Natural disaster-related prenatal maternal stress is associated with alterations in placental glucocorticoid signaling pathway: A QF2011 Queensland Flood Study

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INTRODUCTION

- There is growing evidence that prenatal maternal stress (PNMS) due to a natural disaster impacts on fetal development and child outcomes [1-3].
- Cortisol, a glucocorticoid (GC) is thought to be the main hormone in linking PNMS and adverse development [4].
- The placenta expresses the type 2 11-beta-hydroxysteroid dehydrogenase (11β-HSD2, *HSD11B2* gene) enzyme. This enzyme converts cortisol into inactive cortisone and is known to be reduced by PNMS [5].
- Cortisol exerts its action by binding to glucocorticoid receptor alpha (GR-α, *NR3C1-α* gene) that acts as a transcription factor which regulate the expression of several placental genes, such as type 1 glucose transporter (GLUT1, *SLC2A1* gene) [5,6].
- The cortisol response is inhibited by the inactive GR-β receptor [7].
- Placental sex is a known factor to moderate the effect of stress on fetal development [8].

HYPOTHESIS AND OBJECTIVE

We **hypothesize** that increased PNMS will be associated with a :

- decrease in placental genes associated with reducing glucocorticoid effects (*HSD11B2, NR3C1*-β).
- increase in genes associated with promoting glucocorticoid effects (*HSD11B1, NR3C1-α, CRH*).
- decrease in GLUT1 (*SLC2A1*) and increase in *SLC2A3* and *SLC2A4*

The specific **objective** is to determine if the placenta mediates the effects of disaster-related PNMS on children's early development.

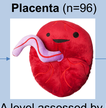
METHOD

Cohort : QF2011: The effects of the Queensland Flood (Australia) on pregnant women, their pregnancies, and their children's early development.

Mother

PNMS from the flood:

- Objective hardship (**QFOSS**)
- Subjective distress (**COSMOSS**)
  - **IES-R:** Impact of Event Scale-Revised
  - **PDI:** Peritraumatic Dissociative Experiences Questionnaire
  - **PDEQ:** Peritraumatic Dissociative Experiences Questionnaire



Placenta (n=96)

- mRNA level assessed by **RT-qPCR**
- 11β-HSD2 and GLUT1 proteins evaluated by **Western blot**
- 11β-HSD2 activity evaluated by **radioenzymatic conversion**

Placental mRNA level evaluated

GC promoting	GC inhibiting	Glucose transporters
CRH (CRH)	*HSD11B2* (11β-HSD2)	*SLC2A1* (GLUT1)
NR3C1-α (GR-α)	*NR3C1-β* (GR-β)	*SLC2A3* (GLUT3)
HSD11B1 (11β-HSD1)		*SLC2A4* (GLUT4)

Children

- Bayley mental (N=48)
- Bayley motor (N=47)
- Autism spectrum rating scale (ASRS) (N=57)

- Statistical models used were Student's T test, Pearson's product moment correlation, hierarchical multiple regression, moderation and mediation.
- Were included in the regression model if significant:
  - Depression (**EDPS:** edinburgh postnatal depression scale)
  - Anxiety (**STAI:** State-Trait Anxiety Inventory)
  - Socio-economic status (**SEIFA:** Socio-Economic Indexes for Areas)
- **Fetal sex** and **timing of the flood** were tested as moderators
- Mediation was used to link PNMS, placental biomarkers and child development
- Statistical analysis were performed using SPSS Statistics software (IBM) where p<0.10 was considered marginally significant and p<0.05 statistically significant

RESULTS

Table 1: Descriptive statistics for stress, anxiety, depression, maternal factors and child outcome measures by child sex (Student's T test, * p<0.05).

	All Mean (n, SD)	Boys Mean (n, SD)	Girls Mean (n, SD)	Sig.
Predictor variables				
QFOSS	17.27 (96, 15.09)	16.45 (51, 15.51)	18.20 (45, 14.72)	0.574
COSMOSS	-0.16 (96, 0.85)	-0.08 (51, 1.02)	-0.25 (45, 0.59)	0.324
IES-R	5.34 (96, 9.28)	6.30 (51, 10.86)	4.25 (45, 7.07)	0.282
PDI	10.67 (96, 7.91)	11.50 (51, 9.03)	9.72 (45, 6.38)	0.273
PDEQ	4.64 (96, 5.92)	4.85 (51, 7.18)	4.41 (45, 4.10)	0.722
Covariates				
STAI	36.45 (96, 9.06)	38.26 (51, 8.45)	34.40 (45, 9.37)	**0.036***
EPDS	4.98 (83, 3.95)	5.07 (44, 3.55)	4.87 (39, 4.41)	0.823
SEIFA	1050.01 (96, 60.56)	1043.43 (51, 67.95)	1057.47 (45, 50.65)	0.259
Days of pregnancy before the flood	82.30 (96, 48.26)	78.34 (51, 46.58)	86.79 (45, 50.25)	0.395
Outcome variables				
Bayley Mental	106.67 (48, 10.33)	108.15 (27, 10.30)	104.76 (21, 10.31)	0.264
Bayley Motor	105.98 (47, 15.22)	104.15 (26, 19.44)	108.24 (21, 7.13)	0.366
ASRS	14.95 (57, 6.95)	13.72 (29, 6.53)	16.21 (28, 7.25)	0.179
Gestation length (weeks)	39.45 (96, 5.36)	39.37 (51, 1.25)	39.53 (45, 1.12)	0.510
Birth weight (Kg)	3.60 (96, 0.40)	3.62 (51, 0.43)	3.58 (45, 0.38)	0.635
Birth weight for gestational age	0.33 (96, 0.73)	0.26 (51, 0.78)	0.40 (45, 0.67)	0.335
Placental weight (kg)	0.65 (93, 0.12)	0.65 (50, 0.12)	0.65 (43, 0.13)	0.889
Placental index	0.18 (93, 0.03)	0.18 (50, 0.03)	0.18 (43, 0.03)	0.973
Mothers' characteristics				
Previous pregnancies	0.74 (94, 0.97)	0.67 (51, 0.88)	0.84 (43, 1.07)	0.400
BMI	24.54 (96, 5.08)	24.57 (51, 4.46)	24.50 (44, 5.77)	0.943
Age at birth	30.98 (96, 5.36)	30.84 (51, 5.11)	31.15 (45, 5.69)	0.779

Table legend:
QFOSS: Queensland flood objective stress score; **COSMOSS**: Composite score of the mother's subjective stress; **EDPS**: edinburgh postnatal depression scale; **STAI**: State-Trait Anxiety Inventory **SEIFA**: Socio-Economic Indexes for Areas; ***CRH***: Corticotropin-releasing hormone; ***NR3C1-α***, ***-β***: Nuclear Receptor Subfamily 3 Group C Member 1-α, -β; ***HSD11B1***: Hydroxysteroid 11-Beta dehydrogenase type 1; **11β-HSD2** and ***HSD11B2***: Hydroxysteroid 11-Beta dehydrogenase type 2; **GLUT1** and ***SLC2A1***: glucose transporter type 1; : Solute Carrier Family 2 type 1; ***SLC2A3***: Solute Carrier Family 2 type 3; ***SLC2A4***: Solute Carrier Family 2 type 4.

[a] Adjusted for Sex and Timing. [b] Adjusted for Sex, Timing, and QFOSS; [c] Adjusted for Timing; [d] Adjusted for Timing and QFOSS; [e] Also adjusted for SES; [f] Also adjusted for maternal anxiety; [g] Also adjusted for maternal depression.

Underline ***p* < 0.10; * *p* < 0.05; ** P<0.01.**

RESULTS

Table 2: 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 all placentas, and in placentas for boys and girls. Underline: p<0.10; * p<0.05; **p<0.01

		GC promoting			GC inhibiting			Glucose transporters		
		CRH	NR3C1-α	HSD11B1	HSD11B2	NR3C1-β	NR3C1	SLC2A1	SLC2A3	SLC2A4
QFOSS	All	0.007	-0.050	0.019	0.078	-0.065	-0.155	**-0.185**	0.068	-0.017
	Boys	-0.027	-0.036	-0.027	0.074	0.004	-0.176	-0.076	0.097	-0.061
	Girls	0.010	-0.066	0.071	0.087	-0.137	-0.131	**-0.304***	0.056	0.008
COSMOSS	All	-0.152	-0.091	-0.045	0.052	**-0.232***	-0.149	-0.074	0.103	0.161
	Boys	-0.166	-0.124	-0.098	0.018	**-0.257**	-0.167	-0.007	0.117	0.159
	Girls	-0.113	-0.026	0.076	0.112	-0.235	-0.131	**-0.254**	0.053	0.231
Timing	All	0.163	**0.173**	**0.221***	-0.110	-0.025	0.001	0.072	-0.088	-0.049
	Boys	0.184	0.028	0.116	-0.187	-0.167	-0.138	0.034	0.053	-0.035
	Girls	0.121	**.366***	**0.341***	-0.036	0.172	0.145	0.134	-0.224	-0.087
Fetal sex	All	**0.176**	-0.024	0.031	-0.023	-0.117	-0.003	-0.099	-0.107	0.130
Seifa	All	**0.195**	0.164	0.088	0.037	-0.021	-0.038	0.016	0.038	0.062
	Boys	0.151	0.06	-0.075	-0.037	0.003	-0.122	0.005	0.089	0.066
	Girls	0.224	**.366***	**.357***	0.144	-0.028	0.086	0.066	-0.007	0.023
STAI	All	-0.088	-0.064	-0.016	0.099	-0.111	-0.128	-0.167	0.038	0.108
	Boys	0.055	-0.068	-0.169	0.051	-0.039	-0.110	-0.168	0.011	0.110
	Girls	-0.144	-0.077	0.172	0.137	**-0.264**	-0.153	-0.223	0.021	0.170
EPDS	All	-0.156	-0.154	-0.139	0.010	0.006	-0.137	-0.071	0.085	0.110
	Boys	-0.100	-0.196	-0.212	-0.095	-0.223	-0.207	0.058	0.202	0.226
	Girls	-0.193	-0.116	-0.067	0.086	0.244	-0.079	-0.197	-0.019	0.024

Table 3: Standardized coefficients (adjusted for covariates) from hierarchical multiple linear regression of stress measures effect on placental glucocorticoid system and glucose transporters. Underline: p<0.10; * p<0.05; **p<0.01

mRNA	All		Boy		Girl	
	QFOSS[a]	COSMOSS[b]	QFOSS[c]	COSMOSS[d]	QFOSS[c]	COSMOSS[d]
CRH	.025	-.159	.035	-.159	.022	-.202
NR3C1-α	-.012	-.062	-.030	-.159	-.134[e]	-.037[e]
HSD11B1	.066	-.025	.011	-.082	-.101[e,f]	-.107[e,f]
HSD11B2	.060	-.012	.017	-.111	.085	.097
NR3C1-β	-.062	**-.323****	-.054	**-.491****	-.125[g]	-.286[g]
NR3C1	-.163	-.104	-.244	-.205	-.118	-.099
SLC2A1	-.169	.028	-.072	.058	**-.293**	-.126
SLC2A3	.064	.060	.127	.142	.033	.058
SLC2A4	-.043	**.266***	-.081	.281	-.001	**.366**
Protein						
11β-HSD2	-.071	-.119	-.113	-.179	.064[e]	-.040[e]
GLUT1	.056	-.025	.071	.068	.003[g]	-.305[g]
Activity						
11β-HSD2	-.115	.126	-.140	.168	-.053[g]	.194[g]

Table 4: Significant hierarchical multiple linear regression results of prenatal stress effects on placental mRNA level of genes tested for either all placentas or for boy placentas only. Underline: p<0.10; * p<0.05; **p<0.01

Predictor variables	*B*	*Std. Error*	*β*	*R*	*R²*	*ΔR²*	*F*	*ΔF*
NR3C1- β All								
Step 1				.118	.014	.014	.659	.659
Sex	-.076	.068	-.116					
Timing	.000	.001	-.015					
Step 2				.133	.018	.004	.548	.336
Sex	-.072	.069	-.110					
Timing	.000	.001	-.028					
QFOSS	-.027	.046	-.062					
Step 3				.296	.088	**.070****	**2.192**	**7.013****
Sex	-.100	.067	-.152					
Timing	-.001	.001	-.078					
QFOSS	.045	.052	.103					
COSMOSS	-.126	.048	**-.323****					
NR3C1-β Boys								
Step 1				.167	.028	.028	1.398	1.398
Timing	-.001	.001	-.167					
Step 2				.174	.030	.003	.751	.129
Timing	-.001	.001	-.184					
QFOSS	-.025	.068	-.054					
Step 3				.426	.182	**.151****	**3.476***	**8.685****
Timing	-.002	.001	**-.327***					
QFOSS	.077	.072	.168					
COSMOSS	-.169	.058	**-.491****					
SLC2A4 All								
Step 1				.143	.020	.020	.969	.969
Timing	-.000	.001	-.060					
Sex	.076	.058	.135					
Step 2				.149	.022	.002	.695	.166
Timing	.000	.001	-.070					
Sex	.078	.058	.139					
QFOSS	-.016	.039	-.043					
Step 3				.264	.070	**.048***	1.708	**4.662***
Timing	.000	.001	-.028					
Sex	.098	.058	**.174**					
QFOSS	-.066	.045	-.179					
COSMOSS	.088	.041	**.266***					

Table 5: Interaction effect (R2 change) of the timing of the stressful event on the effect of stress on placental glucocorticoid system and glucose transporters separated by fetal sex or taken together. Underline p<0.10; * p<0.05; **p<0.01

Gene	Interaction timing					
	All		Boys		Girls	
	QFOSS[a]	COSMOSS[b]	QFOSS[c]	COSMOSS[d]	QFOSS[c]	COSMOSS[d]
CRH	.001	.020	.027	.000	.023	**.070**
NR3C1-α	.000	**.035**	.007	**.082***	.012[e]	.002[e]
HSD11B1	.006	.024	.007	**.080***	.008[e,f]	.000[e,f]
HSD11B2	.005	.003	.000	.028	.018	.025
NR3C1-β	.001	.000	.001	.000	.021[g]	.009[g]
NR3C1	.005	.015	.007	.016	**.063**	.032
SLC2A1	.002	.022	.006	.006	.030	.033
SLC2A3	.011	.010	.014	.018	.021	.010
SLC2A4	.001	**.035**	.011	.012	.001	.053
Protein						
11β-HSD2	.013	.006	.035	.005	.000[e]	.000[e]
GLUT1	.001	.014	.015	.001	.001[g]	.048[g]
Activity						
11β-HSD2	.005	.000	.023	.005	.025[g]	.031[g]

Table 6: Significant hierarchical multiple linear regression results of prenatal stress and timing interaction effects on placental mRNA level of genes tested for boy placentas only. * p<0.05; **p<0.01

Predictor variables	*B*	*Std. Error*	*β*	*R*	*R²*	*ΔR²*	*F*	*ΔF*
NR3C1-α Boys								
Step 1				.028	.001	.001	.039	.039
Timing	.000	.001	.028					
Step 2				.040	.002	.001	.039	.039
Timing	.000	.001	.018					
QFOSS	-.016	.078	-.030					
Step 3				.132	.017	.016	.277	.755
Timing	.000	.001	-.028					
QFOSS	.021	.089	.041					
COSMOSS	-.062	.071	-.159					
Step 4				.316	.100	**.082***	1.274	**4.208***
Timing	-.001	.001	-.113					
QFOSS	.042	.087	.083					
COSMOSS	.077	.096	.198					
COSMOSS X Timing	-.003	.002	**-.513***					
HSD11B1 Boys								
Step 1				.116	.014	.014	.674	.674
Timing	.001	.002	.116					
Step 2				.117	.014	.000	.333	.005
Timing	.001	.002	.120					
QFOSS	.007	.101	.011					
Step 3				.134	.018	.004	.286	.203
Timing	.001	.002	.096					
QFOSS	.032	.115	.048					
COSMOSS	-.041	.092	-.082					
Step 4				.313	.098	**.080***	1.248	**4.078***
Timing	.000	.002	.013					
QFOSS	.059	.113	.089					
COSMOSS	.135	.125	.269					
COSMOSS X Timing	-.004	.002	**-.505***					

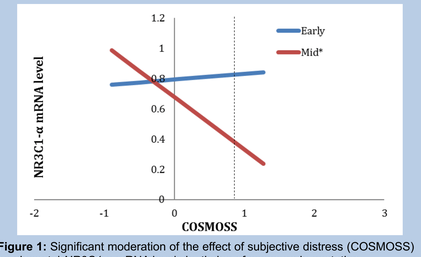


Figure 1: Significant moderation of the effect of subjective distress (COSMOSS) on placental *NR3C1-α* mRNA levels by timing of exposure in gestation.

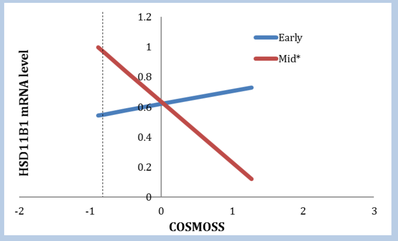


Figure 2: Significant moderation of the effect of subjective distress (COSMOSS) on placental HSD11B1 mRNA levels by timing of exposure in gestation.

Figure legend: Vertical lines represent the statistical threshold of the interaction effect (dashed: P < 0.10). Early (represented at 2 weeks of pregnancy) and mid (represented at 20 weeks of pregnancy) gestation. * P < 0.05 significant conditional effect of COSMOSS on placental mRNA level at mid-gestation exposed level.

Table 7: Standardized coefficients from hierarchical multiple linear regression of subjective stress measures effect on placental NR3C1-β, NR3Cl –α and SLC2A4 either for all or for boy placentas only following significant COSMOSS effect. Underline: p<0.10; * p<0.05; **p<0.01

Predictor	*NR3C1-β* all	*NR3C1-β* boys	*SLC2A4* all
IES-R	**-.336****	**-.363***	**.350****
PDI	-.096	**-.305**	**.254***
PDEQ	-.166	**-.303**	**.215**

Table 8: Interaction effect (R^2 change) of the timing of the stressful event on the effect of stress on NR3C1 –α and HSD11B1 for boy placentas. Underline: p<0.10; * p<0.05; **p<0.01

Predictor	*NR3C1-α* boys	*HSD11B1* boys
IES-R	**.142****	**.144****
PDI	.018	.045
PDEQ	.038	**.075**

DISCUSSION AND CONCLUSION

- PNMS from a natural disaster is linked with:
  - a reduction in placental *NR3C1-β* mRNA, especially for boys, but not *HSD11B2,* suggesting an increase in sensitivity to cortisol by the reduction in GR-β
  - a reduction in *SLC2A1* mRNA in girls placentas and an increase in placental *SLC2A4* mRNA, suggestion a shift towards insulin-sensitive glucose transport in the placenta
- Timing of the flood has a significant moderating effect on placental *NR3C1-α* and *HSD11B1* mRNA
  - *NR3C1-α:* marginally significant difference between early and mid gestation for high subjective distress
  - *HSD11B1*: marginally significant difference between early and mid gestation for low subjective distress
- No significant:
  - interaction of fetal sex on the effect of PNMS on placental mRNA level
  - moderation link between PNMS, placental biomarkers and children assessment tested
- This is the first study to show the effect of PNMS originating from a natural disaster on placental mRNA level of genes implicated in glucocorticoid response and glucose transport suggesting placental adaptation to PNMS.
- Further studies are needed to determine if these alterations in placental biomarkers could be linked to programming effect in the children

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