in mRNA level of the genes, protein expression and activity analyzed were found between male and female placentas (Table S3).

3.2. Intercorrelations among placental glucocorticoid system and glucose transporter mRNA levels

Table 2 shows intercorrelations of mRNA levels for all of genes tested as a function of male and female placentas.

3.2.1. For male placentas

mRNA level of genes promoting glucocorticoid effects (*CRH*, *NR3C1*-α, and *HSD11B1*) were highly correlated with each other ($p = 0.014$ to $p < 0.001$) as well as the mRNA levels of the two glucocorticoid inhibiting genes (*HSD11B2* and *NR3C1-β*) ($p = 0.011$). For the glucose transporter genes, only *SLC2A3* and *SLC2A4* levels were correlated ($p < 0.001$). *SLC2A4* mRNA levels were also negatively associated with the mRNA levels of NR3C1–*β* ($p = 0.037$). *NR3C1* mRNA levels were positively correlated with the levels of both NR3C1–α ($p < 0.001$) and NR3C1–*β* ($p = 0.049$) as well as with *HSD11B1* ($p < 0.001$). Furthermore, the glucose transport *SLC2A1* mRNA levels were positively associated with the mRNA levels of *CRH* ($p = 0.034$) and *NR3C1–α* ($p = 0.042$) and negatively associated with the mRNA levels of *HSD11B2* ($p = 0.015$).

3.2.2. For female placentas

Similarly to the male, mRNA level of genes promoting glucocorticoid effects (*CRH, NR3C1-α,* and *HSD11B1*) were highly correlated ($p < 0.011$ to $p < 0.001$) as well as the mRNA levels of the two glucocorticoid inhibiting genes ($p = 0.014$). For the glucose transporter genes, only *SLC2A3* and *SLC2A4* levels were correlated ($p < 0.001$). *NR3C1* mRNA levels were positively correlated with the mRNA levels of glucocorticoid promoting genes *CRH* ($p = 0.004$), *NR3C1–α* ($p < 0.001$) and *HSD11B1* ($p = 0.015$), but were not associated with any of the glucocorticoid inhibiting genes. *NR3C1* mRNA levels were also positively correlated to the mRNA levels of *SLC2A1* ($p = 0.018$). Moreover, the mRNA levels of SLC2A1 levels were positively associated with the mRNA levels of *CRH* ($p = 0.034$) and *NR3C1–α* ($p = 0.001$) and negatively associated with the mRNA levels of *HSD11B2* ($p < 0.001$).

3.3. Association between PNMS and placental mRNA level

Table 3 shows the correlations between predictors (stress measures and covariates) and placental mRNA levels.

For male placentas, only one marginally significant association was observed: Higher composite subjective distress levels were associated with lower *NR3C1-β* mRNA levels ($p = 0.068$).

For female placentas, higher objective hardship and composite subjective distress levels were associated with lower mRNA levels of the *SLC2A1* ($p = 0.042$ and $p = 0.092$, respectively). Timing of exposure to the flood, regardless of the level of PNMS severity, was associated with higher mRNA levels of the glucocorticoid promoting genes *NR3C1–α* ($p = 0.013$) and *HSD11B1* ($p = 0.022$). Higher parental SEIFA scores were associated with higher mRNA levels of *NR3C1–α* ($p = 0.013$) and *HSD11B1* ($p = 0.016$). Finally, higher recruitment maternal anxiety levels were marginally associated with lower mRNA levels of the *NR3C1*-β ($p = 0.079$). Significant correlations observed in Table 3 between placental biomarkers mRNA level and predictors (stress measures and covariates) are presented in Fig. S2.

3.4. Hierarchical multiple linear regression of PNMS on placental mRNA level for male placentas

The results of the significant regression models are presented in Table 4 and Fig. 1.

3.4.1. Glucocorticoid promoting genes (CRH, NR3C1-α, HSD11B1)

After controlling for the non-significant effects of timing of exposure

Table 2
Pearson's product moment correlations between placental mRNA level of genes tested separated by fetal sex.

		GC promoting			GC inhibiting			Glucose transporters		
		CRH	*NR3C1-α*	*HSD11B1*	*HSD11B2*	*NR3C1-β*	*NR3C1*	*SLC2A1*	*SLC2A3*	*SLC2A4*
CRH	**Male**									
	Female									
NR3C1-α	**Male**	**0.590****								
	Female	**0.490****								
HSD11B1	**Male**	**0.341***	**0.816****							
	Female	**0.375***	**0.645****							
HSD11B2	**Male**	0.145	0.102	0.175						
	Female	0.241	0.089	**0.361***						
NR3C1-β	**Male**	0.129	0.187	0.175	**0.353***					
	Female	−0.057	0.147	**0.311***	**0.363***					
NR3C1	**Male**	0.261	**0.729****	**0.645****	0.165	**0.277***				
	Female	**0.423****	**0.547****	**0.361***	0.099	0.019				
SLC2A1	**Male**	**0.298***	**0.286***	0.014	**−0.338***	−0.203	0.179			
	Female	**0.317***	**0.390****	0.033	**−0.307***	−0.146	**0.351***			
SLC2A3	**Male**	−0.233	0.045	0.089	−0.090	−0.067	−0.087	−0.215		
	Female	−0.242	0.072	−0.008	−0.066	0.066	−0.191	−0.055		
SLC2A4	**Male**	−0.248	−0.040	−0.046	−0.187	**−0.293***	−0.180	−0.097	**0.503****	
	Female	−0.138	0.005	−0.063	−0.082	−0.241	−0.102	0.019	**0.500****	

CRH: Corticotropin-releasing hormone; *NR3C1*: Nuclear Receptor Subfamily 3 Group C Member 1; *NR3C1-α*: Nuclear Receptor Subfamily 3 Group C Member 1-α; NR3C1-β: Nuclear Receptor Subfamily 3 Group C Member 1-β; *HSD11B1*: Hydroxysteroid 11-Beta dehydrogenase type 1; *HSD11B2*: Hydroxysteroid 11-Beta dehydrogenase type 2; *SLC2A1*: Solute Carrier Family 2 type 1; *SLC2A3*: Solute Carrier Family 2 type 3; *SLC2A4*: Solute Carrier Family 2 type 4. Underline: $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.

to the flood, objective hardship, and composite subjective distress, the composite subjective distress × timing of exposure interaction was significantly associated with the mRNA levels of the *NR3C1*-α in male placentas accounting for 8.2% of the variance (Table 4A). The effect of composite subjective distress on male placental *NR3C1-α* mRNA levels was significant if the flood occurred after pregnancy Day 80 (marginal between Days 64 and 79) at which point the higher the composite subjective distress level the lower the mRNA level (Fig. 1A). Interpreting the interaction in the alternate direction (Fig. 1B), timing of exposure to the flood during pregnancy was a marginally significant predictor of *NR3C1*-α when the composite subjective distress level was 0.78 standard deviation (SD) above the mean; the later in pregnancy

Table 3
Pearson's product moment correlations (r) between predictors and placental mRNA level of genes implicated in glucocorticoid (GC) promoting and inhibiting signal, and glucose transport in placentas for males (n = 51) and females (n = 45).

		GC promoting			GC inhibiting			Glucose transporters		
		CRH	*NR3C1-α*	*HSD11B1*	*HSD11B2*	*NR3C1-β*	*NR3C1*	*SLC2A1*	*SLC2A3*	*SLC2A4*
QFOSS	**Male**	−0.027	−0.036	−0.027	0.074	0.004	−0.176	−0.076	0.097	−0.061
	Female	0.010	−0.066	0.071	0.087	−0.137	−0.131	**−0.304***	0.056	0.008
COSMOSS	**Male**	−0.166	−0.124	−0.098	0.018	**−0.257**	−0.167	−0.007	0.117	0.159
	Female	−0.113	−0.026	0.076	0.112	−0.235	−0.131	**−0.254**	0.053	0.231
Timing	**Male**	0.184	0.028	0.116	−0.187	−0.167	−0.138	0.034	0.053	−0.035
	Female	0.121	**0.366***	**0.341***	−0.036	0.172	0.145	0.134	−0.224	−0.087
SEIFA	**Male**	0.151	0.060	−0.075	−0.037	0.003	−0.122	0.005	0.089	0.066
	Female	0.224	**0.366***	**0.357***	0.144	−0.028	0.086	0.066	−0.007	0.023
STAI	**Male**	0.055	−0.068	−0.169	0.051	−0.039	−0.110	−0.168	0.011	0.110
	Female	−0.144	−0.077	0.172	0.137	**−0.264**	−0.153	−0.223	0.021	0.170
EDS	**Male**	−0.100	−0.196	−0.212	−0.095	−0.223	−0.207	0.058	0.202	0.226
	Female	−0.193	−0.116	−0.067	0.086	0.244	−0.079	−0.197	−0.019	0.024

QFOSS: Queensland Flood Objective stress score; COSMOSS: Composite score of the mother's subjective stress; STAI: State-trait anxiety inventory; EDS: Edinburgh depression scale; SEIFA: Socio-Economic Indexes for Areas. *CRH*: Corticotropin-releasing hormone; *NR3C1*: Nuclear Receptor Subfamily 3 Group C Member 1; *NR3C1-α*: Nuclear Receptor Subfamily 3 Group C Member 1-α; *NR3C1-β*: Nuclear Receptor Subfamily 3 Group C Member 1-β *HSD11B1*: Hydroxysteroid 11-Beta dehydrogenase type 1; *HSD11B2*: Hydroxysteroid 11-Beta dehydrogenase type 2; *SLC2A1*: Solute Carrier Family 2 type 1; *SLC2A3:* Solute Carrier Family 2 type 3; *SLC2A4*: Solute Carrier Family 2 type 4. Underline: $p < 0.10$; *: $p < 0.05$.

Table 4
Significant hierarchical multiple linear regression results of prenatal stress effects and timing of exposure to flood on placental mRNA level of genes tested for male placentas.

Predictor variables	*B*	*Std.Error*	*β*	*R*	R^2	ΔR^2	*F*	*ΔF*
***A) NR3C1-α* Males**								
Step 1				0.028	0.001	0.001	0.039	0.039
Timing	0.028	0.143	0.028					
Step 2				0.040	0.002	0.001	0.039	0.039
Timing	0.018	0.152	0.018					
QFOSS	−0.030	0.152	−0.030					
Step 3				0.132	0.017	0.016	0.277	0.755
Timing	−0.028	0.162	−0.028					
QFOSS	0.041	0.173	0.041					
COMOSS	−0.159	0.183	−0.159					
Step 4				0.316	0.100	**0.082***	1.274	**4.208***
Timing	−0.083	0.159	−0.083					
QFOSS	0.083	0.169	0.083					
COSMOSS	−0.449	0.226	**−0.449**					
COSMOSS X Timing	−0.385	0.188	**−0.382***					
***B) HSD11B1* Males**								
Step 1				0.116	0.014	0.014	0.674	0.674
Timing	0.116	0.142	0.116					
Step 2				0.117	0.014	0.000	0.333	0.005
Timing	0.120	0.151	0.120					
QFOSS	0.011	0.151	0.011					
Step 3				0.134	0.018	0.004	0.286	0.203
Timing	0.096	0.162	0.096					
QFOSS	0.048	0.173	0.048					
COSMOSS	−0.082	0.183	−0.082					
Step 4				0.313	0.098	**0.080***	1.248	**4.078***
Timing	0.042	0.159	0.042					
QFOSS	0.089	0.169	0.089					
COSMOSS	−0.368	0.227	−0.368					
COSMOSS X Timing	−0.379	0.188	**−0.376***					
***C) NR3C1-β* Males**								
Step 1				0.167	0.028	0.028	1.398	1.398
Timing	−0.001	0.001	−0.167					
Step 2				0.174	0.030	0.003	0.751	0.129
Timing	−0.001	0.001	−0.184					
QFOSS	−0.025	0.068	−0.054					
Step 3				0.426	0.182	**0.151****	**3.476***	**8.685****
Timing	−0.002	0.001	**−0.327***					
QFOSS	0.077	0.072	0.168					
COSMOSS	−0.169	0.058	**−0.491****					

NR3C1-α: Nuclear Receptor Subfamily 3 Group C Member-α; *NR3C1-β*: Nuclear Receptor Subfamily 3 Group C Member-β; *HSD11B1*: Hydroxysteroid 11-Beta Dehydrogenase type 1; QFOSS: Queensland flood objective stress score; COSMOSS: Combined stress measure of the mother's subjective stress. Underline: $p < 0.10$; * $p < 0.05$, ** $p < 0.01$ (See also Tables S4–S6 and Fig. 1).

flood occurred the lower the placental mRNA levels of this gene. In order to determine which aspect of the mothers' composite subjective distress was related to the mRNA levels of *NR3C1*-α, separate regression analyses were conducted by replacing maternal composite subjective distress with PTSD-like symptom, peritraumatic distress, and peritraumatic dissociation levels in separate analyses. After controlling for the non-significant effects of timing of exposure, objective hardship, and PTSD-like symptom levels, the PTSD-like symptoms × timing of exposure interaction was significantly associated with the mRNA levels of *NR3C1*-α, accounting for 14.2% of the variance in male placentas (Table S4A). The effect of PTSD-like symptoms on male placental mRNA was significant if the flood occurred after pregnancy Day 104 (marginal between Days 92 and 103) (Fig. S3A) at which point the higher the level of PTSD-like symptoms the lower the mRNA level. Interpreting the interaction in the alternate direction (Fig. S3B), timing of exposure was a significant predictor of *NR3C1*-α when maternal PTSD-like symptoms log-transformed levels were above 2.34: the later in pregnancy the flood occurred the lower the placental mRNA levels of this gene. The regression analyses conducted using peritraumatic distress and peritraumatic distress levels revealed that these two predictor variables and their interaction terms with timing of exposure were not significantly associated with the mRNA levels of *NR3C1*-α (Table S4A).

After controlling for the non-significant effects of timing of exposure, objective hardship and composite subjective distress, the composite subjective distress × timing of exposure interaction was significantly associated with the mRNA levels of *HSD11B1* and accounted for 8.0% of the variance in male placentas (Table 4B). The effect of composite subjective distress levels on mRNA levels in male placentas were significant when the flood occurred after pregnancy Day 130 (marginal between Days 82 and 129) (Fig. 1C) with greater levels of composite subjective distress associated with lower placental mRNA levels. Interpreting the interaction in the alternate direction (Fig. 1D), timing of exposure was found to have a marginally significant effect on placental mRNA level of *HSD11B1* when composite subjective distress was lower than −0.84, or nearly 1 SD below the mean, indicating that at low levels of composite subjective distress, there was a trend for placental mRNA levels to be lower the earlier in pregnancy the flood occurred. In order to determine which aspect of the mothers' composite subjective distress was related to the mRNA levels of *HSD11B1*, separate regression analyses were conducted by replacing levels of maternal composite subjective distress levels with PTSD-like symptom, peritraumatic distress, and peritraumatic dissociation levels in separate analyses. After controlling for the non-significant effects of timing of exposure, objective hardship, and PTSD-like symptoms, the PTSD-like

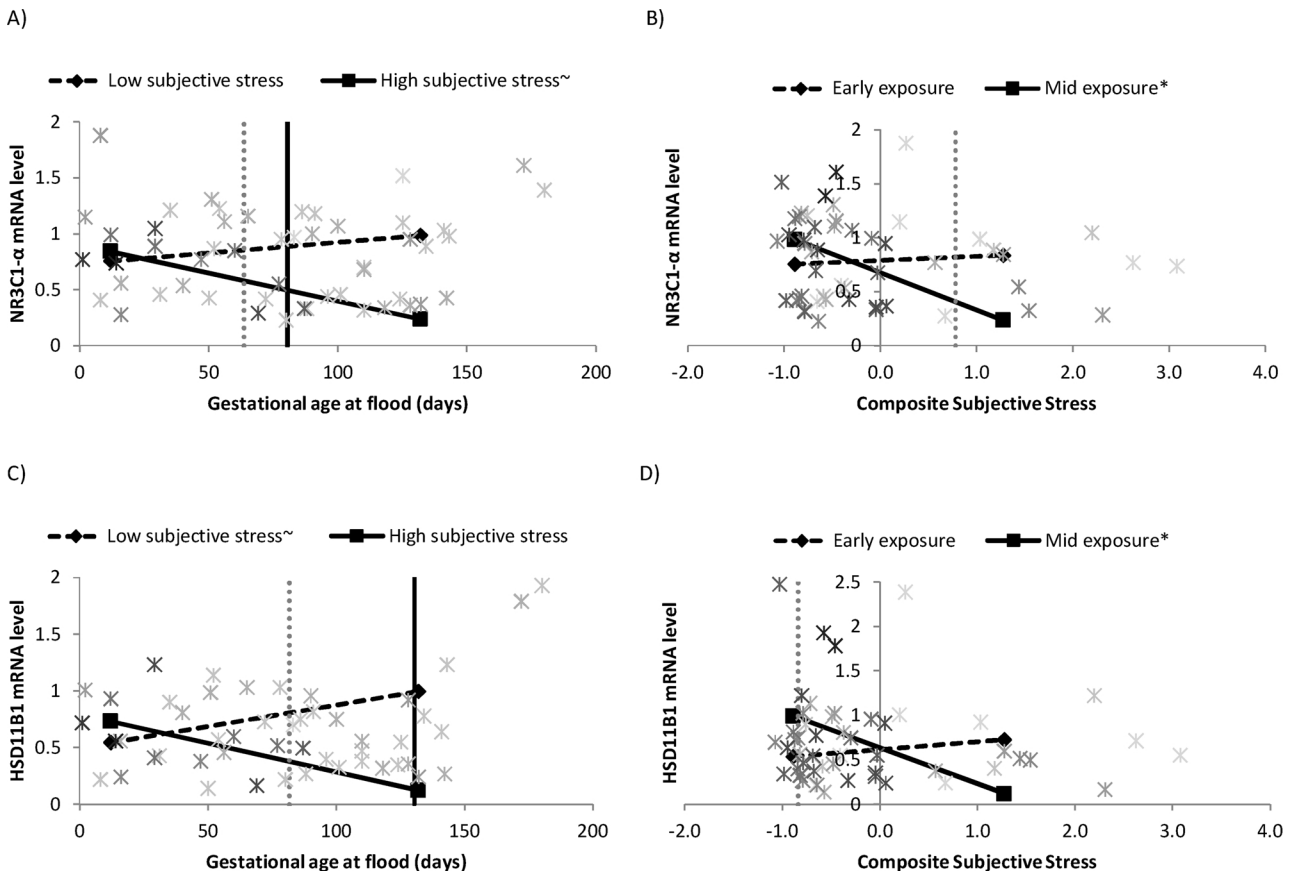

Fig. 1. Significant moderation of the effect of subjective distress (COSMOSS) on placental (A and B) glucocorticoid receptor α (*NR3C1-α*), and (C and D) 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*) mRNA levels by timing of exposure in gestation. Vertical solid (or dashed) lines represent the significance threshold of the region of significance showing significant (or marginal) subjective stress effects at later timing of exposure in A) and C); and significant (or marginal) timing effects at B) higher COSMOSS and D) lower COSMOSS. "Early" pregnancy is represented at 2 weeks of pregnancy, and "mid" is represented at 20 weeks of pregnancy. "Low" subjective stress is represented by 0.89 SD below the mean and "high" subjective stress is represented by 1.27 SD above mean. Those levels represent the 10th and 90th percentiles of the continuous-level variables and were only used for graphical representations. Their slope and significance were computed as conditional effects using the PROCESS macro for SPSS (Hayes, 2013). In panels A and C, the darker the shade of grey of a data point, the higher the composite subjective stress level of the participant. In panels B and D, the darker the shade of grey of a data point, the later the timing of exposure in gestation for the participant. Slope significance legend: $^{*}p < 0.05$; ~p < 0.1. See also Table 4.

symptoms × timing of exposure interaction was significantly associated with the mRNA levels of HSD11B1 and accounted for 14.4% of the variance in male placentas (Table S4A). The effect of IES-R on *HSD11B1* mRNA levels in male placentas was significant when the flood occurred after pregnancy Day 102 (marginal between Days 91 and 101) (Fig. S3C) with higher levels of PTSD-like symptoms being associated with lower *HSD11B1* placental mRNA level. Interpreting the interaction in the alternate direction (Fig. S3D), timing of exposure was found to have a significant effect on placental mRNA level of *HSD11B* when PTSD-like symptom log-transformed levels were lower than 0.44: this effect indicates that at low levels of PTSD-like symptoms, placental mRNA levels of *HSD11B1* were lower the earlier in pregnancy the flood occurred. Timing also had a marginally significant effect on placental mRNA level of *HSD11B1* when PTSD-like symptoms log-transformed levels were higher than 2.44, such that *HSD11B1* mRNA levels were lower the later in pregnancy the flood occurred.

After controlling for the non-significant effects of timing of exposure, objective hardship and peritraumatic dissociation levels, the peritraumatic dissociation × timing of exposure interaction tended to be associated with the placental mRNA levels of *HSD11B1* ($p = 0.053$), accounting for 7.5% of the variance in male placentas (Table S4A). The effect of the level of peritraumatic dissociation on mRNA levels of *HSD11B1* in male placentas was significant when the flood occurred after pregnancy Day 88 (marginal between Days 76 and 87) with higher levels of peritraumatic experiences being associated with lower *HSD11B1* placental mRNA level (data not shown). Interpreting the interaction in the alternate direction, timing of exposure was found to have a marginally significant effect on placental mRNA levels when peritraumatic dissociation log-transformed levels were lower than 0.13: this effect indicates that at low levels of peritraumatic dissociation there was a trend for placental mRNA levels to be lower the earlier in pregnancy the flood occurred (data not shown). The regression analyses conducted using levels of peritraumatic distress revealed that this predictor variable and its interaction term with timing of exposure to the flood were not significantly related to the placental mRNA levels *HSD11B1* (Table S4A).

3.4.2. Glucocorticoid inhibiting genes (HSD11B2 and NR3C1-β)

After controlling for timing of exposure, objective hardship, and composite subjective distress did not significantly account for any of the variance of mRNA levels of the *HSD11B2* (Table S5). After controlling for the non-significant effects of timing of exposure and objective hardship, composite subjective distress levels were significantly related to the mRNA levels of *NR3C1-β*, accounting for 15.1% of the variance in male placentas. Increased levels of maternal composite subjective distress were related to lower mRNA levels of this gene (Table 4C). In order to determine which aspect of the mothers' subjective distress was related to the mRNA levels of *NR3C1-β*, separate regression analyses

were conducted by replacing composite subjective distress levels with PTSD-like symptoms, peritraumatic distress and peritraumatic dissociation levels. After controlling for the non-significant effects of timing of exposure and objective hardship, PTSD-like symptoms levels were significantly related to the mRNA levels of *NR3C1-β*, accounting for 8.3% of additional variance in male placentas. Increased levels of maternal PTSD-like symptoms were related to lower mRNA levels of this gene (Table S4B). Likewise, after controlling for the non-significant effects of timing of exposure and objective hardship, both peritraumatic distress ($p = 0.070$) and peritraumatic dissociation ($p = 0.063$) levels tended to be associated with lower mRNA levels *NR3C1-β*, accounting for 6.6% and 6.9% of the variance in male placentas, respectively (Table S4B).

3.4.3. Glucose transporter genes (SLC2A1, SLC2A3, SLC2A4)

Timing of exposure, objective hardship, and composite subjective distress did not significantly account for any of the variance of mRNA levels of any of the glucose transporter genes in male placentas (Table S5).

3.5. Hierarchical multiple linear regression of PNMS on placental mRNA level for female placentas

3.5.1. Glucocorticoid promoting genes (CRH, NR3C1-α, HSD11B1)

Timing of exposure, objective hardship, and composite subjective distress did not significantly account for any of the variance of mRNA levels of any of the three glucocorticoid promoting genes (*CRH, NR3C1-α, HSD11B1*) in female placentas (Table S5). However, there was a marginally significant timing x subjective distress interaction for CRH mRNA level with a significant reduction in CRH with higher composite subjective distress if the flood occurred later in pregnancy (Table S6).

3.5.2. Glucocorticoid inhibiting genes (HSD11B2 and NR3C1-β)

After controlling for timing of exposure, objective hardship, and composite subjective distress did not significantly account for any of the variance of mRNA levels of the *HSD11B2* nor *NR3C1-β* in female placentas (Table S5).

3.5.3. Glucose transporter genes (SLC2A1, SLC2A3, SLC2A4)

After controlling for the non-significant effects of timing of exposure, there was a trend for higher objective hardship levels to be associated with lower *SLC2A1* gene mRNA levels in female placentas ($p = 0.053$), explaining 8.5% of the variance (Table S5). After controlling for the non-significant effects of timing of exposure and objective hardship, there was a trend ($p = 0.057$) for higher composite subjective distress levels to be associated with higher *SLC2A4* gene mRNA levels in female placentas, explaining 8.5% of the variance (Table S5).

4. Discussion

This study is the first to assess the effect of natural disaster-related PNMS on the placental glucocorticoid system and glucose transporters. We hypothesized that greater PNMS would be associated with a decrease in placental gene expression levels associated with reducing glucocorticoid effects, and an increase in the expression levels of the genes associated with promoting glucocorticoid effects. We also hypothesized a reduction in the placental level of GLUT1 and an increased level of GLUT4 with increased PNMS exposure. By studying the placentas from women exposed to a major flood with sudden onset in either their first or second trimester of pregnancy, we could study the relative effects of two distinct elements of PNMS: the objective severity of the pregnant women's exposure and the severity of their subjective stress response. Suspecting that the timing of the stress in pregnancy might moderate PNMS effects, we tested relevant interactions.

4.1. Placental glucocorticoid system

We found that the objective hardship (QFOSS) from a natural disaster did not predict placental mRNA level of genes implicated in the glucocorticoid system. However, in accord with our hypothesis, we found that, after controlling for objective hardship, exposure to high composite subjective distress (COSMOSS) levels were significantly related to a reduction in *NR3C1-β* mRNA levels in male placentas. GR-β is a cortisol inhibiting protein as it binds to GR-α in the nucleus and inhibits its transcription factor function (Oakley and Cidlowski, 2013). Placental GR-β has been neglected in anxiety, depression and PNMS studies. Our results suggest that the reduction in *NR3C1-β* mRNA level would be associated with a lower GR-β protein level leading to a higher sensitivity to glucocorticoids for male placentas.

The timing of exposure to the Queensland floods during pregnancy was a significant moderator on the effect of composite subjective distress, controlling for objective stress, on placental levels of two glucocorticoid-promoting genes in male placentas: *HSD11B1* and *NR3C1*-α. We found that for early exposure, there was no significant effect of composite subjective distress, but for women exposed to the flood during mid-pregnancy (beyond 3.6 months or beyond 5.3 months, respectively) there was a significant reduction in placental *NR3C1-α* and *HSD11B1* mRNA associated with high maternal composite subjective distress levels. We also observed that the timing of exposure effect was especially strong for PTSD-like symptoms from 104 days of gestation onwards, compared to peritraumatic distress or dissociation levels at the time of the flood. Thus, placental functioning in months 3–5 of pregnancy appear to be particularly susceptible to heightened levels of maternal subjective distress.

While *NR3C1-α* expression is highly studied in the placenta, to our knowledge, this is the first study demonstrating an association between natural disaster-related PNMS and an alteration in placental *NR3C1-β* mRNA levels, as well as an effect of timing of exposure as a moderator of PNMS on placental *NR3C1-α* and *HSD11B1*. Furthermore, we observed a marginally significant interaction between composite subjective distress and timing of exposure for *CRH* mRNA level for females only, with a significant reduction in *CRH* mRNA with higher composite subjective distress if the flood occurred later in pregnancy (data not shown). These results suggest that different types of PNMS influence placental glucocorticoid sensitivity differently depending on the timing of the stressor *in utero,* with early- (months 1–2) or mid- (months 3–5) pregnancy stress associated respectively with no effect, or with a decrease in sensitivity to glucocorticoids in the term placentas. This moderating effect of timing of exposure on placental function could explain why prior research has found that timing of exposure to PNMS has different programming effects on child neurodevelopment (Cao et al., 2014; Simcock et al., 2016).

The human *NR3C1* gene can produce GR-α, GR-β, GR-ϒ, GR-A and GR-P isoforms through alternative splicing or translation initiation (Oakley and Cidlowski, 2013). Moreover, there are eight isoforms originating from the *NR3C1*-α mRNA (Mina et al., 2015; Saif et al., 2014). The difference in isoform expression was thought to mediate in part the sexually dimorphic placental response to PNMS (Clifton, 2010; Saif et al., 2014; St-Pierre et al., 2016b). This difference is seen in our results as placental *NR3C1-β* mRNA levels of female placentas were not significantly reduced by composite subjective distress levels, unlike the reduced *NR3C1-β* mRNA levels observed in male placentas. However, it is also possible that other GR isoforms would be implicated in placental glucocorticoid sensitivity or resistance. Further studies are needed to assess the effect on different expression level of GR in the placenta and possibly associate the change in GR isoform level to fetal programming.

Interestingly, natural disaster-related PNMS did not affect placental 11β-HSD2 expression or activity for either male or female placentas, which is not concordant with the current literature. O'Donnell et al. observed a reduction in *HSD11B2* mRNA level and 11β-HSD2 activity with higher maternal anxiety one day before elective cesarean section

(O'Donnell et al., 2011). Another study also found a negative (but weak) correlation of *HSD11B2* with both anxiety and depression in the third trimester (Seth et al., 2015). Similar results on the activity and mRNA level were also observed for restraint stress on rats in late gestation (Mairesse et al., 2007). Another study found lower *HSD11B2* mRNA levels in placentas of male infants were associated with higher maternal life satisfaction at pregnancy week 17, but observed higher *HSD11B2* mRNA levels for placentas from female infants with a higher maternal life satisfaction in mothers at 36 weeks of pregnancy (Mina et al., 2015). In the same study, depression at pregnancy week 36 was also linked to lower placental *HSD11B2* mRNA levels. These studies have in common the fact that maternal stress was assessed in late gestation, near the time of delivery, unlike our study where late-gestation placentas were unavailable. While these results conflict with those obtained in the present study, stress from a natural disaster has a sudden in onset, while in other studies, anxiety, depression and life satisfaction is usually a prolonged state and/or even a stable trait in people. As such, our 'stress' method used in the present study more closely resembles that used in experimental animal studies (randomly assigned prenatal stress) than it does of human studies of maternal psychological functioning. Further studies are needed to confirm our observations on the effect of natural disaster-related PNMS on placental 11β-HSD2 expression and activity.

Altogether, our data suggests another mechanism by which PNMS increases placental glucocorticoid sensitivity, while not compromising cortisol transfer to the fetus, for example, changing 11β-HSD-1, −2 expression, and demonstrates the importance of considering other components of the placental glucocorticoid system in further studies.

4.2. Placental glucose transporters

In accord with our hypothesis, higher levels of composite subjective distress were marginally associated with higher *SLC2A4* mRNA levels for female placentas, while higher objective hardship was correlated with lower placental *SLC2A1* mRNA levels also for female placentas (with no effect of GLUT1 protein level). This suggests that PNMS could modify the *SLC2A1*: *SLC2A4* ratio, and thus the glucose transfer from the mother to the female fetus, suggesting a potentially different adaptation strategy for glucose transport in the placenta according to fetal sex.

These results suggest that when stressed in pregnancy, the glucose transport across the placenta seems to promote insulin-sensitive GLUT4 instead of the omnipresent GLUT1 in female placentas. GLUT1 is highly expressed in the human third trimester placentas while GLUT4 is expressed at a higher level in early gestation (Baumann et al., 2002; Ericsson et al., 2005), suggesting that GLUT1 is the major glucose transporter to meet the fetal glucose needs in late gestation. Our data are in accordance with the literature showing that glucocorticoids reduce the placental *SLC2A1* mRNA level in primary trophoblast cell *in vitro* and *in vivo* in rat models (Hahn et al., 1999). PNMS also has been shown to reduce placental GLUT1 protein level in rats and was associated with lower plasma glucose level in the fetus (Mairesse et al., 2007). The latter study also showed an increase in placental GLUT4 protein level at term when dams were stressed which complements our findings that composite subjective distress levels were positively associated with placental *SLC2A4* mRNA levels. However, the same study also reported a slightly higher level of GLUT3 in the placentas of stressed rats while no alteration of *SLC2A3* was observed in our study. Further investigations are necessary to determine if these effects of PNMS on glucose transporters are linked with child metabolic programming.

4.3. Strengths

The use of a natural disaster as a stressor has clear advantages for studying the psychobiological mechanisms of fetal programming in humans. The objective severity of exposure to many natural disasters is quasi-randomly distributed in the population, typically has a sudden onset that affects pregnant women at various times in gestation, and occurs independently from the pregnant women's mental health or personality traits (King et al., 2005). The use of multiple maternal stress assessments (objective hardship, PTSD-like symptoms, peritraumatic distress and dissociative experiences) is also a strength of this study. Furthermore, because we analyzed the effect of objective hardship independently from the mother's subjective distress, we were able to untangle their effects on placental mRNA levels of target genes.

4.4. Limitations

Limitations include the relatively small number of placental samples, and the lack of placentas from women not exposed to the flood, as well as the missing placentas of women exposed to the flood in the third trimester who gave birth before ethics approval for the study was obtained. This is a common challenge for this kind of study where we are dealing with a sudden and unpredictable natural disaster. We did not account for multiple testing in our analysis of data and thus the risk of type I errors has not been accounted for. While we do use a scale to measure PNMS from little to extremely stressed, we did not use a non-exposed similar cohort for biomarkers comparison. Finally, we were not able to control for maternal factors such as, drug use, alcohol consumption, and smoking that might influence placental biomarkers prior to or following PNMS exposure because of too many missing data points.

5. Conclusion

Although results did not show any effects of PNMS on placental 11β-HSD2 mRNA, protein and activity, we showed a reduction in placental *NR3C1-β* mRNA in male placentas. Thus, our data shows evidence of distinct placental glucocorticoid and glucose systems adaptations to PNMS as a function of fetal sex and timing of exposure during pregnancy, with high composite subjective distress levels in mid-pregnancy associated with lower levels of expression of GC-inhibiting genes in male placentas, suggesting that in the QF2011 cohort males were sensitive to glucocorticoids while female fetuses induced strategies to that promoted normal growth. Another major finding was that pregnancy months 3–6 appear to be a period of susceptibility to the effects of natural disaster-related PNMS on the placenta relative to earlier months; we can make no conclusions about third trimester exposure. While the results presented here confirm that natural disaster-related PNMS affects the placental glucocorticoid and glucose systems, the exact mechanism by which natural disaster-related PNMS acts on the placenta and impacts fetal growth and programming requires further investigation. A thorough understanding of how natural disaster-related PNMS influences the placental glucocorticoid system and glucose transport will provide a better appreciation of the role of the placenta in the fetal adaptive response to *in utero* exposure to stress. Since the placenta is easily accessible at birth, our study has high translational potential and may be useful in predicting the effect of natural disaster-related PNMS exposure on programming.

Disclosure statement and conflict of interest

The authors declare no conflict of interest and have nothing to disclose.

Contributors

C. Vaillancourt, S. King, S. Kildea, P.A. Dawson, and D.P. Laplante participated in study conception and design as well as revising critically the manuscript. J. St-Pierre and P. Dawson were involved in the collection, preparation and storage of the placentas. J. St-Pierre drafted the

manuscript and did the placental experimental work and the data acquisition, and did the statistical analysis under supervision of G. Elgbeili. J St-Pierre and C. Vaillancourt contributed to the analysis and interpretation of data. All authors have approved the final version of the article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.04.027.

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