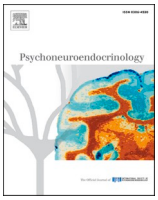
Psychoneuroendocrinology 135 (2022) 105574

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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



Changes in maternal cortisol, cortisol binding globulin and cortisone levels following diagnosis of fetal anomaly

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ARTICLE INFO

Keywords:
maternal stress
cortisol
cortisone
pregnancy
fetal anomaly

ABSTRACT

The diagnosis of fetal anomaly can be a major stressor to the expectant mother. Current understanding of the relationship between psychological stress and cortisol in pregnancy is limited. This study examined: (1) differences in the ratio of serum cortisol to cortisol binding globulin (SC/CBG) and cortisone levels among women with and without a diagnosis of fetal anomaly, (2) the association between self-reported stress and cortisol from mid to late pregnancy, and (3) the agreement between two different techniques for analyzing cortisol: liquid chromatography-tandem mass spectrometry (LC-MS/MS) and radioimmunoassay (RIA). Thirty-six pregnant women with a diagnosis of fetal anomaly (study group) and 101 women with healthy pregnancies (comparison group) provided blood samples and completed self-report questionnaires at gestational weeks 18–24 (T1) and 30 (T2). In the comparison group, mean SC/CBG increased from 0.341 nmol/L at T1 to 0.415 at T2 ($p < .001$), whereas in the study group there was no change (0.342 nmol/L at T1, 0.343 at T2). There was no difference in cortisone levels between the groups at either timepoints. There was a negative association between both depression and traumatic stress at T1, and SC/CBG at T2 ($p < .05$). There was no association between general distress and SC/CBG. The two methods for analyzing cortisol gave similar results, but with LC-MS/MS showing a lower detection limit than RIA. Increased cortisol with advancing gestational age is expected, thus these findings indicate that under certain conditions of severe stress there may be a suppression of maternal cortisol increase from mid to late gestation. The discrepancy does not seem to be due to differences in the metabolization of cortisol, as indicated by the similar levels of cortisone. Further research is needed in order to understand the potential underlying mechanisms limiting the expression of cortisol in response to certain types of stress in pregnancy.

1. Introduction

Diagnosis of fetal anomalies affects 2–4% of parents who undergo ultrasound screening during pregnancy (Dolk et al., 2010). The detection of a fetal anomaly may cause significant distress to the expectant mother. Ample research indicates that the diagnosis of fetal anomaly is accompanied by intense feelings of loss, grief, depression, worry, shock and sometimes anger (Cole et al., 2016; Kaasen et al., 2010). Psychological stress can trigger a cascade of physiological reactions in the body, including activation of the hypothalamic-pituitary-adrenal (HPA) axis (Miller et al., 2007). The hypothalamus responds to stressors by releasing corticotrophin-releasing hormone (CRH), which ultimately triggers the secretion of cortisol. Pregnancy is a transient period of hypercortisolism, with total cortisol levels raising up to four times non-pregnant levels by the third trimester (Allolio et al., 1990). This increase in cortisol is essential for fetal development and the physiological changes necessary for labor (Benfield et al., 2014). However, elevated cortisol during pregnancy has been linked to a wide range of

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https://doi.org/10.1016/j.psyneuen.2021.105574
Received 18 June 2021; Received in revised form 18 October 2021; Accepted 18 October 2021
Available online 23 October 2021

adverse obstetric and neonatal outcomes, including reduced fetal growth, preterm birth, and poorer developmental outcomes in infancy and beyond (Dunkel Schetter, 2011; Mancuso et al., 2004). Yet, to date, our knowledge on how a diagnosis of fetal anomaly affects the physiological stress response is limited.

While the diagnosis of a fetal anomaly certainly constitutes a major stressor, some research indicates that the maternal HPA axis becomes insensitive to stress in mid to late gestation (Kammerer et al., 2002). In an earlier study, Kaasen and colleagues found no difference in cortisol between women with healthy pregnancies and women with a diagnosis of fetal anomaly at 18–22 weeks gestation, despite extreme differences in self-reported distress (Kaasen et al., 2012). The few other studies that have examined the impact of real-life stressors on maternal cortisol levels are inconclusive. Of three studies that have examined the physiological stress response to medical procedures in mid-to-late gestation, one found increased cortisol in response to the procedure (Lilliecreutz et al., 2011), while two found no effect on cortisol levels (Gitau et al., 2001; La Marca-Ghaemmaghami et al., 2013). Studies that have tested the effect of more severe stress on levels of cortisol in mid-to-late pregnancy, such as partner violence or a natural disaster, have found similarly mixed results (D'Anna et al., 2012; Glynn et al., 2001; Valladares et al., 2009). With this study, we add to this existing literature by examining the relationship between stress and cortisol longitudinally. Most previous studies only measure cortisol at one timepoint (Gitau et al., 2001; Kammerer et al., 2002; Kaasen et al., 2012; La Marca-Ghaemmaghami et al., 2013; Lilliecreutz et al., 2011). A longitudinal approach may be necessary as the effect of stress on stress hormones during pregnancy may depend on timing of stress exposure. Additionally, how the diagnosis of fetal anomaly is experienced and the feelings it elicits may change over time.

A major challenge to studying the relationship between stress and cortisol in pregnancy is that during this time, cortisol levels are influenced by two physiological mechanisms: the HPA axis and the placenta. It is plausible that the high levels of cortisol during pregnancy could act via a negative feedback mechanism to block the release of CRH from the hypothalamus, thus blunting the maternal HPA-axis responsivity to stress. However, if the whole HPA axis became desensitized, it is difficult to explain the associations between stress and cortisol found in some studies (D'Anna et al., 2012; Lilliecreutz et al., 2011; Valladares et al., 2009). Due to these inconsistencies it has been suggested that researchers should begin searching for potential mechanisms outside the HPA axis (O'donnell et al., 2009). The placenta is the primary driver of hypercortisolism during pregnancy and is involved both in secreting CRH and metabolizing cortisol (Blanford and Murphy, 1977). Previous research suggests that the functioning of the placenta is sensitive to maternal emotional states (Glover et al., 2009; Helbig et al., 2013). Importantly, the enzyme 11-β-Hydroxy Steroid Dehydrogenase (HSD11B) converts cortisol to cortisone, and in the placenta HSD11B2 plays a crucial role in limiting fetal exposure to maternal cortisol (Benediktsson et al., 1997). Recent evidence suggests that emotional distress can affect the activity of placental enzymes that result in an altered metabolism of cortisol to cortisone (Galbally et al., 2021). However, to date the relationship between stress and cortisone is much unexplored, thus, we aim to examine the effect of stress on cortisone, as well as on cortisol, during pregnancy.

Another possible explanation for previous inconsistent findings may relate to methodological differences in analyzing cortisol. Historically cortisol has been measured directly from biological samples using immunoassays, including radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) (Holder, 2006). Analytical disadvantages have become increasingly apparent in these methods (Kushnir et al., 2011). In addition, cortisol and cortisone are two very similar molecules and it is analytically challenging to measure both simultaneously. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method is one of the most sensitive and selective analyses available in clinical laboratories. LC-MS/MS also provides a robust platform for simultaneous measurements of cortisol and cortisone (Broccardo et al., 2013; Kushnir et al., 2011).

The purpose of the current study is to examine cortisol, cortisol binding globulin (CBG), cortisone levels, and self-report measures of distress, among women with and without a diagnosis of fetal anomaly at two timepoints during pregnancy. Our specific aims are threefold. The first aim is to conduct a longitudinal analysis of both cortisol and cortisone. The inclusion of cortisone in addition to cortisol will allow us to explore whether a lack of cortisol response to stress could be explained by increased metabolism of cortisol to cortisone, rather than a lack of cortisol responsiveness. Secondly, we aim to examine the relation between self-reported depression, traumatic stress and general distress, and cortisol over time. This will allow us to explore whether subjective feelings of distress predict cortisol. The last aim is to validate our previous cortisol assay (RIA) with a more specific analysis (LC-MS/MS) in order to examine if a more accurate analysis of cortisol can influence our results.

2. Material and methods

2.1. Procedures

The present study is part of a larger, ongoing longitudinal study examining parental stress reactions following the detection of fetal anomalies (the SOFUS study). Data was collected between May 2006 and February 2009. Participant recruitment occurred among pregnant women receiving obstetric care at Oslo University Hospital, Rikshospitalet. Participants in the study group were recruited following the identification of a suspected structural fetal anomaly during obstetric ultrasound examination. In the comparison group, participants were recruited following normal findings on routine ultrasound scan. We used convenience sampling dependent on workload (i.e. limited inclusion during periods of vacation or heavy clinical workload). The flow chart in Fig. 1 details inclusion and exclusion of eligible participants.

Data from two assessments carried out during pregnancy are included. The first assessment (T1) was completed within 48 h of the diagnosis of a fetal anomaly or normal ultrasound findings. The second assessment (T2) occurred at gestational age 30 weeks, which was six to twelve weeks after T1. Both assessments included self-report questionnaires on psychological distress, as well as blood sampling for biological stress markers. We collected sociodemographic variables as well as medical and obstetric history using self-report questionnaires and electronic charts.

2.2. Participants

Thirty-six pregnant women who had received a diagnosis of an ultrasound-detected fetal structural anomaly were included (study group). The anomalies included all types of fetal structural malformations, which ranged from minor (e.g. club foot) to severe (e.g. skeletal dysplasia). 52.5% of the women received a diagnosis categorized as severe while the remaining 47.5% received diagnoses characterized as mild to moderate. Further details regarding the types of diagnoses, diagnostic severity and prognosis have been reported elsewhere (Kaasen et al., 2010). A comparison group of 101 women with normal ultrasound findings and no history of fetal anomalies were also included. Gestational age at inclusion ranged from 18 to 24 weeks in the study group and 18–22 weeks in the comparison group. We excluded women with multiple pregnancies, who were under the age of 18 years, not fluent in Norwegian, or who were not legally competent to provide informed consent.

2.3. Blood samples

Blood samples were taken between 08:30 and 09:00 a.m. on the same day as the questionnaires were completed. Peripheral venous

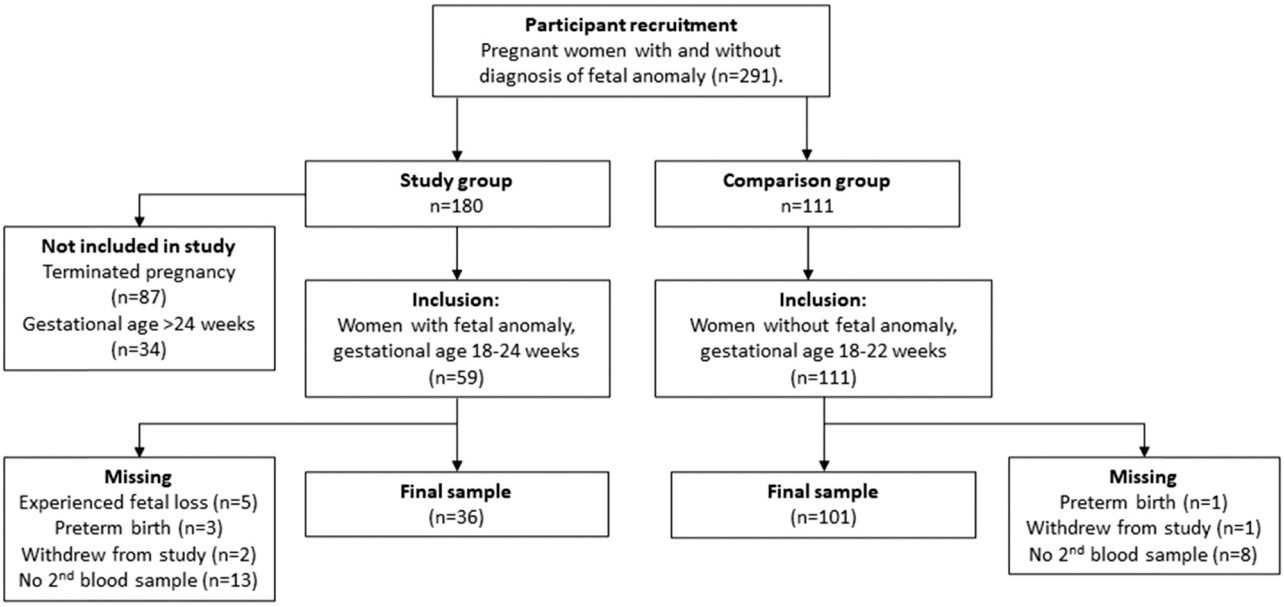

Fig. 1. Participant flowchart.

blood was drawn into sterile vacuum collection tubes and allowed to clot before being centrifuged at 2000G for 10 min at room temperature. Serum was aliquoted and stored at −80 °C until it was thawed for analysis. Serum concentrations of cortisol and cortisone were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by a method developed at the Hormone Laboratory at Oslo University Hospital, Aker (CV 11% at 149 nmol/l, LOQ 0,5 nmol/l). The methods were accredited according to NS-EN ISO/IEC 17025:2017. Analyses were performed between August and December 2020. Cortisol-binding globulin (CBG; BioSource Inc., Worcester, MA, USA) as well as the previous analysis of cortisol published by Kaasen et al. (2012) were analyzed by RIA (Orion Diagnostica, Epsoo, Finland) in 2012. Intra- and interassay coefficients of variation were < 10% for both assays. We used the ratio of LC-MS/MS serum cortisol to cortisol binding globulin (SC/CBG) as a measure of free cortisol levels which gives the most accurate representation of biologically active cortisol (Westphal, 1983).

2.4. Psychometric measures

The Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987) is a 10-item scale to assess symptoms of depression. It has been validated for use during pregnancy (Murray and Cox, 1990) and with Norwegian populations (Eberhard-Gran et al., 2001). Five of the items measure dysphoric mood, two measure anxiety, and one each measures guilt, suicidal ideas, and incidence of 'not coping' in the past week. Items were summarized based on answers to a Likert scale from 0 "not at all" to 3 "most of the time" (total range 0–30). Cronbach's alpha for the measure was 0.90.

The Impact of Event Scale (IES) (Horowitz et al., 1979) measures psychological reactions to a defined stressful or traumatic event. The questions were posed with reference to "the child's condition". The original IES scale has 15 items across two subscales: intrusion (seven items) and avoidance (eight items). Intrusion is characterized by repeated unwanted thoughts and images and strong waves of emotion. Avoidance is characterized by blunted sensation, denial of meaning and consequences of the event, and behavioral inhibition. Each item is scored between 0 and 5. The revised IES-22 version (Weiss, 2007) used in this study contains one additional item measuring intrusion and six additional items measuring arousal. Arousal is characterized by sleep disturbance, irritability, and hypervigilance. In the revised IES-22 items are scored from 0 "not at all" to 4 "extremely", however, in our study the original 0–5 scoring was kept. Scores for the items in each subscale were summarized. The total ranges for the three subscales were 0–40 for intrusion and avoidance, and 0–30 for arousal. The IES has been validated for use in Norwegian (Eid et al., 2009), and has previously been used to assess traumatic stress during pregnancy (Rychik et al., 2013). Cronbach's alpha for the measure ranged from 0.84 to 0.90 for the three subscales.

The General Health Questionnaire (GHQ) (Goldberg and Hillier, 1979) is a 28-item measure of distress and wellbeing during the last two weeks. It includes four subscales with seven items each: somatization, anxiety and insomnia, social dysfunction, and depression. Each item is scored between 0 and 3 (total range 0–84), where 0 denotes "better than" or "not more than" usual, and 3 denotes "much worse than usual". The items in each subscale were summarized. The GHQ-28 has been used previously to assesses distress during pregnancy (Prady et al., 2013), including in Norwegian populations (Skreden et al., 2010). Cronbach's alpha ranged from 0.75 to 0.82 for the various subscales.

2.5. Statistical analysis

2.5.1. Preliminary analyses

A power analysis was conducted in order to determine the minimum number of participants needed to detect a difference in cortisol between the study and comparison group. The estimate was based on data from Severi et al. (2005) observing changes in cortisol among pregnant women with high and low anxiety. Using the nomogram provided by Altman and Gore (1982) we found that 30 people in each group (study and comparison group) should be sufficient to detect a difference of one SD with a power of 90% and $\alpha = 0.01$.

2.5.2. Hypothesis testing

All analyses were performed using IBM SPSS version 27 (Statistical Package for the Social Sciences, IBM, Armonk, NY, USA) for Windows OS. For descriptive statistics we used parametric and non-parametric analyses as appropriate. Next, four mixed-design ANOVAs were conducted in order to compare the mean levels of LC-MS/MS serum cortisol (SC), CBG, SC/CBG ratio, and cortisone in the study group and the comparison group over time. The ANOVAs had one within-subjects variable with two levels: time (T1 and T2) and one between-group variable with two levels: group (anomaly, no anomaly). Third, in order to examine the relationship between distress and cortisol, four

linear regression analyses using self-reported distress at T1 to predict SC/CBG at T2 were performed. Lastly, we compared RIA and LC-MS/MS- determined serum cortisol levels using correlation and tested for proportional bias using linear regression with the mean of the two measurements as the predictor variable and the difference between the measurements as the dependent variable. The agreement of cortisol measures was also examined using a Bland-Altman plot.

2.5.3. Missing data

Missing data was handled using listwise deletion. At T2 32% of participants in the study group and 9% in the comparison group were missing blood samples, and these participants were excluded from the study (see Fig. 1). Within the study group women with previous children were more likely to be excluded from the sample ($p < .01$). Attrition analyses indicated no other significant differences between excluded and included participants. For example, there was no difference in maternal age ($p = .439$ in the study group, $p = .657$ in the comparison group), years of education ($p = .727$ in the study group, $p = .452$ in the comparison), or smoking ($p = .950$ in the study group, $p = .653$ in the comparison).

2.6. Ethics

The study was approved by the Regional Committee for Medical Research Ethics, Southern Norway, Oslo, Norway (reference number S-05281). All participants gave their written informed consent prior to participation.

3. Results

3.1. Description of sample

The sociodemographic characteristics of the participants are shown in Table 1. There were significant differences between women in the study group and the comparison group in age, education, smoking status and gestational age at inclusion, such that women in the comparison group were older, more educated, less likely to smoke, and were included earlier in the pregnancy than women in the study group.

The mean levels of psychological distress and biological stress markers in each group at T1 and T2 are shown in Table 2. At both timepoints the study group showed significantly higher scores on all self-report measures of distress than the comparison group.

Table 1
Sociodemographic characteristics of women in the study group and comparison group. Significant differences between the groups are highlighted in bold.

	Study group (n = 36) *Mean (SD)*	**Comparison group** (n = 101) *Mean (SD)*	*p-value*
Maternal age (years)	29.74 (4.76)	31.64 (4.16)	**<0.05**
Gestational age at inclusion (weeks)	20.04 (3.71)	18.8 (2.05)	**<0.05**
Education, n (%)			**<0.001**
High school or less	15 (41.7)	15 (14.7)	
More than high school	21 (58.3)	86 (85.3)	
Previous children, n (%)			.56
No previous children	17 (47.2)	53 (52.9)	
Previous children	19 (52.8)	48 (47.1)	
Smoking, n (%)			**<0.05**
Yes	4 (11.1)	2 (2)	
No	32 (88.9)	99 (98)	
Chronic disease, n (%)	8 (22.2)	19 (18.6)	.64
Medication use, n (%)	2 (5.6)	1 (1)	.17

Table 2
Psychometric self-report scores and physiological stress markers among women in the study and comparison group at time 1 (18–24 weeks gestation) and time 2 (30 weeks gestation).

	Study group (n = 36)		**Comparison group (n = 101)**	
	Mean (SD)	**Median (range)**	**Mean (SD)**	**Median (range)**
Time 1				
Psychometrics				
EPDS	11.57 (6.34)	11 (1–21)	3.18 (3.14)	2 (0–17)
GHQ total	29.95 (11.79)	27.5 (10–59)	19.98 (8.94)	18.5 (8–59)
IES intrusion	22.94 (10.65)	24 (1–40)	9.49 (6.59)	8 (0–29)
IES avoidance	9.38 (7.65)	8.5 (0–26)	2.45 (4.05)	1 (0–26)
IES arousal	12.50 (8.12)	10.5 (0–28)	3.68 (4.25)	3 (0–25)
Biological stress markers				
SC/CBG	0.342 (0.085)	0.332 (0.04–0.51)	0.341 (0.058)	0.341 (0.17–0.54)
Cortisol (nmol/L)	665 (195.9)	668 (70–995)	634 (147.9)	628 (266–976)
CBG (nmol/L)	1961 (422.7)	1935 (1234–2863)	1873 (390.4)	1872 (852–2821)
Cortisone (nmol/L)	94.2 (27.11)	93.5 (17–184)	77.7 (15.41)	75 (47–119)
Time 2				
Psychometrics				
EPDS	4.91 (3.39)	4.5 (0–12)	3.13 (3.46)	2 (0–16)
GHQ total	22.16 (8.97)	21 (9–48)	18.67 (7.82)	17.5 (7–49)
IES intrusion	10.63 (8.63)	7 (1–30)	6.91 (6.81)	5 (0–31)
IES avoidance	4.39 (6.26)	3 (0–27)	1.38 (3.28)	0 (0–22)
IES arousal	5.63 (5.49)	4 (0–18)	3.53 (3.71)	2 (0–20)
Biological stress markers				
SC/CBG	0.343 (0.083)	0.337 (0.14–0.52)	0.415 (0.080)	0.407 (0.20–0.65)
Cortisol (nmol/L)	744 (160.07)	737 (322–1195)	815 (180.81)	826 (184–1203)
CBG (nmol/L)	2228 (415.14)	2219 (1422–3275)	2007 (475.66)	1961 (921–3515)
Cortisone (nmol/L)	95.4 (16.73)	96 (59–130)	106.6 (80.46)	98.5 (57–897)

Abbreviations: EPDS = Edinburgh Postnatal Depression Scale; GHQ = General Health Questionnaire; IES = Impact of Events Scale; SC = serum cortisol; CBG = cortisol binding globulin.

3.2. Comparison of stress hormones over time among women with and without fetal anomaly

3.2.1. SC/CBG ratio

The mixed-design ANOVA indicated that there was a main effect of time such that unbound cortisol increased from T1 to T2; $F(1136) = 13.08$, $p < .001$; $\eta^2 = .088$, and with 95% CI [.014, .048] for the mean difference (see Fig. 2). There was also a time x group interaction, such that SC/CBG increased only in the comparison group, but not in the study group; $F(1136) = 21.55$, $p < .001$; $\eta^2 = .138$. At T1 the mean SC/CBG in the study group was.349 nmol/L, with 95% CI [.328, 370], and the comparison group had a mean of.343 nmol/L, with 95% CI [.331, .356]. At T2 the mean in the study group remained similar, $M = 0.340$ nmol/L, with 95% CI [.313, .367], while the mean in the comparison group increased to.414 nmol/L with 95% CI [.399, .430].

3.2.2. Serum cortisol

In a similar matter, total serum cortisol increased in the sample as a whole from T1 to T2 ($p < .001$); $F(1136) = 81.01$, $\eta^2 = .375$ and with 95% CI [112.6, 176.0] for the mean difference. There was also a time x group interaction, such that serum cortisol increased more in the comparison group than in the study group; $F(1136) = 5.63$, $p < .05$, η^2

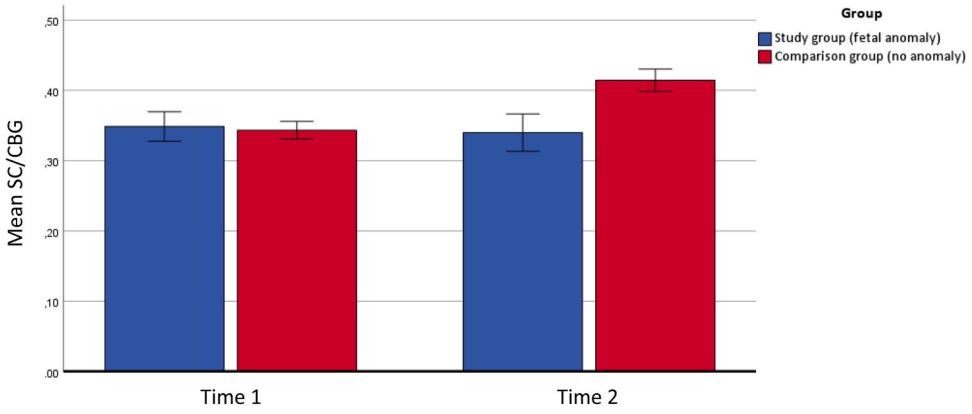

Fig. 2. Mean serum cortisol/cortisol binding globulin (SC/CBG) ratio in the study group and the comparison group at time 1 (gestational age 18–24 weeks) and time 2 (gestational age 30 weeks). Error bars = 95% confidence intervals.

= .040. The groups were similar at T1: in the study group $M = 619.9$ nmol/L, 95% CI [570.3, 669.6] and in the comparison group $M = 633.0$ nmol/L, 95% CI [603.3, 662.6]. At T2 the mean in the study group was 726.1 nmol/L with 95% CI [669.2, 783.1], and in the comparison group the mean was 815.2 nmol/L with 95% CI [781.3, 849.2].

3.2.3. Cortisol binding globulin

There was an increase of CBG over time; $F(1136) = 46.41$, $p < .001$, with $\eta^2 = .254$% and 95% CI [192.5, 350.0] for the mean difference. There was also an interaction effect, such that CBG increased more in the study group than in the comparison group over time; $F(1136) = 10.40$, $p < .01$, $\eta^2 = .071$. At T1 the study group had a mean of 1794 nmol/L with 95% CI [1668,1921], and the comparison group had a mean of 1863 nmol/L with 95% CI [1788,1939]. At T2 the study group had a mean of 2194 nmol/L with a 95% CI [2044,2344] and in the comparison group, $M = 2006$ nmol/L with 95% CI [1918,2095].

3.2.4. Cortisone

There was a significant increase in cortisone over time; $F(1136) = 8.06$, $p < .01$, with $\eta^2 = .056$% and 95% CI [5.84, 32.66]. There was no time x group interaction, $p = .152$.

We repeated the analyses of variance while controlling for gestational age at inclusion, smoking status, and years of education, and all effects remained significant at $\alpha = 0.05$.

3.3. The relationship between psychological stress and cortisol

Across groups, depression at T1 predicted SC/CBG at T2 ($p < .01$), with unstandardized $b = -0.004$% and 95% CI [−0.007, −001]. All subscales of traumatic stress also predicted SC/CBG: intrusion ($p < .05$), with $b = -0.002$% and 95% CI [−0.003, −0.001], avoidance ($p < .05$), with $b = -0.003$% and 95% CI [−0.005, −0.001] and arousal, ($p < .05$), with $b = -0.003$% and 95% CI [−0.005, .000]. There was no significant association between general distress at T1 and SC/CBG at T2 ($p = .312$). The correlation between different variables at T1 and T2 are presented in Table 3.

3.4. Comparison of agreement between cortisol measurements

There was high correlation between serum cortisol measures using the RIA method and the LC-MS/MS method, $r(311) = 0.875$, p < .001. The Bland-Altman plot (Fig. 3) indicated that there was a systematic difference between the measurements, with a tendency for the difference to be greater when cortisol levels were higher. Linear regression analysis showed that there was proportional bias between the two measurements ($p < .001$), with $b = 0.170$% and 95% CI from.113 to.227. This indicates that the LC-MS/MS cortisol method has a lower detection limit than RIA.

4. Discussion

4.1. Interpretations of main findings

Our main finding is that among women in the study group free cortisol levels did not increase with length of gestation, while in the comparison group free cortisol did increase. Contributing to this difference was both a significantly lower increase in total serum cortisol levels, as well as a higher increase in CBG, in the study group relative to the comparison group. The difference did not appear to be due to differences in metabolization of cortisol, as there was no difference in cortisone levels between the study and control group. In addition, we found that higher levels of depression and traumatic stress at 18–24 weeks gestation predicted lower cortisol at 30 weeks gestation. Lastly, we found reasonably high agreement between RIA and LC-MS/MS analysis technique, but with a tendency for LC-MS/MS to yield consistently lower serum cortisol measurements than RIA.

Cortisol is known to increase with advancing gestational age (Allolio et al., 1990; Mastorakos and Ilias, 2003), thus the lack of change in SC/CBG from T1 to T2 among women in the study group represents a deviation from what is expected during pregnancy. A potential explanation for this discrepancy could be that the detection of fetal anomaly may be associated with a suppression of maternal cortisol from mid to late gestation. We also found a negative association between depression, traumatic stress and later free cortisol levels, but no relationship between general distress and SC/CBG. Previous research have found inconsistent results regarding the association between stress and cortisol in pregnancy, and the relationship appears to depend, at least to some degree, on the type of stressor and the subjective feelings that are elicited (Dunkel Schetter, 2011).

A possible mechanism explaining the low cortisol levels observed in the study group could relate to altered HPA-axis regulation in response to trauma. In non-pregnant populations many studies have reported an association between post-traumatic stress disorder (PTSD) and lower cortisol awakening response (Speer et al., 2019). One potential hypothesis is that PTSD may be associated with enhanced negative feedback regulation of the HPA-axis, resulting in blocking of the release of CRH from the hypothalamus. While our study did not assess for diagnosis of PTSD, more than half the women in the study group reported clinically significant levels of traumatic stress symptoms. It could be that the trauma of receiving a diagnosis of fetal anomaly is associated with HPA-axis dysregulation, resulting in reduced cortisol output in the study group.

Perinatal depression may also be associated with reduced cortisol awakening response, although the literature is not conclusive (Seth

Table 3
Bivariate Pearson Correlation of the combined data in the study and comparison groups between variables at Time 1 (T1; variables 1–9 at 18–24 weeks gestation) and Time 2 (T2; variables 10–18 at 30 weeks gestation), as well as links between measurements in time.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. EPDS, T1	–																	
2. GHQ, T1	.710**	–																
3. IES intrusion, T1	.714**	.549**	–															
4. IES avoidance, T1	.684**	.553**	.674**	–														
5. IES arousal, T1	.860**	.712**	.728**	.713**	–													
6. SC/CBG, T1	-0.056	-0.070	-0.014	.004	-0.040	–												
7. Cortisol, T1	.096	.044	.113	-0.036	.071	.551**	–											
8. CBG, T1	.177*	.129	.179*	-0.027	.139	-0.250**	.656**	–										
9. Cortisone, T1	.436**	.357**	.352**	.332**	.420**	.173*	.424**	.334**	–									
10. EPDS, T2	.563**	.541**	.358**	.479**	.540**	-.112	-0.110	-0.039	.150	–								
11. GHQ, T2	.409**	.587**	.259**	.285**	.444**	.009	.018	.016	.143	.668**	–							
12. IES Intrusion, T2	.428**	.274**	.522**	.377**	.489**	.022	.033	.032	.025	.518**	.443**	–						
13. IES Avoidance, T2	.487**	.406**	.501**	.552**	.587**	-.008	-0.067	-0.057	.193*	.456**	.363**	.592**	–					
14. IES Arousal, T2	.488**	.481**	.352**	.407**	.588**	-.025	-0.007	.014	.118	.715**	.631**	.651**	.544**	–				
15. SC/CBG, T2	-0.238**	-.101	-0.191*	-0.175*	-0.190*	.203**	.170**	-0.009	.000	.003	.114	-0.021	-0.090	.062	–			
16. Cortisol, T2	-0.062	-0.062	-0.113	-0.157	-0.019	.098	.481**	.459**	.083	-0.019	-0.021	.058	-0.073	.132	.482**	–		
17. CBG, T2	.195*	.035	.106	.037	.168*	-0.154	.326**	.508**	.071	.012	-0.093	.118	.016	.098	-0.471**	.510**	–	
18. Cortisone, T2	-0.011	-0.087	-0.131	-0.073	-0.069	-0.127	.003	.131	.089	-0.079	-0.079	-0.101	-0.067	-0.073	.046	.281**	.125	–

Abbreviations: EPDS = Edinburgh Postnatal Depression Scale; GHQ = General Health Questionnaire; IES = Impact of Events Scale; SC = serum cortisol; CBG = cortisol binding globulin
* Correlation significant at α = 0.05 (2-tailed).
** Correlation significant at α = 0.01 (2-tailed).

et al., 2016). Of six previous studies that have investigated the relationship between waking cortisol and depression in pregnancy, three studies found no relationship (Cheng and Pickler, 2010; Peer et al., 2013; Pluess et al., 2010), while three studies identified negative associations (Giesbrecht et al., 2012; O'Connor et al., 2014; Taylor et al., 2009). The studies by O'Connor et al. (2014) and Taylor et al. (2009) both found lower awakening cortisol levels among women experiencing major depression, but no relationship between negative mood and cortisol among healthy participants. These studies suggest that more severe symptoms of depression, rather than transient negative mood states, may be associated with blunted cortisol in pregnancy. Among women in our study who received a diagnosis of fetal anomaly, 56% scored above the clinical cut-off for likely diagnosis of depression. Thus, it may be that these women exhibited a blunted wakening cortisol response and are driving the observed relationship between depressive symptoms and lower cortisol.

We also found a greater increase in CBG in the study group relative to the control group. Elevated levels of CBG has been observed in PTSD (Kanter et al., 2001), although not consistently (De Kloet et al., 2007). Maternal depression may also influence CBG levels during pregnancy (Gemmel et al., 2018). CBG is secreted by the liver and raises during gestation mainly due to rising estrogen levels (Edwards and Boonstra, 2018). Increased CBG during pregnancy protects maternal tissues from excessive free cortisol exposure. CBG also plays a role in modulating steroid signals at the maternal-fetal interface by binding to specific membrane receptors, where it is thought to downregulate placental CRH production (Benassayag et al., 2001; Edwards and Boonstra, 2018). One can speculate whether increased binding of CBG to the placenta reduced the production of placental CRH, which could potentially contribute to the lower cortisol levels observed in the study group. Hence, CBG may influence total cortisol levels and HPA feedback in pregnancy, however, to our knowledge, there are no published studies on this, and therefore we can only speculate on the implication.

Another novel finding in our study is that there was no difference in cortisone levels between the study and control group. Thus, there does not appear to be a significant difference in the metabolism of cortisol to cortisone between the groups. A key regulator of fetal exposure to cortisol is the placental enzyme HSD11B2, which converts cortisol to cortisone. The expression of HSD11B2 has been shown to respond to maternal stress levels (Cottrell et al., 2014; Galbally et al., 2021). However, in our study we found no difference in cortisone, which may be an indication that the activity of HSD11B2 was not affected by high stress levels in the study group. Follow-up studies are needed to further understand the role of the placenta in the relationship between stress and cortisol during pregnancy.

4.2. Strengths and limitations

A major strength of this study is that we prospectively and longitudinally measured both distress and cortisol. Distress was measured using three different, previously validated questionnaires. We also included a comparison group with considerably lower levels of distress. The inclusion of mothers with healthy pregnancies, while using the exact same psychometric tools and study design, increases the validity of our findings.

Our study is also among the few to compare the same cortisol samples measured using both LC-MS/MS and RIA. We found a high degree of agreement between the measurements, which supports the reliability of our findings. However, it appeared that using LC-MS/MS to measure cortisol resulted in systematically lower estimates than those from RIA. This is in accordance with earlier findings, which show that laboratories that use LC-MS/MS report consistently lower values than laboratories using other methods, such as RIA (Turpeinen and Hämäläinen, 2013). LC-MS/MS is one of the most specific techniques available in clinical laboratories to simultaneously measure cortisol and cortisone. Immunoassays such as RIA and ELISA can suffer from weaknesses, including

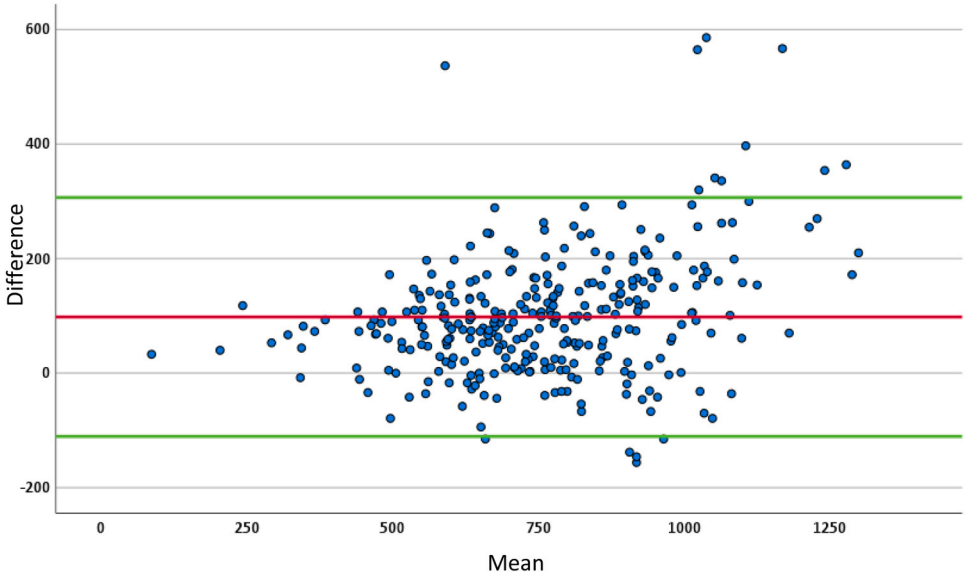

Fig. 3. Bland-Altman plot displaying the agreement between serum cortisol (nmol/L) measured using radioimmunoassay (RIA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The X-axis displays the mean value of the two measurements for each participant, and the Y-axis displays the difference between the two measurements for each participant (RIA minus LC-MS/MS). The red line on the Y-axis indicates the mean difference, and the two green lines indicate the upper and lower 95% confidence interval of the mean difference. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

sample matrix effects and a lack of specificity resulting from cross-reactivity with structurally related endogenous steroids such as metabolites of lipids (Kushnir et al., 2011). This may explain the observed difference between the measures.

A limitation of the study is that we only measured cortisol at one timepoint at each assessment, which does not allow for an examination of the pattern of cortisol secretion throughout the day. Variability of cortisol throughout the day has become increasingly central to our understanding of the relationship between psychological stress and cortisol (Miller et al., 2007), and the effect of maternal prenatal stress on diurnal cortisol should be further explored. Further, it would have been interesting to examine the separate impact of traumatic stress and depression on cortisol levels, by controlling for the effect of each variable on the other in the regression analysis. However, given that traumatic stress and depression were highly correlated a much larger sample size would be needed to achieve adequate numbers of women with traumatic stress and no depression, and depression but no traumatic stress.

A second limitation is that our study and comparison group were not matched and that they differed in terms of several sociodemographic variables. Due to the relative rarity of fetal anomaly diagnosis, it was not methodologically feasible to collect blood samples prior to the diagnosis. There was also greater attrition and more missing data in the study group than in the comparison group, which inevitably raises the possibility of bias. However, these differences between groups may not necessarily affect associations between study variables (Nilsen et al., 2009; Wolke et al., 2009).

Another methodological issue is the heterogeneity of congenital malformations. Due to the low prevalence of different anomalies, we chose to include all diagnosed malformations. Previous research indicates that maternal psychological distress is comparable across diagnoses (Kaasen et al., 2010; Skreden et al., 2010). One can also question whether the presence of a fetal anomaly could directly affect maternal cortisol levels, independent of psychological distress. While there is strong correlation between maternal and fetal cortisol, there is little evidence that fetal development directly influences maternal endocrine regulation (Talge et al., 2007).

4.3. Implications for practice

Maternal prenatal distress has been related to a variety of adverse obstetric and neonatal outcomes (Mancuso et al., 2004). It has been hypothesized that a key pathway underlying these associations involves fetal exposure to maternal cortisol (Dunkel Schetter, 2011). Cortisol plays an essential role in mediating fetal organ maturation and the physiological changes necessary for labor (Benfield et al., 2014). Both too high and too low cortisol levels appear to be harmful (Mancuso et al., 2004). It is therefore important for both practitioners and researchers to understand more about the mechanisms driving variations in cortisol during pregnancy, and in specific the mechanisms underlying the relationship between stress and cortisol.

5. Conclusion

Overall, our findings suggest that there may be a suppression of maternal cortisol in mid to late gestation under certain conditions of severe stress. This effect appears to depend, at least in part, on the type of the experienced stress, as self-reported depression and traumatic stress predicted cortisol, but not general distress. We found no difference in cortisone levels between the groups, indicating that the lack of cortisol responsiveness was not due to increased metabolization of cortisol. We speculate whether the observed lack of increased cortisol with advancing gestation in the study group may be due to increased CBG and HPA-axis dysregulation in response to trauma.

Funding

This work was supported by the Research Council of Norway (RCN; grant number 288083 and 301004), Norwegian Women's Public Health Association, the Norwegian Association for Children with Congenital Heart Disease, University of Oslo and Oslo University Hospital.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105574.

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