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Perinatal hypothalamic-pituitary-adrenal axis regulation among women with eating disorders and their infants

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Abstract

Background: Psychiatric illness is associated with heightened hypothalamic-pituitary-adrenal (HPA) axis activity during pregnancy which may have long term effects on infant stress regulation. HPA axis regulation has not previously been investigated in women with eating disorders (ED) or their infants during the perinatal period.

Methods: Women were recruited to a prospective longitudinal study in three groups: 1) current or active ED (C-ED= 31), 2) past ED (P-ED= 29) and healthy control (HC = 57). Maternal psychopathology, diurnal cortisol levels, corticotropin-releasing hormone (CRH) and CRH binding protein (CRH-BP) were measured during the third trimester of pregnancy. At eight weeks postpartum infant cortisol was obtained before and after routine immunisations to determine infant hormonal response to a stressful situation.

Results: Women with current ED had a significantly lower cortisol decline throughout the day compared to HC, in both adjusted and unadjusted analyses. Lower cortisol decline among women with a current ED were associated with higher levels of psychopathology during pregnancy. Women's cortisol awakening response, CRH and CRH-BP levels did not differ across the three groups. Infants' stress response was also significantly higher among those in the C-ED group, although this effect was attenuated after controlling for confounders.

Conclusions: During pregnancy women with ED have lower cortisol declines, suggestive of blunted diurnal cortisol rhythms. Postnatally, their infants also have a heightened response to stress. This is the first study to identify HPA axis dysfunction in pregnancy in women with ED, and to show an intergenerational effect. Since dysfunctions in HPA activity during childhood may represent a risk factor for psychological and physical health problems later in life, further investigation of the potential long-term implications of these findings is crucial.

Key words: Perinatal, eating disorders, stress, pregnancy, hypothalamic-pituitary-adrenal axis, cortisol, infant

1. Introduction

Maternal psychiatric disorders commonly occur during pregnancy and in the postnatal period The implications are not limited to the mother, and perinatal mental illness (PNMI) can have long-term consequences for child development (Stein et al, 2014). During early pregnancy, 7.5% of women have been reported to have an eating disorder (ED) (Easter et al., 2013), and, despite some reductions women with ED continue to experience high levels of psychopathology throughout the perinatal period (Easter et al., 2014). Maternal ED have been associated with an increased risk of pregnancy and obstetric complications, in particular increased risk of miscarriage, intrauterine growth restriction and low birth weight (Micali et al., 2007; Solmi et al., 2013). There is also evidence of increased levels of emotional, conduct and hyperactivity disorders among children of women with ED (Micali et al., 2014). Nevertheless, ED have received substantially less research attention compared to other PNMI.

The exact mechanisms for associations between maternal psychiatric illness and childhood psychological problems are currently unknown, but are likely to involve a combination of psychological, social and biological factors (Goodman and Gotlib, 1999). The role of fetal programming (Godfrey and Barker, 2001), (i.e. the effect of the *in utero* environment on health and development across the lifespan) as a potential mechanism has become the focus of a great deal of research. There is a growing body of literature indicating that maternal mood and anxiety can affect the intrauterine environment, increasing the risk of obstetric complications, and have enduring effects on the psychological development of the offspring (O'Connor et al., 2002; Stein et al., 2014).

The Hypothalamic Pituitary Adrenal (HPA) axis is a major biological system involved in modulating stress and increasing research suggests that fetal exposure to excess glucocorticoids represents a critical mechanism for fetal programming (Meaney et al., 2007; Sandman et al., 2012). Under normal circumstances the HPA axis has a diurnal pattern,

characterised by high levels of stress hormones in the morning, which reach a nadir in the evening. The HPA axis is programmed to respond rapidly to stressful situations and return to homeostasis once the fear of threat has passed.

Psychiatric illness appears to shape the physiology of the stress system and different psychopathology is associated with different physiological profiles. For example, whilst elevated cortisol levels have been associated with most forms of depression (Stetler and Miller, 2011), low cortisol levels are associated with post-traumatic stress disorder (Yehuda and Seckl, 2011) and have also been reported in atypical depression (Stetker and Miller, 2011).

ED have also been associated with abnormalities of the HPA axis (Ginty et al., 2012). Although findings are somewhat mixed, plasma cortisol and corticotropin-releasing hormone (CRH) levels have both been shown to be elevated in women with anorexia nervosa (AN) (Boyar et al., 1977; Hotta et al., 1986; Kaye et al., 1987; Favaro et al., 2008) and bulimia nervosa (BN) (Monteleone et al., 2001).

Recent studies have indicated that that different ED subtypes might be characterised by different abnormalities of the HPA axis and dependent on the patient's stage of recovery. Cortisol Awakening Response (CAR) has been found to be significantly elevated among adult with AN (Monteleone et al., 2014, 2015), specifically during the acute phase of the illness (Monteleone et al., 2016); whereas, studies of BN have shown more contrasting findings, with evidence of both normal and increased circadian rhythm (Lo Sauro et al., 2008).

Patients with bulimic symptoms have also shown greater cortisol suppression to the dexamethasone suppression test (DST), a frequently used test to assess adrenal gland function, compared to both controls and patients with restrictive AN (Díaz-Marsá et al, 2008). Therefore, unlike patients with AN who have been found more consistently to display hyper-reactivity, the activity in the HPA axis in BN is more varied.

Increased cortisol feedback inhibition has previously been associated with post-traumatic stress (Yehuda , 2004), since trauma history is common among patients with ED it has been postulated that hypersensitive DST maybe related to trauma among patients with BN (Díaz-Marsá et al, 2008).

Despite increasing understanding of HPA axis activity among patients with ED, no studies have reported the pattern of biological markers of stress among women with ED during pregnancy or the longitudinal effects on the offspring.

Although stress hormones are necessary for fetal maturation, if excessive levels reach fetal circulation they can potentially have adverse effects on development (Sandman et al., 2011) and affect their infants' ability to respond appropriately to stressful situations. Stress paradigms in humans are only recently emerging, but studies have generally indicated that high levels of self-reported stress or maternal cortisol levels in pregnancy are associated with a larger infant cortisol reaction (Gutteling et al., 2004; Gutteling et al., 2005; Tollenaar et al., 2011; Davis et al., 2011).

Micali and Treasure (2009) propose a conceptual biological model of risk of fetal programming among women with ED. This model highlights the possible mediating roles of nutrition and stress during pregnancy among women with ED and the potential interaction of these factors via hyperactivity of the maternal and fetal HPA axis. Two potential pathways are implicated in this model: poor nutrition (e.g. protein restriction) and co-morbid anxiety and depression in women with ED during pregnancy. These pathways are mediated by increased levels of maternal CRH and consequentially elevated levels of glucocorticoids in the fetal circulation. It is hypothesised that elevated levels of glucocorticoids in fetal compartments in turn increases the risk of obstetric complications and alterations in fetal development in women with ED. Micali and Treasure (2009) highlight that under-nutrition during pregnancy may be particularly relevant to HPA axis dysfunction in women with a history of AN, whereas other pathways might be more relevant to BN.

In the postnatal period, the way in which infants respond to stressful situations is indicative of how they regulate their emotions and behaviour, and is crucial for healthy psychological development. Infant stress regulation may therefore be an early risk factor for developmental problems. Recently, regulation of stress has been implicated as a risk factor for the development of AN (Favaro et al., 2008; Fararo et al., 2010). Favaro and colleagues (2008; 2010) found a combined effect of obstetric complications and childhood abuse on the risk of developing an ED later in life. These preliminarily findings suggest a potential role of perinatal complications and prenatal programming of the stress response in the pathogenesis of ED.

In light of this literature the aims of this paper were threefold: 1) to investigate antenatal and postnatal biological markers of stress among women with and without ED, 2) to assess the relationship between biological markers of stress and psychopathology among women with and without ED and 3) to investigate infants' stress response at eight weeks postpartum and its relationship with maternal measures of stress and psychopathology in a longitudinal study of infants of women with ED and controls.

We aimed to investigate the effect of maternal active and past ED to try disentangling the direct effect of ED (state) on relevant outcomes, vs. any effect due to a past ED (residual effect) or that might index endophenotype/intermediate phenotype markers (trait).

2. Materials and measures

2.1 Design and participants

The Nutrition and Stress in Pregnancy (NEST-p) study is an observational prospective study of pregnant women and their infants (Easter et al., 2013).

Three groups of women were recruited for this study during the first or second trimester: women with an active ED (C-ED), women with a past ED (P-ED) and a healthy control (HC) group. Women were recruited via three recruitment methods: 1. Women attending their first or second routine ultrasound scan (see Easter et al, 2013, for further details), 2. Women

referred during pregnancy to a specialist psychiatric service for treatment for an ED and 3. Recruitment posters and online information.

Inclusion criteria for the index groups were an active or past DSM-IV diagnosis of ED, ages between 18-45 years, and within the first or second trimester of pregnancy. Additional inclusion criteria for the HC group were no active or past full or partial psychiatric disorder including an ED. Exclusion criteria were: a comorbid psychotic illness or an active psychiatric disorder other than ED among women with a past ED. Women were excluded if they suffered from any chronic medical disorder or were unable to communicate in English.

The C-ED group included women meeting DSM-IV diagnostic criteria at the time of recruitment or within the three months prior to pregnancy. The amenorrhea criterion for a diagnosis of AN was not used due to the nature of the sample. Women were considered to have recovered from their ED if they did not meet criteria for an ED in the year prior to their current pregnancy (P-ED).

A total of 137 eligible women were recruited and grouped according to their current ED status: C-ED (n=37), P-ED (n=39) and healthy controls (n=57). Women were included in the present study if they provided biological samples during pregnancy and biological measures of infant stress were obtained ((n= 91, 66%; C-ED: n=21, P-ED: n=26, HC: n=44)). Within the C-ED group 12 (57%) women met criteria for AN, 6 (29%) for BN and 3 (14%) for BED. Within the P-ED group 15 (58%) met criteria for AN, 9 (35%) for BN and 2 (8%) for BED.

2.2 Measures

Diagnostic status: Lifetime and current ED diagnoses were determined at recruitment using the Structured Clinical Interview for Axis I DSM-IV-TR Disorders (SCID-I) (First et al., 2002), which was administered and scored by trained researchers and diagnosis determined with the senior author.

Past experiences of trauma were assessed using the initial screening question for post-traumatic stress disorder (PTSD) from the SCID-I (First et al., 2002). Participants were

classified as having history of a traumatic experience if they answered yes to this question and provided a description of event(s) fitting the criteria of a traumatic event.

Maternal psychological measures: The following self-report measures were used to assess maternal psychopathology and stress between 25 and 32 weeks gestation: Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn and Beglin, 1994), Beck Depression Inventory (BDI) (Beck et al., 1961), Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983), Perceived Stress Scale (PSS) (Cohen et al., 1983), Pregnancy Related Anxiety Questionnaire Revised (PRAQ-R) (Huizink et al., 2004).

Maternal biological markers of stress: During the third trimester and at eight weeks postpartum participants were provided with an assessment pack and six labelled tubes (Salivettes®) containing oral swabs for saliva collection, which they were asked to complete at home the same week as psychometric measures were completed. Detailed written and verbal instructions were given and participants were asked to keep a detailed record sheet, including any difficulties obtaining samples, disturbed sleep or stressful events around the time of obtaining the sample. During pregnancy and at eight weeks postpartum, diurnal salivary cortisol was measured on three occasions throughout the day (on awakening, 30 minutes following awakening and 8pm), on two consecutive days. For the purpose of the analysis mean cortisol values per time-point (i.e. time of day) across the two days were calculated. Samples were stored by participants in their fridge until all swabs had been completed and then posted back to the research centre in a pre-paid envelope. Once received samples were immediately frozen and stored at -20°C until analysed.

CRH and CRH-BP were obtained via blood samples drawn from women between 25 and 32 weeks gestation by a trained phlebotomist at the Maudsley Hospital Outpatients department. Samples were transferred in an ice pack and immediately centrifuged at -4°C. Plasma was pipetted from the sample and stored in cryovials at -80°C until analysed.

Infant stress response paradigm: infant cortisol levels were measured immediately prior to and 20 minutes following routine immunisations at approximately eight weeks postpartum. A delay of 20 minutes was selected for this study as it is thought to represent typical peak cortisol levels in infants following a stressor (Gunnar et al., 2009). A researcher attended all immunizations, recorded details of the timings, and obtained the saliva swab from the infants. On the following day mothers collected a sample from their infant on awakening in the morning and between 6-8pm in the evening. Participants were instructed to avoid feeding their infant for 30 minutes prior to taking the samples. Samples were stored by participants in their fridges, and returned by post to the research centre once all samples had been collected. When received samples were frozen at -20°C until they were analysed.

Oral swabs provided for maternal saliva collection were not suitable for children under the age of six years; therefore Sorbettes were used as recommended by Salimetrics at the time of the study.

Sociodemographic data: Maternal age, marital status, ethnicity and education were obtained via self-report at recruitment into the study.

2.3 Analysis of biological samples

Determination of cortisol levels was carried out using the High Sensitivity Salivary Cortisol ELISA KIT from Salimetrics, and following the recommended procedure. Magnetic Radioimmunoassay (RIA) kits supplied by Phoenix Pharmaceuticals, were used to analyse the samples for CRH, using the standard procedures. Unfortunately there is no commercially available CRHBP assay. For the purpose of this study a 25 amino acid peptide corresponding to the C-terminus of the CRHBP (recombinant expressed in CHO) was used as the standard peptide. Monoclonal mouse anti-human CRHBP antibody (MAB2796) was from R&D Systems (Abingdon, UK).

2.4 Data analysis

Two frequently used measures of HPA axis functioning were calculated separately from diurnal cortisol measurements taken during pregnancy and at eight weeks postpartum: cortisol awakening response (CAR) and cortisol decline (CD) (Ice and James, 2006). The CAR represents the natural increase in cortisol in response to wakening, and is a delta score calculated by subtracting cortisol levels at awakening from cortisol levels 30 minutes following awakening. The CAR is a marker of subtle changes in HPA axis activity and associations between the CAR during pregnancy and birth outcomes have been shown (Obel et al., 2005). CD was calculated by subtracting evening cortisol levels from cortisol levels on awakening. These markers were calculated from the average cortisol values taken across the two days.

Initial analyses were undertaken to investigate the characteristics of the data and to check the underlying assumptions of the statistical tests. Logarithmic transformations were applied to skewed data when appropriate, and the transformed variables were used in the main analyses.

Multiple regression analysis was used to assess differences between groups in HPA axis measures. Unadjusted models were initially run and then adjusted for confounding variables. Potential covariates (maternal age, pre-pregnancy smoking, maternal education level and parity) that were likely to influence the outcomes were included: (i) following a literature review of shown associations with the outcomes under study; and (ii) if they were associated with the predictor and outcome, and not with the causal pathway. Mothers' time of awakening and the time of morning sample were not associated with the main outcomes and therefore not adjusted for in the regression models. A post-hoc sensitivity analysis was undertaken to see if these differences in diurnal cortisol patterns were associated with a history of trauma.

Pearson's correlations were undertaken to assess potential associations between maternal psychopathology during pregnancy, maternal and infant cortisol levels and CRH and CRH-BP levels.

2.5 Missing data and attrition

In order to maximise the data, women are included in analyses if they had complete data on all measures included in the regression model, therefore the sample size in each statistical model varies slightly throughout this paper.

Women who provided samples were significantly older compared to those who didn't (32 years vs. 30 years, p=0.02). There were no differences in the employment status, ethnicity, education or relationship status of women who provided saliva samples during pregnancy compared to those who did not. Women with a valid blood sample were more likely to have completed higher/further education compared to women who did not (64% vs 26%, p=0.003), but there were no differences in age, or ethnicity employment or relationship status.

2.6 Ethical approval

This study was approved by the Joint South London and the Institute of Psychiatry NHS Research Ethics Committee' (Ref. 09/H0807/12).

3. Results

3.1 Socio-demographic characteristics

Women in the C-ED group were significantly younger, less likely to be employed and reported a higher rate of past traumatic experiences, compared to women in the P-ED and healthy control groups, Table 1

3.2 Biological markers of stress during pregnancy

During pregnancy mean cortisol levels at awakening and thirty minutes after awakening were lower among women in the C-ED group, compared to the HC group. Evening cortisol levels were comparable across the three groups. See Figure 1.

Regression analyses indicated a significantly lower CD from morning to evening among women with C-ED compared to the HC group, suggestive of a flatter diurnal cortisol rhythm, see Figure 1. There were no significant differences in the CAR of women in the C-ED or P-ED groups, compared to the HC group. Although diurnal cortisol levels among women in the P-ED group were slightly elevated compared to the HC, these differences were not significantly different when tested in regression models (Table 2).

Secondary sensitivity analyses were undertaken to examine whether current ED diagnosis affected diurnal cortisol rhythms. Due to the small number of women with BED providing cortisol samples during pregnancy women with BED were grouped with women with BN. There were no significant differences in CAR among women with active AN or BN/BED, compared to the HC group. Cortisol decline was significantly lower among women with active BN/BED compared to both the HC group (log β coefficient= -.19, 95%CI=-.26/-.12, p<0.0001) and the current AN group (log β coefficient= -.16, 95%CI=-.25/-.08, p<0.0001). There were no significant differences in cortisol decline among women with current AN and the HC group (log β coefficient=-.03, 95%CI=-.09-.03, p=0.330).

No significant differences in maternal CRH levels during pregnancy were found between the three groups in either unadjusted or adjusted analyses.

Maternal levels of CRH-BP were lower in the C-ED (124.3 ng/L) and P-ED (120.6 ng/L) groups compared to the HC group (160.5 ng/L), although these differences were not statistically significant. See Table 2.

Table 2 here

Figure 1 here

A post-hoc sensitivity analysis was undertaken to determine the potential role of increased trauma among women in the index groups. Cortisol decline (but no other cortisol measures) was significantly lower among women who had experienced a past trauma, but trauma did not have a significant effect, nor did it reduce estimates, when included as a covariate in the regression models.

3.3 Biological markers of stress postnatally

Diurnal patterns of maternal cortisol at eight weeks postnatal were similar across the three groups as with during pregnancy, with lower morning cortisol levels observed among women with past and current ED. Results indicated that there were no significant differences in the CAR or cortisol decline at eight weeks postnatal among women with C-ED or P-ED, compared to the HC group, although the trend remained similar to that observed during pregnancy.

3.4 Infant cortisol and stress response

A greater infant stress response was observed in the C-ED group (median 3.8 nmol/L, IQR -1.3-9.6), compared to the HC group (median 1.4 nmol/L, IQR -3.1-5.4). In adjusted analyses this finding just failed to reach statistical significance, although the trend remained. There were no differences in infant stress response between the P-ED (median 3.9 nmol/L, IQR -3.1-5.4) and HC group. See Table 3 and Figure 2.

On the day following the immunisations there were no differences in the morning cortisol of infants in the C-ED (median 0.8 nmol/L, IQR 0.5-1.2) and P-ED (0.7 nmol/L, IQR 0.5-8.5) groups, compared to the HC group (median 0.7 nmol/L, IQR 0.5-0.9). Evening cortisol levels were also comparable across the three groups: C-ED (median 0.5, IQR 0.1-0.9); P-ED (median 0.4, IQR 0.2-0.7); HC (median 0.3, IQR 0.1-0.8).

Figure 2 about here

3.5 Correlations with psychopathology

Subsequent analyses were undertaken to investigate whether there were any correlations between antenatal and postnatal biological measures of stress and maternal psychopathology during pregnancy. The results of these analyses are shown in Table 3.

Significant negative correlations were found between maternal cortisol decline and total EDE-Q, BDI and PSS scores during pregnancy, indicating that high ED symptoms, depressive symptoms and perceived stress levels during pregnancy were associated with lower cortisol declines. No significant associations between maternal psychopathology or biological markers of stress during pregnancy and infant stress response were found.

Table 3 here

4. Discussion

In this investigation, different patterns of circadian salivary cortisol were observed in women with active ED during pregnancy. Specifically, low morning cortisol levels, suggestive of a flatter cortisol decline throughout the day, were apparent in women with active ED during pregnancy, compared to women who had recovered from an ED prior to pregnancy and women without ED. Although CRH and CRH-BP were lower among women with active ED during pregnancy, they were statistically comparable to women in the healthy control group.

ED, particularly AN, are typically associated with hyperactivity of the HPA axis (Favero et al., 2008), therefore, findings of flattened cortisol rhythms among women in the present investigation, indicating a blunted response, was surprising. However, few studies have examined circadian rhythms of salivary cortisol in women with ED, and no previous investigations have done so during pregnancy.

As such, it is important to consider the potential mechanisms underlying blunted cortisol rhythms among women with ED during the antenatal period. It has been postulated that prolonged periods of hypercortisolism may result in a blunting or ‘burn out’ of the HPA axis (Heim et al., 2000). A previous investigation of circadian salivary cortisol rhythms in a

general population sample, found that high levels of ED-related attitudes and behaviours (such as restraint, hunger, binge eating and body esteem) were negatively related to the CAR (Therrien et al., 2008), indicating a blunting of HPA axis reactivity. It has also previously been reported that flattened diurnal cortisol rhythm during pregnancy are associated with higher levels of stress and anxiety (Obel et al., 2005; Kivlighan et al., 2008). Similarly in the present study lower cortisol declines were associated with higher ED symptoms, depression and stress during pregnancy; suggesting that a combination of ED symptoms, depression and stress may be contributing to the cortisol patterns observed.

The question arises as to whether these findings are specific to ED psychopathology, dysfunctional eating patterns and weight, or whether they are reflective of more general psychopathology in this group. A systematic review of perinatal maternal depression and cortisol function in pregnancy concluded that hypercortisolemia may be associated with immediate antenatal momentary mood states, while hypocortisolemia is likely to be associated with chronic maternal depressive states (Seth et al., 2016). A recent investigation, using the same study design as the present study, found that major depressive disorder was associated with hyperactivity of the HPA axis during the third trimester (Osbourne, 2016). Further investigation, comparing these outcomes across different psychiatric disorders will help elucidate the role of depression in HPA axis function among women with ED.

Reduced cortisol declines observed among women with current ED were largely driven by low morning cortisol profiles, which is in contrast to recent findings of enhanced CAR among women with active AN (Monteleone et al., 2016). This may in part be due to women with AN and BN being grouped together in the present study. Subset analysis suggested that lower cortisol declines may be more typical in women with BN and BED, and therefore in line with previous findings of increased cortisol feedback inhibition in bulimic patients (Díaz-Marsá et al, 2008). These initial findings suggest that different ED pathologies may have divergent effects on HPA axis functioning during pregnancy, which warrants further investigation.

As hypothesised, infant cortisol response to a stressor was found to be higher in infants of women with active ED during pregnancy, compared to infants of mothers with no ED. However, this finding just failed to reach statistical significance after controlling for confounders. This is likely to be due to the relatively small sample size, resulting in lack of statistical power to detect differences once other variables were included in the model. Child gender may affect infant stress response, however given that stress response did not differ between male and female babies in the present study and the relatively small sample size we decided not to stratify the analysis by gender. This interesting aspect might be the focus of a future study.

No previous studies have investigated cortisol levels in infants of women with ED, therefore further investigation and replication with larger samples is required. Although there was no evidence that infant stress response was predicted by maternal self-reported psychopathology or biomarkers of stress during pregnancy, the findings of elevated stress response in the infants are in line with previous investigations which suggest that higher levels of psychopathology during pregnancy are associated with altered stress response in their infants (Gutteling et al., 2004; Gutteling et al., 2005; Tollenaar et al., 2011; Davis et al., 2011).

The finding of elevated cortisol levels in infants of women with ED is important since previous research has indicated that altered functioning of the HPA axis in infancy is linked to deficits in cognitive performance (Gue et al, 2004), heightened emotionality (Weinstock, 1997), and behavioural problems (Griffin et al., 2003). Furthermore, infant stress regulation may be an early risk factor for developmental problems and psychopathology. Recently, stress regulation and interactions with obstetric complications, have also been implicated risk factors for the development of AN (Favaro et al., 2008; Favaro et al., 2010). Therefore, alterations in HPA axis functioning might be an important mechanism of intergenerational transmission (Micali and Treasure, 2009).

It is difficult to separate antenatal and postnatal effects in investigations of infant cortisol levels, as well as other environmental and genetic contributions. Mothers who are stressed or anxious during pregnancy are also likely to be stressed during the postnatal period. Furthermore, postnatal effects have been found to play a 'buffering' role in infants' reactions to stressful situations (Albers et al., 2008). Other environmental influences, such as social support and paternal factors are all likely to contribute.

In order to gain a more in-depth understanding of HPA axis activity during pregnancy among women with ED and their infants further investigation and replications of these findings in larger samples will be crucial. Similarly, understanding whether maternal HPA axis dysfunction in women with ED is confined to pregnancy or simply a reflection of persistent dysfunction (i.e. state vs. trait) will be important.

4.1 Strengths and limitations

The main strength of this study is its uniqueness in investigating both psychological and physiological markers of maternal anxiety and stress during pregnancy in women with and without ED, and the inclusion of a longitudinal design. The methodology utilised in the present investigation has a number of advantages over previous investigations in this area: women were followed up prospectively, extensive validated measures of maternal psychopathology were employed and ED classification and other psychiatric diagnoses were made on the basis of diagnostic interview. The inclusion of two index groups current vs past ED aimed to disentangle the role of active ED symptomatology in pregnancy.

The study is not without limitations, which need to be considered within the context of the findings. The overall sample size and number of women in each group was relatively small and a proportion of the women did not complete biological measures resulting in some attrition, which further reduced the sample size and may have introduced bias into the results and reduced the power to detect potential differences.

There was some indication that women with BN and BED were driving cortisol decline findings in this study, given the small number of women in each diagnostic group, further exploration of the impact of ED diagnosis on ED is needed. We sought to determine the potential role of increased trauma among women in the index groups. However, this analysis was not able to account for the severity, time and duration of traumatic experiences. Although efforts were taken to ensure that women followed the protocol when obtaining saliva samples strict adherence cannot be guaranteed.

Furthermore, we only report on markers of HPA activity at one time-point during pregnancy. Typically a progressive increase in cortisol levels is observed between the second and third trimester of pregnancy. It would be advantageous to have a pre-pregnancy assessment in order to compare changes in psychopathology and cortisol levels before and during pregnancy, however this would be extremely difficult to achieve.

5. Conclusion

In conclusion, this study confirms and extends on previous findings in this field of research. This is the first study to identify HPA axis dysfunction in pregnancy in women with ED, and to show an intergenerational effect. Since dysfunctions in HPA activity during childhood may represent a risk factor for psychological and physical health problems later in life, further investigation of the potential long-term implications of these findings is crucial. Recent National Institute for Health and Clinical Excellence (NICE, 2014) guidelines emphasise the need for close monitoring and consistency of care for women with mental illness during the perinatal period.

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Table 1: Sample and sociodemographic characteristics of women with complete salivary cortisol data in pregnancy

	C-ED n=21	P-ED n=26	Healthy Control n=44	*p*-value
Age: mean (s.d.)	27.7 (5.1)	33.1 (5.4)	33.6 (4.1)	*p*<0.0001
Ethnicity: n(%) White Ethnicity	13 (68%)	20 (87%)	38 (90%)	*p*=0.08
Education: n(%) Higher/Further Education	12 (63%)	14 (82%)	35 (88%)	*p*=0.08
Employment status: n(%) Full/part time employed	14 (74%)	19 (86%)	41 (93%)	*p*=0.005
Past trauma history: n(%)	16 (62%)	7 (27%)	3 (12%)	*p*<0.0001

**sociodemographic comparisons between women in the C-ED and HC groups.*

Table 2: Antenatal biological markers of stress among women with and without eating disorders

		N	Median (IQR)	Unadjusted analysis				Adjusted analysis			
				β coefficient (log)	95% CI Lower	95% CI Upper	p-value	β coefficient (log)	95% CI Lower	95% CI Upper	p-value
Maternal CAR* (nmol/L)	**C-ED**	21	0.5 (-2.4-4.3)	-.011	-.11	.08	.81	-.004	-.13	.12	.94
	P-ED	26	0.3 (-0.5-2.1)	-.007	-.09	.08	.87	-.018	-.09	.12	.73
	HC	44	1.7 (0.2-3.8)	Reference group				Reference group			
Maternal cortisol* Decline (nmol/L)	**C-ED**	21	6.8 (2.9-8.4)	**-.11**	**-.16**	**-.06**	**<.0001**	**-.11**	**-.18**	**-.04**	**.002**
	P-ED	26	9.8 (7.8-9.8)	.011	-.04	.06	.66	.02	-.03	.08	.46
	HC	44	9.6 (8.2-11.3)	Reference group				Reference group			
Maternal CRH* (ng/L)	**C-ED**	18	119.7 (98.0-181.4)	-10.2	-55.9	35.5	.66	-10.1	-55.9	35.5	.66
	P-ED	20	129.5 (106.3-193.1)	-.84	-44.9	43.3	.97	-.84	-44.9	43.3	.97
	HC	38	154.6 (118.4-195.9)	Reference group				Reference group			
Maternal CRH-BP* (pmol/L)	**C-ED**	14	98.5 (58.3-147.8)	-36.2	-97.1	24.7	.24	-19.1	-101.3	63.1	.64
	P-ED	15	184.4 (87.8-116.4)	-39.9	-99.5	19.7	.19	-50.6	-118.7	17.5	.14
	HC	26	130.3 (75.9-219.9)	Reference group				Reference group			
Infant stress response (nmol/L)**	**C-ED**	17	3.8 (-1.3-9.6)	**.027**	**.005**		**.016**	.021	-.004	.047	.099
	P-ED	23	3.9 (-0.9-7.4)	.015	-.005		.13	.017	-.006	.038	.151
	HC	38	1.4 (-3.1-5.4)	Reference group							

*Maternal analysis adjusted for: Mother's age, ethnicity and employment status; **Infant analysis adjusted for: Mothers' age, ethnicity and employment status

IQR = Interquartile Range

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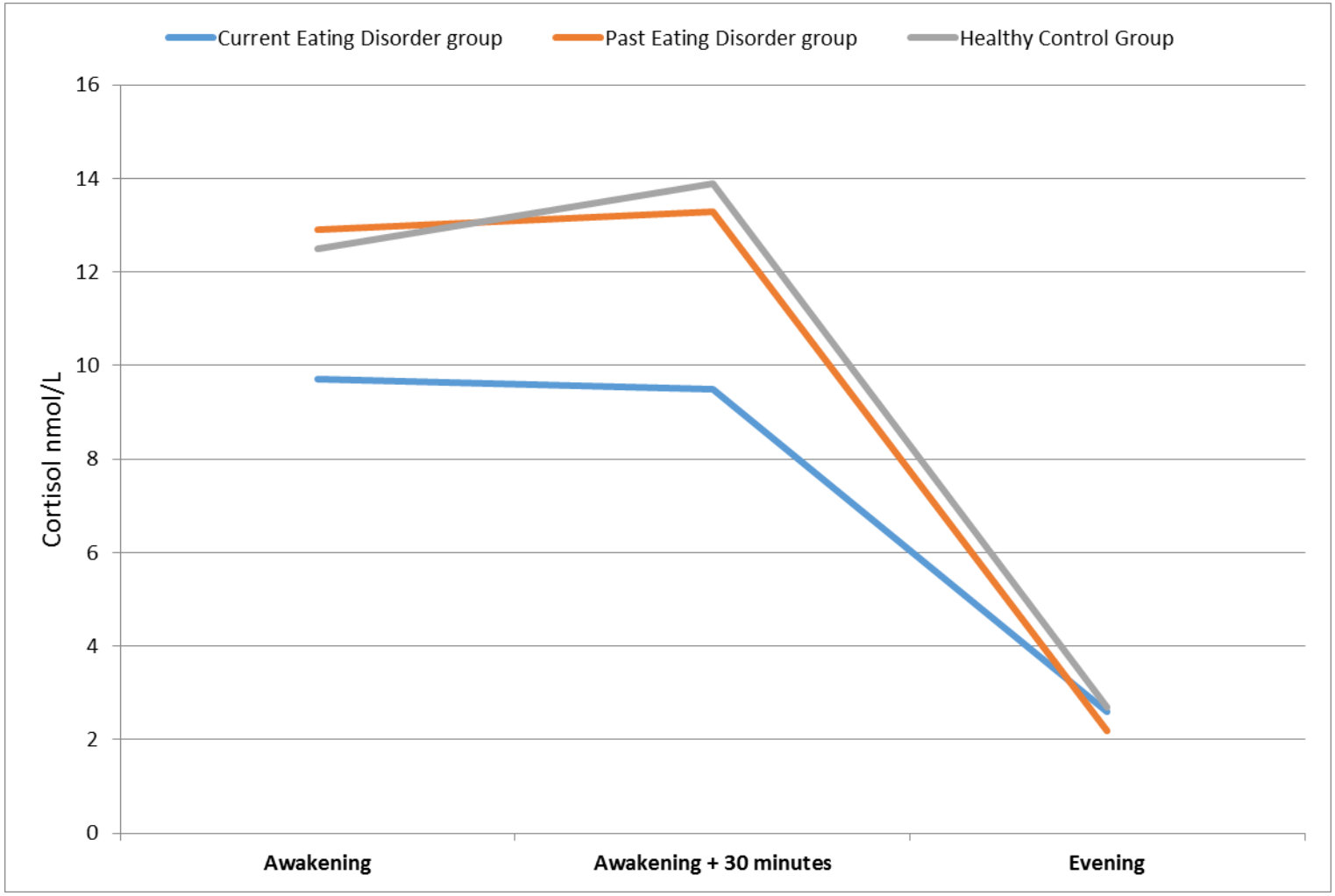
Figure 1: Median cortisol levels for women with current eating disorders, past eating disorders and healthy controls



Eating Disorders and HPA axis regulation
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Figure 2: Median cortisol levels for infants of women with current eating disorders, past eating disorders and healthy controls at eight weeks postnatal

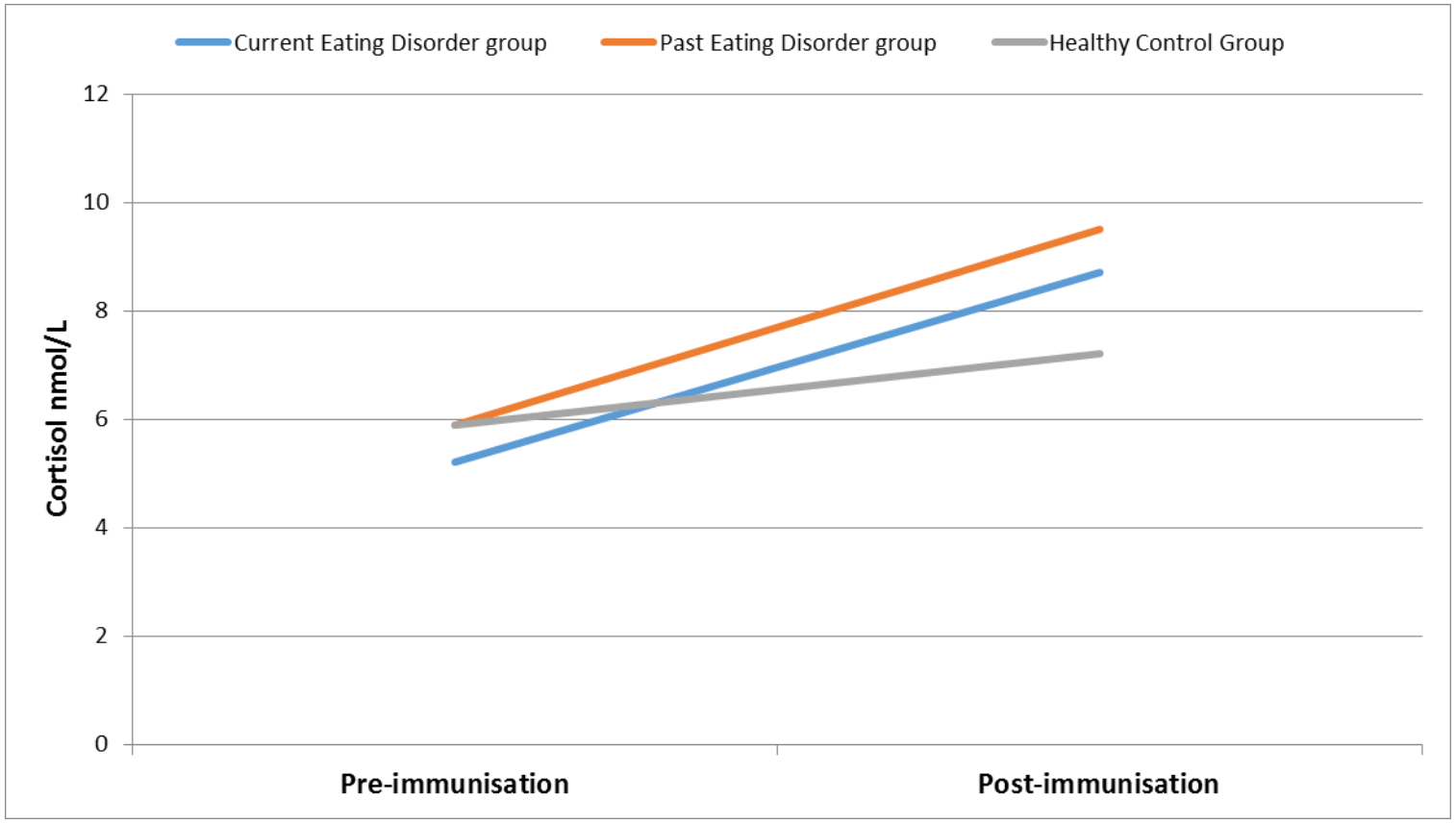

Table 3: Correlation matrix of antenatal maternal psychopathology and biological markers of stress and infant stress response

	Maternal CAR (n=91)	Maternal cortisol decline (n=91)	Maternal CRH (n=76)	Maternal CRH-BP (n=55)	Infant stress response (n=78)
Eating disorder symptoms (n=82)	-.037	-.230*	-.033	-.051	.141
Depression (n=82)	-.135	-.258*	-.067	.067	.010
State anxiety (n=88)	-.089	-.164	-.197	-.072	-.136
Trait anxiety (n=87)	-.062	-.179	-.147	-.068	-.043
Pregnancy related anxiety (n=87)	.016	-.184	-.086	-.149	.055
Perceived stress (n=78)	-.210	-.289*	-.073	-.185	-.042
Infant stress response (n=78)	.012	-.035	-.011	-.075	1

* correlation is significant at the 0.05 level

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