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**Associations between maternal prenatal cortisol and fetal growth are specific to
infant sex: Findings from the Wirral Child Health and Development Study**

Short Title: Prenatal cortisol and infant birth weight

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Abstract

Recent findings highlight that there are prenatal risks for affective disorders that are mediated by glucocorticoid mechanisms, and may be specific to females. There is also evidence of sex differences in prenatal programming mechanisms and developmental psychopathology, whereby effects are in opposite directions in males and females. As birth weight is a risk for affective disorders, we sought to investigate whether maternal prenatal cortisol may have sex-specific effects on fetal growth. Participants were 241 mothers selected from the Wirral Child Health and Development Study (WCHADS) cohort (n=1233) using a psychosocial risk stratifier, so that responses could be weighted back to the general population. Mothers provided saliva samples, which were assayed for cortisol, at home over two days at 32 weeks gestation (on waking, 30-minutes post-waking, and during the evening). Measures of infant birth weight (corrected for gestational age) were taken from hospital records. General population estimates of associations between variables were obtained using inverse probability weights. Maternal LogAUC cortisol predicted infant birth weight in a sex-dependent manner (interaction term $p=0.029$). There was a positive and statistically significant association between prenatal cortisol in males, and a negative association in females that was not statistically significant. A sex-interaction in the same direction was evident when using the waking ($p=0.015$), and 30-minute post waking ($p=0.013$) cortisol, but not the evening measure. There was no interaction between prenatal cortisol and sex to predict gestational age. Our findings add to an emerging literature that suggests that there may be sex-specific mechanisms that underpin fetal programming.

Key words: fetal programming; prenatal stress; glucocorticoids; HPA axis; birth weight

Introduction

According to the developmental origins of health and disease (DOHaD) hypothesis, low birth weight (LBW) reflects poor intrauterine nutrition, which leads to fetal changes that are adaptive in subsequent environments of food scarcity. In environments where food is plentiful, however, LBW individuals are susceptible to obesity, diabetes, and cardiovascular disease.^{1,2} There is also a large body of evidence that the DOHaD hypothesis has implications for understanding the early origins of mental health disorders.³ Poor fetal growth, which is commonly measured as birth weight, gestational age, or birth weight corrected for gestational age, is associated with increased risk for a range of later psychopathological disorders, including; ADHD^{4,5} psychosis,⁶ and substance disorders.⁷ Such reports are from observational studies, and therefore it is difficult to draw conclusions regarding mechanisms and causality. For example, low fetal growth may not directly mediate associations with later outcomes, but could instead be a marker of another pathway that mediates the association, such as glucocorticoid mechanisms. Alternatively, LBW could directly reflect the risk, for example differences in neuronal maturation and connections.

The possibility of a link between low birth weight and later depression has been extensively investigated. Several studies have demonstrated a relationship between low birth weight and increased rates of depression⁸⁻¹⁰ however there have also been reports of no association.¹¹⁻¹³ A meta-analysis conducted in 2013 demonstrated a weak relationship between LBW and depression, but effects diminished when publication bias was controlled for.¹⁴ One possible explanation is differential effects by sex. For example, two large population cohorts have demonstrated significant interactions between the sex of the child and LBW in the prediction of adolescent depression; in each case LBW was associated with greater depressive symptoms in females only.^{15,16} These findings compliment an emerging

body of literature, which suggests that, more broadly, prenatal risks for offspring psychopathology may be sex-dependent, and that risks for affective outcomes are specific to females. For example, exposure to maternal prenatal anxiety is associated with a flattened diurnal cortisol profile and depressive symptoms in female adolescents,¹⁷ and maternal prenatal depression has been shown to specifically predict adolescent depression in females in a very large population cohort.¹⁸ Similarly, exposure to high levels of maternal prenatal cortisol is associated with a more fearful temperament in childhood,¹⁹ increased amygdala volume, and affective problems²⁰ in females only.

While these studies report effects in females but not in males, sex dependent effects may also arise from effects in males in the opposite direction from those in females. We have previously reported that LBW predicted increased vagal reactivity at 29 weeks in girls but decreased vagal reactivity in boys,²¹ and that elevated vagal reactivity predicted increasing oppositional defiant disorder (ODD) symptoms up to age 5 years in girls, but decreasing in boys.²² ODD symptoms in children predict later depression in girls but later conduct problems in boys,^{23,24} and so these associations may reflect different autonomic mechanisms linking LBW to different adolescent outcomes in males and females. Effects of LBW may reflect glucocorticoid mechanisms. In animal studies both prenatal stress and low birth weight are associated with elevated fetal exposure to maternal glucocorticoids. Consequently, glucocorticoid receptor expression in the hippocampus and hypothalamus is decreased, which results in impaired feedback regulation of the HPA axis.²⁵ We have recently reported opposite effects in females and males of maternal cortisol in pregnancy on infant temperament in two independent samples. High cortisol was associated with increased negative emotionality in female infants, and decreased negative emotionality in male infants.^{26,27} Consistent with these findings from the Wirral Child Health and Development

Study (WCHADS) cohort of opposite effects of autonomic and glucocorticoid functioning in males and females, associations between increasing vagal reactivity or cortisol with increasing behavioral symptoms in girls, but with decreasing in boys, have been reported in several publications.^{22,28-32}

In light of the evidence reviewed here we sought to investigate whether elevated prenatal cortisol may have sex-specific effects on fetal growth. We examined two predictions, first that increased maternal cortisol would be associated with decreased birth weight in females with no effect in males, and the second that there would be associations between maternal cortisol and birth weight in opposite directions in males and females. In order to index *in utero* growth we operationalized fetal growth as birth weight controlled for gestational age. We also tested for effects of prenatal cortisol on gestational age, as birth weight and gestational age may reflect different underlying mechanisms with independent effects on specific mental health outcomes.³ To our knowledge there are no existing reports of associations between maternal prenatal cortisol and infant birth weight from population samples.

Methods*Design*

Participants were members of the Wirral Child Health and Development Study (WCHADS), a prospective epidemiological longitudinal study of first time mothers starting in pregnancy with multiple follow-up assessments after birth. The Wirral is a geographic peninsular, bounded on three sides by water, located in the North West of England. For some phases requiring more detailed and expensive measurement, data collection was restricted to a randomly drawn sub-sample stratified by risk based on reports of partner psychological abuse. The stratified design allows general population estimates of means and associations to be derived for measures from all phases. Recruitment of the cohort has been described in detail previously.³³ Approval for the procedures was obtained from the Cheshire North and West Research Ethics Committee (UK).

Sample

The WCHADS cohort consists of 1233 first-time mothers with a mean age of 26.8 years (SD=8.5, range=18-51) at recruitment. Using the revised English Index of Multiple Deprivation (IMD),³⁴ based on data collected from the UK census in 2001, 41.8% fell in the most deprived UK quintile, consistent with high levels of deprivation in some parts of the Wirral. Only 48 women (3.9%) described themselves as other than White British. The measures used in this report were obtained for the whole cohort from questionnaires at 20 weeks gestation, and administrative records at birth. The stratified sub-sample of mothers (N=316) provided interviews at 32 weeks gestation (mean=32.1. SD=2.0). Diurnal saliva samples were also collected at 32 weeks gestation, and were available for 241 participants.

Measures

Maternal cortisol. At 32 weeks gestation, mothers collected saliva samples at home over two consecutive working days. Saliva was collected on waking, 30 min post-waking, and during the evening (approx. 12 h after waking (mean=12h 10min, SD=1h 15min)). Participants stored the samples in their freezer until a research assistant collected them 1-2 weeks later. Samples were stored at -20°C before transportation to Imperial College London on dry ice for analysis. All samples were assayed for salivary cortisol using a commercially available immunoassay (Salimetrics, UK). Inter- and intra-assay variation were 7.9% and 8.9% respectively. Salivary assays were run in duplicate except for a small minority of cases with minimal volume (n=3). In this study we used the log of the area under the curve (LogAUC) as an index of diurnal cortisol release. The area under the curve was calculated using the trapezoid method with respect to ground, taking the mean (over 2 days) of the awakening, 30 minutes and 12 hour post-awakening measures.

A full set of maternal saliva samples over two days was available for 231 participants. Ten participants provided saliva samples over one day, and their raw scores were used in analyses instead of a mean score. Cortisol measured across the two days was correlated at the three time-points (waking cortisol $r=0.485$, $p<0.001$, 30-min post-waking $r=0.473$, $p<0.001$, 12-h post-waking $r=0.157$, $p=0.02$). A full set of saliva samples collected over at least one day were not available for the remaining 75 participants of the intensive sample. Participants of the intensive sample who did and did not provide saliva samples did not differ on any demographic measures (all p 's >0.05 , data not shown).

Infant birth weight and gestational age. Infant birth weight and gestational age measures were obtained from hospital records. In this study we used a birth weight variable that was

corrected for gestational age, which was calculated by dividing birth weight (g) by gestational age (weeks).³⁵

Stratification factor. Maternal responses to questions about psychological abuse in their current or recent partner relationship³⁶ were used to generate the stratified intensive sample of mothers for more detailed study. The stratification variable was chosen for its known association with a variety of risk factors for early child development. This variable was effective in selecting two groups that differed on levels of maternal prenatal anxiety; the mean State Anxiety scores³⁷ in the low- v. high-risk strata were 31.5 (S.D.=8.45) v. 35.5 (S.D.=11.17) (Cohen's $d=0.40$, $p=0.001$), for comparison of transformed scores. Partner psychological abuse was assessed using a 20 binary-item questionnaire covering humiliating, demeaning or threatening utterances in the partner relationship during pregnancy and over the previous year,³⁶ and has been described previously.³⁸ Participants first rated these items about their own behavior towards their partner, and then about their partner's behavior towards them. The stratification was based upon the highest scores of the partner to participant or participant to partner scores for each family, and this variable was used to stratify the sample and create the population weights for analysis.

Statistical analysis

The two-phase stratified sample design allows estimates to be reported for the general population by applying weights. Inverse probability weights were constructed that took account of the sample design stratification factor and variables associated with response and attrition: maternal age, depression, and smoking in pregnancy, years of education, marital status and the deprivation index for the mother's home neighborhood. The analysis method compensates for differential selection and response, the stratified sampling and the

weighting working as a pair, to give population estimates. Test statistics and confidence intervals for weighted means, and regression estimates, were based on survey adjusted Wald tests (t-tests if single degrees of freedom (df) or F-tests if multiple df) using the robust 'sandwich' estimator of the parameter covariance matrix.³⁹ Models used for inference analyzed the birth weight or gestational age variables using linear regression. Maternal prenatal cortisol, infant sex, and a prenatal cortisol*infant sex interaction were used as main predictors in analyses. Interaction terms were created using standardized, centered variables. Maternal prenatal smoking was included as a confounder in all regression analyses. Smoking status was included as a binary variable, and participants who did smoke reported whether they smoked more or less than 10 cigarettes per day. 65 participants (27%) reported smoking in pregnancy, however of these only 6 women reported smoking 10 or more cigarettes per day, which was too few to examine effects of how much participants smoked on birth weight. We also considered the following maternal variables as confounders in initial analyses: body-mass index, age, education and area deprivation. However, none of these confounders were significant predictors of birth weight (corrected for gestational age), and were therefore removed from the remaining analyses because the continued inclusion of so many non-significant confounders was not considered helpful in a sample of this size. Weights were calculated for each model separately and a form of stabilized weight was used that removed weight variability associated with the conditioning covariates. To assist in interpretation, associations estimated by simple unweighted regressions have been displayed graphically. In previous publications using data from the same cohort and another independent cohort, we reported that maternal LogAUC cortisol and cortisol sampled on waking predicted infant negative emotionality in a sex-dependent manner, however samples taken 30 minutes after waking and during the evening did not.²⁶ In the current study we examined whether such time of day effects were also evident in

relation to birth weight by testing the prediction from maternal cortisol sampled at the three time points to birth weight and gestational age, in interaction with infant sex. Analyses were undertaken in STATA 14.

Results

Demographics

Means and percentages split by sex for the birth weight, gestational age, maternal cortisol and confounding variables for the intensive sample with maternal cortisol data are shown in **Table 1**. None of the variables differed significantly by infant sex (all p -values > 0.05).

Prenatal cortisol and birth weight

The results of the weighted regression model used to predict the birth weight variable, corrected for gestational age, are summarized in **Table 2**. In regression model 1, there was no significant effect of infant sex on birth weight (Beta=-0.121, $p=0.093$), and maternal log AUC cortisol did not individually predict infant birth weight (Beta=0.026, $p=0.707$). However, there was a significant prediction from the maternal cortisol interaction with infant sex (Beta=-0.151, $p=0.029$). Model 2 shows the results of the analyses for males and females separately. For males, maternal prenatal cortisol significantly predicted birth weight, and the association was positive (Beta=0.167, $p=0.036$), indicating that increased cortisol was associated with increased birth weight. In females there was a negative association between prenatal cortisol and birth weight, however this did not reach statistical significance.

In the current study we also tested whether time-of-day effects were evident in the prediction of infant birth weight, and these results are summarized in **Table 3**. The maternal cortisol measures taken on awakening and 30 minutes after awakening predicted infant birth weight in interaction with sex (Beta=-0.150, $p=0.015$ and Beta=-0.172, $p=0.036$ respectively). When considered individually, both maternal cortisol on waking and cortisol sampled 30 minutes after awakening significantly predicted male birth weight, and the

associations were positive (Beta=0.206, p=0.030 and Beta=0.214, p=0.021 respectively), indicating higher cortisol values were associated with higher birth weights. In females, the predictions to birth weight from maternal awakening and 30 minutes post-awakening measures were in a negative direction, indicating higher cortisol was associated with reduced birth weight, but did not reach statistical significance. Maternal cortisol taken during the evening did not predict birth weight in interaction with infant sex.

Prenatal cortisol and gestational age

We also tested whether maternal LogAUC cortisol predicted gestational age in interaction with infant sex, and these results are summarized in **Table 4**. In this analysis, neither maternal prenatal cortisol, infant sex, nor an interaction between LogAUC cortisol and infant sex predicted gestational age. Similarly, analyses using maternal cortisol measures taken on awakening, 30 minutes post awakening and during the evening did not predict gestational age, either individually or in interaction with infant sex (data not shown).

We tested for sensitivity by re-analyzing our data with the 10 participants who only provided saliva samples over one day removed. However, this did not change the results of the study (data not shown).

Discussion

We conducted analysis of a longitudinal cohort, stratified by risk, to examine the prospective relationship between maternal cortisol sampled during the third trimester of pregnancy and fetal growth, operationalized as birth weight corrected for gestational age. There was a sex-dependent effect of maternal cortisol on infant birth weight. This sex-dependent effect was evident when using each of the morning cortisol samples in the analysis, and when using the log of the area under the curve as an index of diurnal cortisol release. The interaction arose from a statistically significant positive association between maternal prenatal cortisol and birth weight in boys, and a negative association that did not reach statistical significance in girls.

This finding can be interpreted in two ways. First, if the consequences of low birth weight for later development are the same for girls and boys, with lower birth weight associated with greater risk, then elevated maternal cortisol confers vulnerability in girls but resilience in boys. This would imply that sex differences in outcomes associated with prenatal stress arise from the combination of risk effects in females and protective effects in males. Indeed, prenatal risks associated with depressive and internalizing symptoms are often reported for females but not males, as outlined in the introduction. The second possibility is that the opposite effects on birth weight represent increased risk both in girls and in boys. This would be the case if later outcomes associated with birth weight were sex-dependent. Previous findings from WCHADS are consistent with this possibility. Low birth weight for gestational age, the indicator of sub-optimal in utero growth used also in this study, predicted increased infant vagal reactivity at 29 weeks of age in girls²¹ which in turn predicted increased oppositional defiant (ODD) symptoms in girls.²² Alternatively, LBW predicted reduced vagal reactivity²¹ and reduced ODD symptoms²² in boys. ODD symptoms

in children predict later depression in girls but later conduct problems in boys,^{23,24} and so these associations may reflect different autonomic mechanisms linking low birth weight to different adolescent outcomes in males and females. There have also been reports from several other cohorts of opposite effects of autonomic and glucocorticoid functioning representing risk in males and females. For example, increased vagal reactivity has been associated with increased externalizing and internalizing symptoms in females, whereas this association was true for boys with decreased vagal reactivity.²⁸ Whether variations in vagal and cortisol reactivity directly influence child behaviors or are a marker for other influences remains to be established.

Inconsistencies in the available evidence mean that it is not yet clear which of these possible explanations is more likely. On the one hand, there is a literature linking low birth weight with a variety of psychopathological outcomes, as outlined in the introduction. There is also evidence linking poor fetal growth with reduced cognitive functioning⁴⁰ and behavioral problems,^{41,42} in particular inattention/hyperactivity.^{4,43} On the other hand, associations between poor fetal growth and cognitive/attention difficulties may not be as simple as previously thought. A U-shaped relationship between fetal growth and cognitive function,⁴⁴ intellectual disability,⁴⁵ attention problems⁴⁶ and ADHD⁵ has been demonstrated, highlighting that both reduced and increased fetal growth may represent risks for poor cognitive and attention outcomes. There is also evidence to suggest, the U-shaped relationship is specific to outcomes predicted by fetal growth (i.e. birth weight corrected for gestational age), and is also specific to male infants.⁵ This highlights that an increased rate of inter-uterine growth is a risk for cognitive difficulties independent of gestational length, and also highlights potential sex differences in effects of fetal growth on cognitive outcomes. Thus, the accelerated fetal development in males in the presence of high maternal cortisol

demonstrated in the current study may be part of a mechanism leading to poor cognitive and attention outcomes in later life. Very recent findings demonstrate that maternal prenatal cortisol also has sex-specific associations with child neuronal connectivity, whereby females demonstrate an adaptive response via increased neuronal connectivity, but no adaptive response was evident in males.⁴⁷ However, this study was conducted in a small sample of mother-child dyads (N=49), and requires replication.⁴⁷

In the current study we also report an interaction between maternal cortisol measures taken on waking and 30 minutes after waking and infant sex to predict birth weight, and the direction of effects was the same as for the LogAUC cortisol variable. However, there was no interaction effect when the evening cortisol measure was used in the regression analysis. This finding is consistent with reports from the obstetric literature that various indices of morning cortisol more strongly predict obstetric outcomes, such as birth weight, than those taken throughout the day.⁴⁸⁻⁵⁰ It is currently unclear why maternal cortisol sampled in the morning, but not during the evening, may be particularly relevant for fetal growth, but this is an interesting direction for future research.

We report a significant interaction between prenatal cortisol and infant sex to predict birth weight, but not gestational age. This suggests that maternal prenatal cortisol may be particularly relevant for the speed of *in utero* growth, but not duration of *in utero* growth. It has been suggested that birth weight, gestational length and also birth length may represent different underlying mechanisms with different effects on mental health.³ A comprehensive understanding of such biological mechanisms remains to be elucidated, however an emerging animal and human literature provides evidence for sex differences in placental functioning in the context of maternal prenatal stress. The placenta is a highly dynamic

endocrine tissue that produces hormones and nutrients to support fetal development, and also acts as a protective barrier against potential insults. Fetal sex is a key determinant in the functional responses of the placental to maternal perturbations during pregnancy.⁵¹ Sex-differences in placental mechanisms are evident throughout gestation, and therefore could provide a plausible mechanism by which the maternal milieu could have sex-dependent effects on fetal growth and development.^{51,52}

A significant strength of this study is the epidemiological sample recruited during pregnancy with a subsample stratified by psychosocial risk for more detailed assessment. This enabled data from the time consuming and costly measures of maternal prenatal cortisol to be weighted back to the general population. Our measures were assessed prospectively, and included relevant confounding variables. Limitations of the study include that participants self-reported the timing of the cortisol sample collection, which could potentially be inaccurate and introduce error. We also had no information on time spent asleep before the first morning sample, or information on the time of the last meal/drink before each cortisol sample, which could affect the cortisol measurement.^{53,54} While the sample size of 241 was adequate to identify the interaction between sex of fetus and birth weight, and the effects of maternal cortisol in boys, in the subsample of girls the 95% CIs for the smaller effect sizes included zero and so may have arisen by chance. Our findings generalize to the Wirral population, however our sample is a somewhat more ethnically homogeneous and deprived population than the UK as a whole.

To conclude, we report an interaction between maternal prenatal cortisol and infant sex to predict birth weight. This interaction arose from different effects of prenatal cortisol on birth weight in males and females: there was a statistically significant positive association in

males, and a negative association in females that did not reach statistical significance. This finding adds to an emerging body of literature that suggests that there may be sex-specific fetal programming mechanisms in the context of prenatal risks. These results have implications for understanding the early origins of sex differences in developmental psychopathology, which are well established but poorly understood.

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Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (UK) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (Cheshire North and West Research Ethics Committee, UK).

Conflicts of Interest

None

Tables**Table 1** Birth weight, gestational age, maternal cortisol and confounding variables, by infant sex

Variable type	Measure	Males (n=115)			Females (n=126)		
		Mean	SD	%	Mean	SD	%
Dependent	Birth weight by gestational age (g/weeks)	88.10	2.41	-	85.26	10.06	-
	Gestational age (weeks)	39.75	1.36	-	39.96	1.22	-
Independent	Maternal LogAUC cortisol	2.03	0.18	-	2.03	0.15	-
	Maternal waking cortisol (nmol/l)	12.07	5.22	-	12.61	4.59	-
	Maternal 30-min post-waking cortisol (nmol/l)	14.20	5.56	-	14.65	5.69	-
	Maternal evening cortisol (nmol/l)	4.28	2.98	-	3.91	2.28	-
Confounder	Maternal prenatal smoking	-	-	30.17	-	-	23.02

Table 2 Summary of regression models using maternal prenatal LogAUC cortisol and infant sex to predict birth weight corrected for gestational age

	Regression Model 1			Regression Model 2					
	Whole sample (N=241)			Males (n=115)			Females (N=126)		
	Beta	CI's	p	Beta	CI's	p	Beta	CI's	p
Maternal prenatal smoking	-0.166	-7.776 - -0.466	0.027	-0.151	-10.323 - 2.135	0.195	-0.204	-9.583 - 0.187	0.059
Infant sex	-0.121	-2.887 - 0.224	0.093	-	-	-	-	-	-
Maternal LogAUC cortisol	0.026	-7.764 - 11.425	0.707	0.167	0.798 - 23.454	0.036	-0.109	-23.912 - 8.937	0.369
Infant sex X maternal LogAUC cortisol	-0.151	-3.408 - -0.184	0.029	-	-	-	-	-	-

Table 3 Summary of regression models using maternal cortisol sampled at three times of the day, and in interaction with infant sex, to predict birth weight corrected for gestational age

	Whole sample (N=241)				Males (n=115)			Females (N=126)		
Maternal cortisol and infant sex interaction	Beta	CI's	p	Maternal cortisol	Beta	CI's	p	Beta	CI's	p
sex X awakening cortisol	-0.150	-0.614 - -0.067	0.015	awakening cortisol	0.203	-10.326 - 1.981	0.030	-0.065	-9.605 - 0.352	0.508
sex X awakening cortisol + 30minutes	-0.172	-7.395 - -0.189	0.013	awakening cortisol + 30minutes	0.214	-9.445 - 2.270	0.021	-0.111	-0.622 - 0.210	0.329
sex X evening cortisol	-0.057	-0.748 - 0.256	0.335	evening cortisol	-0.06	-9.370 - 2.809	0.358	-0.133	-1.351 - 0.233	0.165

Table 4 Summary of regression model using maternal LogAUC cortisol and infant sex to predict gestational age

	Whole sample (N=241)		
	Beta	CI's	p
Maternal prenatal smoking	0.008	-0.448 - 0.492	0.925
Infant sex	0.114	-0.044 - 0.330	0.133
Maternal LogAUC cortisol	-0.012	-1.137 - 0.954	0.863
Infant sex X maternal LogAUC cortisol	-0.033	-0.218 - 0.128	0.610

Figure legends

Figure 1 Simple, un-weighted regression lines showing association between maternal prenatal LogAUC cortisol and infant birth weight by gestational age for male and female infants

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