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Tandem mass spectrometry determined maternal cortisone to cortisol ratio and psychiatric morbidity during pregnancy—interaction with birth weight

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ABSTRACT

Maternal serum cortisol has been suggested to be influenced by psychiatric morbidity, and may also influence fetal growth. However, several studies found equal cortisol levels in depressed and healthy pregnant women. Placental 11-β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) shields the fetus from maternal cortisol by conversion to cortisone, a function that may be compromised by maternal stress. We aimed to compare the serum ratio of cortisone to cortisol, in women with and without psychiatric morbidity during pregnancy. A secondary aim was to investigate whether fetal growth, approximated by infant birth weight, was associated with the cortisone to cortisol ratio.

We performed tandem mass spectrometry analysis of serum cortisol and cortisone in late pregnancy in 94 women with antenatal psychiatric morbidity and 122 controls (cohort 1). We also compared the placental gene expression of *HSD11B1* and *2* in another group of 69 women with psychiatric morbidity and 47 controls (cohort 2).

There were no group differences in cortisol to cortisone ratio, absolute levels of cortisone and cortisol (cohort 1), or expression of *HSD11B1* or *2* (cohort 2). However, cortisone to cortisol ratio was positively associated with birth weight in women with psychiatric morbidity, also after adjustment for gestational length, fetal sex, maternal height, smoking, SSRI use, and time of blood sampling (standardized $\beta = 0.35$, $p < 0.001$), with no association in the healthy controls

Thus, the maternal serum cortisone to cortisol ratio does not seem to be affected by psychiatric morbidity, but psychiatric morbidity may increase fetal exposure to cortisol or other metabolic factors influencing fetal growth.

1. Introduction

Cortisol metabolism during pregnancy affects the neuroendocrinology of the pregnant woman as well as her fetus. Cortisol is essential for fetal organ maturation (de Fencl and Tulchinsky, 1975; Rog-Zielinska et al., 2013), but excess cortisol exposure can impair fetal growth (Edwards et al., 1993; Stewart et al., 1995), and may have programming effects on the child's hypothalamic-pituitary-adrenal-(HPA) axis reactivity (Alexander et al., 2012; Davis et al., 2011). Maternal serum cortisol concentrations increase over the course of pregnancy (Jung et al., 2011), but the placenta shields the fetus from excess cortisol by expressing 11-β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), an enzyme that converts cortisol into inactive cortisone (Murphy et al., 1974). Its isozyme 11β-HSD1, predominantly converts cortisone to cortisol (Ricketts et al., 1998).

The increased cortisol and corticotropin releasing hormone (CRH) levels often seen in depressed patients has been implicated as a mediator of the association between depression during pregnancy and lower birth weight, as well as shorter gestational length (Field

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et al., 2006; Hannerfors et al., 2015; Sandman et al., 2006). However, a number of studies show similar levels of cortisol in blood, saliva, and urine of women suffering from depression or anxiety during pregnancy compared to healthy controls (Hellgren et al., 2013; see Iliadis et al., 2015 for a review). Certainly, fetal cortisol exposure is affected by other factors than maternal cortisol production. It is known that severe stress, such as undernutrition, can decrease placental 11β-HSD2 activity in rodents (Langley-Evans et al., 1996) and, more recently, lower placental 11β-HSD2 expression and activity has been found in women with higher levels of anxiety or depression (O'Donnell et al., 2012). Thus, fetal cortisol exposure may be increased when the mother is suffering from psychiatric morbidity, even when her own serum cortisol is not markedly different from that of healthy pregnant women.

Since the placenta clears some of the maternal cortisol, the maternal serum cortisone to cortisol ratio is likely to reflect maternal *and* placental 11β-HSD2 activity and may therefore be a better indicator than solely cortisol of the magnitude by which the maternal cortisol levels influence the fetus. Placental 11β-HSD2 activity is influence by a multitude of factors. Based on the suggestion that maternal mood has an effect (O'Donnell et al., 2012), we hypothesized that this would be detected as a decrease in the maternal cortisone to cortisol ratio in women with psychiatric symptoms during pregnancy. There are not many published reports of serum cortisone concentrations in pregnant women, and more importantly, none have used tandem mass spectrometry, a method which has been shown to be superior for determination of cortisol levels in late pregnancy (Jung et al., 2011).

The aim of this study was to test the hypothesis that serum cortisone to cortisol ratio during late pregnancy is lower in women with antenatal psychiatric morbidity compared to healthy controls. Secondary aims were to explore whether the cortisone to cortisol ratio was associated with birth weight and/or gestational age at delivery, and to determine if the ratio interacted with maternal psychiatric morbidity in influencing birth weight and/or gestational length. Lastly, we wanted to test the hypothesis that placental gene expression of *HSD11B1* and *2* is lower in women with psychiatric morbidity compared to controls.

2. Methods

2.1. Setting

Pregnant women were recruited within the 'Biology, Affect, Stress, Imaging, and Cognition in pregnancy and the puerperium' (BASIC) cohort. The BASIC study investigates biological correlates of mood and anxiety disorders during pregnancy and in the postpartum period. A majority of all pregnant women in Uppsala County, Sweden are invited to participate around their routine ultrasound examination in gestational week 17. Exclusion criteria for the BASIC study are (1) inability to adequately communicate in Swedish, (2) confidentially kept personal data, (3) ultrasound findings leading to termination of pregnancy, and (4) age under 18 years. The women who participate (around 23% of the county births) fill out web-based questionnaires, including the Swedish version of the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987; Wickberg and Hwang, 1996) in gestational weeks 17 and 32. A majority of the participating women also consent to the collection of placental biopsies. The study procedures are in accordance with ethical standards for human experimentation and the study was approved by the Regional Ethical Review Board in Uppsala.

The study is based on two cohorts within the BASIC project. Cohort 1 is composed of women who have attended a late pregnancy visit to our laboratory, including serum sampling and a psychiatric interview (see Section 2.2.1). Cohort 2 contains a selection of the participants from whom placental samples were available (see Section 2.2.2).

2.2. Participants and procedure

2.2.1. Primary study population, cortisone to cortisol ratio (cohort 1)

For the current study, we invited BASIC participants with EPDS score ≥13 in gestational week 32, and a random sample of women with EPDS scores <13 at gestational week 32, with the intention of oversampling women with antenatal depressive symptoms (Rubertsson et al., 2011). The participants visited the research laboratory at the Department of Women's and Children's Health, Uppsala University in gestational week 35–39 (according to the ultrasound-estimated date of delivery) between January 2010 and May 2013. The visits were scheduled between 8 AM and 3 PM, with the majority starting either at 9 AM or at 1 PM.

After providing written informed consent, the women were interviewed about ongoing depressive and primary anxiety disorders with the Swedish version of the Mini International Neuropsychiatric Interview (MINI), a structured interview based on DSM-IV criteria (Sheehan et al., 1998). The interview also included questions on previous depressive episodes. All participating women were interviewed about use of medication in the preceding three months and filled out the EPDS and the trait version of the State-Trait Anxiety Inventory (STAI-T) (Spielberger, 1983) during the visit. At the end of the 90 min visit, a venous blood sample was drawn.

In addition, EPDS scores from gestational weeks 17 and 32, medications taken during pregnancy, and educational level, were available from the web questionnaires. Data on smoking during the first trimester, obstetric complications, mean arterial pressure at the last visit to midwife during pregnancy, date of delivery, and infant sex, weight, and length was obtained from the medical records.

Out of a total 234 participating women, serum samples were available from 216 women. Ninety-four of these women were classified as suffering from psychiatric morbidity during pregnancy. Women with an ongoing minor (two to four symptoms persisting for at least two weeks) or an ongoing major depressive episode (at least five symptoms persisting for at least two weeks), or a prior episode in combination with at least one EPDS score of 13 or more during pregnancy, were considered to have experienced a depressive episode during pregnancy ($n = 59$) (with or without psychiatric comorbidities). The remaining women in the psychiatric morbidity group had either a current anxiety disorder ($n = 9$), an ongoing eating disorder ($n = 2$), at least one EPDS score ≥13 during pregnancy ($n = 14$), and/or ongoing treatment with antidepressants ($n = 10$). Women with no ongoing depressive or anxiety disorder according to the MINI interview, no ongoing psychotropic treatment, and EPDS scores <13 in gestational week 17 and 32, and at the visit, were considered as healthy controls ($n = 122$).

2.2.2. Placental gene expression population (cohort 2)

As part of the BASIC study, postpartum placental biopsies were collected for placental gene expression analyses. From a total of 931 placental biopsies, all singleton placentas where the mother was affected by psychiatric morbidity during pregnancy (n = 69), as indicated by SSRI use (n = 44), or non-medicated psychiatric morbidity during pregnancy, according to medical records (or ongoing MINI-diagnosis when available) (n = 25), plus a random selection of healthy controls (n = 47), were selected for placental gene expression analysis. Cohort 2 was only partly overlapping (n = 33) with cohort 1. Group characteristics are found in Supplementary Table 1.

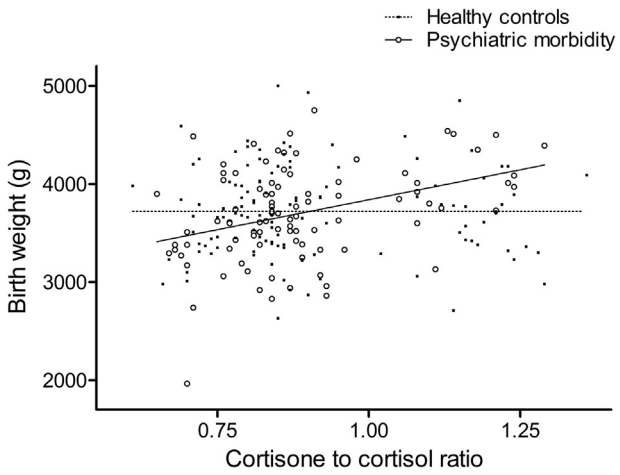

Fig. 1. Scatterplot showing the relationship between the natural logarithm transformed maternal serum cortisone to cortisol ratio and fetal birth weight. The dotted line represents the linear regression of the healthy controls (■, standardized $\beta = -0.001$, $p = 0.991$), and the solid line the linear regression of the women with psychiatric morbidity during pregnancy (◯, standardized $\beta = 0.371$, $p < 0.001$).

2.3. Tandem mass spectrometry determination of total serum cortisol and cortisone (cohort 1)

2.3.1. Sample preparation

A slightly modified method of steroid extraction was used (Liu et al., 2011). 200 μL of serum was mixed with the internal standard mixture. The internal standard mixture was prepared with d_4 cortisol in methanol. After the addition of internal standard to serum samples, each contain 2.5 ng of d_4 cortisol. Extraction of steroids from human serum was done using 2 mL of *tert*butylmethyl ether (MTBE) as the extraction solvent. Samples were gently vortexed for 10 min and centrifuged at 1000 *g* for 10 min. The supernatant was collected and the solvent was evaporated under a stream of nitrogen gas. The dried samples were reconstituted with 80 μL methanol. During the extraction, the lipids were protected against oxidation by the addition of 0.05 mg/mL butylated hydroxytoluene (BHT) to the extraction solvent (MTBE).

2.3.2. Separation of steroids by ultra performance convergence chromatography (UPC^2)

The Acquity UPC^2 system from Waters Corporation, Milford, USA, was equipped with a binary solvent delivery pump, an autosampler, a column oven and a back pressure regulator. The qualitative analysis was performed at 40 °C using an Acquity UPC^2 BEH column (100 mm 3.0 mm, 1.7 μm; Waters, Milford, MA, USA). The mobile phase flow rate was maintained at 3.0 mL/min with a gradient elution (eluent A, CO_2; eluent B, methanol). The gradient program was started with 2% of component B, then, a linear gradient was programmed from 2% to 17% for 2.0 min, followed by a linear gradient down to 2% B in 3.0 min, and finally it was held for 1.0 min which allowed ionic liquids to elute out from the instrument. Isocratic solvent was methanol in 0.1% formic acid with a flow rate of 0.4 mL/min. The back pressure was set at 1800 psi and the injection volume was 2.0 μL.

2.3.3. Identification of serum cortisol and cortisone by tandem mass spectrometry (Xevo TQ-S)

Steroids were identified by using a Waters Xevo TQ-S mass spectrometer (Milford, MA, USA). The data acquisition was in the positive ion electrospray ionization (ESI) mode. The desolvation gas was nitrogen, and the collision gas was argon (0.25 mL/min). The data acquisition range was *m*/*z* 50–800. The capillary voltage was 2.0 kV, the cone voltage was 30.0 V, and the source offset was 20 V. The source temperature was 150 °C and the desolvation temperature was 500 °C with the desolvation gas flow rate of 750.0 L/h. The cone gas flow was 150.0 L/h. The nebulizer gas flow at 7.0 bar. MS data were collected using two separate scan functions. The first scan function was set at low collision energy (5 eV), which provided parent ions, and the second scan function was set a high collision energy (ramped from 15 to 30 eV). The scan time for each function was set at 0.3 s. The MS data acquired in the *m*/*z* range of 100–1200. The precursor/product ion transitions in multiple reactions monitoring (MRM) were used for mass analysis and quantification. The pertinent mass transitions of cortisol, cortisone and internal standard were 363.05/121.2, 327.2; 361.5/163.0 and 367.25/121.0, respectively. The quantification of analytes was accomplished using internal standard and the retention time of the analytes were also compared against standard steroids for peak identification. Moreover, relevant mass transitions were compared with mass transitions obtained from standard steroids. The sample analysis was performed in triplicate. The values of relative standard deviation (RSD) of intra-day repeatability were 2.53 for cortisol and 4.07 for cortisone. The RSD values for inter-day repeatability were 1.07 and 3.78 for cortisol and cortisone, respectively. All data collected in centroid mode were obtained using MasslynxNT4.1 software (Waters Corp., Milford, MA USA).

2.4. Placental expression of HSD11B1 and 2 (cohort 2)

Placental tissue was sampled postpartum, by the midwife's assistants attending the deliveries. Two basal plate biopsy specimens of the maternal–fetal interface, approximately 2 cm in size, were excised from the central part of the placenta in a way that each sample contained the decidua basalis and villous placenta. Areas involving calcification or infarcts were avoided. The biopsies were rinsed in sterile phosphate-buffered saline, frozen on dry ice within 30 min after the delivery, and stored at −70 °C until preparation for mRNA level analysis. Total RNA from placental tissue from the fetal side was isolated with a commercial kit (#74106, Qiagen, Hilden, Germany). The first-strand cDNA was prepared from 250 ng of total RNA with Superscript VILO (Life Technologies, Paisley, UK) following the manufacturer's protocol. For real-time PCR, 100 ng of cDNA was analyzed with custom TaqMan low-density array cards (Applied Biosystems, Carlsbad, CA) covering 48 genes encoding proteins related to monoaminergic signaling, nerve growth factor signaling, and *HSD11B1* (Hs00194153_m1) and *HSD11B2* (Hs00930759_g1) (ABI Prism 7900HT Sequence Detection System, ABI Prism 7900HT SDS Software 2.4, Applied Biosystems). Eight samples were randomly analyzed per card in one run. Thermal cycling conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Four reference genes (*ACTB*, Hs01060665_g1; *TOP1*, Hs00243257_m1; *YWHAZ*, Hs03044281_g1; *GAPDH*, Hs99999905_m1) were initially analyzed. Using NormFinder (Andersen et al., 2004), *YWHAZ* and *GAPDH* were chosen as reference genes based on low intra- and intergroup variation. The relative expression (ΔC_T) was thus calculated as the number of cycles to detection threshold (C_T) for the target gene minus the C_T for *YWHAZ*, Hs03044281_g1 and *GAPDH* (Hs99999905_m1).

2.5. Statistical analyses

A sample size estimation based on previously published cortisone levels in late pregnancy (Seron-Ferre et al., 2001) suggested that equal group sizes of 87 women would render a statistical power of 0.8 at an alpha level of 0.05 to detect a group difference of ten percent.

Owing to the scarcity of previous publications on maternal serum cortisone, we performed bivariate correlations, and cate-

gorical comparisons, of cortisol, cortisone and their ratio against a list of available possible confounders. The inclusion of covariates in the final model was based on previous findings in the literature (Goedhart et al., 2010), and significant associations in the bivariate analyses.

The absolute values of cortisol, cortisone, and the cortisone to cortisol ratio were found not to be normally distributed (Shapiro-Wilk = 0.771, 0.692, and 0.744) respectively. The cortisone to cortisol ratio approached, but did not reach, normal distribution after natural logarithm transformation (Shapiro-Wilk = 0.896). Hence, the non-parametric Spearman's *rho* (r_s) was calculated for bivariate correlations and Mann-Whitney *U*-tests were used for group comparisons of the cortisone to cortisol ratio, using non-transformed values. The natural logarithm transformed ratio was used in the ANOVA and regression models, and the unstandardized residuals from the regression were checked for normality.

To investigate a possible interaction between cortisone to cortisol ratio and group (healthy controls vs. psychiatric morbidity), a univariate ANOVA was performed with infant birth weight as the dependent variable and cortisone to cortisol ratio, group, and a term for the interaction between cortisone to cortisol ratio and group as independent factors. To follow up the results from the ANOVA, a linear regression analysis, stratified for group, was performed. This model was adjusted for known confounders, including factors known to influence birth weight (maternal height (cm), parity (primi-/multiparous), infant sex, gestational age at delivery (days), smoking during the first trimester, and antidepressant use), or known to influence cortisol and cortisone concentrations (gestational age at sampling (days), hour of day at sampling).

For cohort 2, assuming the mean and standard deviation of relative *HSD11B2* mRNA observed by Murphy and Clifton, 2003, group sizes of 51 women gave a power of 0.8 to detect a group difference of 30% (the approximate difference seen between high and low anxiety scorers in O'Donnell et al. (2012)).

The ΔCt-values of *HSD11B1*, but not *HSD11B2*, were normally distributed; analyses were made with Mann-Whitney *U*-tests and non-parametric correlations.

P-values below 0.05 were considered significant and all analyses were performed with the IBM SPSS 20 software. Unless otherwise specified, data is presented as mean ± standard deviation for normally distributed variables (as approximated by a Shapiro-Wilk statistic >0.95), and as median with interquartile range for non-normally distributed data (Shapiro-Wilk statistic <0.95).

3. Results

3.1. Participant characteristics (cohort 1)

All participating women had singleton pregnancies ending in live births. The vast majority of participants ($n = 215$, 99.5%) were of Caucasian ethnicity, and were married or cohabiting ($n = 214$, 99.1%). Table 1 shows the group characteristics of healthy controls and the women with psychiatric morbidity during pregnancy. Study participant characteristics in relation to cortisol, cortisone, and the cortisone to cortisol ratio, are shown in Table 2 (continuous variables) and Table 3 (categorical variables). Women with psychiatric morbidity were on average younger and less likely to have a higher education. Their gestational age was on average two days shorter on the day of blood sampling, but there were no group differences in gestational age at delivery or birth weight.

3.2. Cortisone to cortisol ratio distribution (cohort 1)

The cortisone to cortisol ratio displayed a bimodal distribution, with one larger population with a normally distributed ratio under 1 ($n = 168$, Shapiro-Wilk = 0.985), and a smaller population with a normally distributed ratio above 1 ($n = 48$, Shapiro-Wilk = 0.980) (Fig. 1). None of our recorded demographic variables distinguished between the two populations (Supplementary Table 2).

3.3. Group differences in cortisol metabolism variables (cohort 1)

There were no significant differences in serum cortisone to cortisol ratio, or absolute values between women with psychiatric morbidity and healthy controls (Table 1). Similarly, no differences in serum cortisol, cortisone, or cortisone to cortisol ratio were found between healthy controls and women with a depressive episode during pregnancy ($n = 59$), or women with an ongoing anxiety disorder (n = 30), both *p*-values = 0.6 (Mann-Whitney *U*-tests).

3.4. Cortisol metabolism variables in relation to gestational length and birth weight (cohort 1)

Bivariate analyses in the entire study population showed that the cortisone to cortisol ratio was in a weak but significant correlation with birth weight while neither the gestational length nor the infants' birth length was associated with the cortisone to cortisol ratio (Table 2). The absolute level of cortisone was in borderline significant positive correlation with infant birth weight ($r_s = 0.134$, $p = 0.050$), while cortisol was not ($r_s = 0.050$, $p = 0.5$). Neither cortisol nor cortisone was correlated with gestational age at delivery ($r_s = 0.022$, $p = 0.8$ and $r_s = -0.008$, $p = 1.0$, respectively).

The ANOVA revealed a significant interaction between group and cortisone to cortisol ratio on birth weight ($F(1, 212) = 9.2$, $p = 0.003$). Follow-up stratified regression analyses indicated that the interaction was driven by the psychiatric morbidity group in which the cortisone to cortisol ratio was positively associated with lower birth weight (β=0.35, $p < 0.001$), after adjustment for maternal height, parity, infant sex, gestational age at delivery, smoking, SSRI use, gestational age at sampling, and hour of day at sampling (Table 4). In contrast, no association between the cortisone to cortisol ratio and birth weight was noted among the healthy controls (β=0.06, $p = 0.368$). The unstandardized residuals from the stratified regression model were normally distributed (Shapiro-Wilk = 0.984, and 0.987 for the healthy controls and the psychiatric morbidity group respectively). The unadjusted regression models are displayed in Fig. 1

3.5. . HSD11B1*and* 2 *placental gene expression (cohort 2)*

Placental *HSD11B1* or *2* gene expression did not differ between women with psychiatric morbidity and healthy controls (Supplementary Table 2). Placental ΔC_T-values of *HSD11B1* correlated negatively with birth weight ($r_s = -0.229$, $p = 0.013$), while *HSD11B2* ΔC_T-values correlated positively ($r_s = 0.200$, $p = 0.032$, $n = 116$), indicating higher expression of *HSD11B1* with higher birth weight, and lower birth weight with higher expression of *HSD11B2*. The ΔC_T-values of *HSD11B2*, but not *1*, was correlated with gestational length (*HSD11B1*: $r_s = -0.160$, $p = 0.088$; *HSD11B2*: $r_s = 0.242$, $p = 0.009$, $n = 115$).

In women who were part of both cohorts, cortisone serum concentrations were in significant correlation with the placental *HSD11B2* mRNA ΔC_T-values ($r_s = 0.397$, $p = 0.022$, $n = 33$, i.e. in negative correlation with relative gene expression). No other correlations between the placental gene expression and maternal cortisol, cortisone, or cortisone to cortisol ratio were noted (all *p*-values > 0.3).

Table 1
Characteristics of healthy controls and women with psychiatric morbidity during pregnancy (cohort 1). Data displayed as mean (±SD), n (%), or median (IQR).

	Healthy controls ($n = 122$)	Psychiatric morbidity ($n = 94$)	p[a]
Demographic			
Age, years	32.2 ± 4.1	30.1 ± 4.7	0.001
Education >12 years	105 (86.8%)	68 (73.9%)	0.017
Primipara	51 (41.8%)	47 (50.0%)	0.3
First trimester BMI, kg/m^2	23.2 (21.5–26.2)	23.8 (21.3–26.6)	0.6
Smoking during 1st trimester	2 (1.6%)	6 (6.4%)	0.1
AM blood sampling	65 (53.3%)	54 (57.4%)	0.6
Gestational age at sampling, days	264 ± 5	262 ± 7	0.007
Gestational age at delivery, days	282 ± 7	282 ± 8	0.8
Infant birth weight, g	3721 ± 460	3700 ± 483	0.8
Psychiatric morbidity			
EPDS score gestational week 17	5 (2–8)	11 (7–15)	>0.001
EPDS score gestational week 32	5 (2.75–8.25)	14 (10–16)	>0.001
EPDS score at visit	3 (1–5)	8 (5–12)	>0.001
STAI-T score at visit	32.3 ± 7.6	43.6 ± 9.9	>0.001
SSRI treatment at visit	0	20 (21.3%)	>0.001
Other psychotropic medication	0	1 (1.1%)	n.c.
Depressive episode during pregnancy	0	59 (62.8%)	>0.001
Anxiety disorder during pregnancy	0	30 (31.9%)	>0.001
Eating disorder during pregnancy	0	2 (2.1%)	n.c.
Serum concentrations			
Cortisol, nmol/L	159 (111–231)	174 (121–251)	0.4
Cortisone, nmol/L	88.0 (64.0–225)	86.7 (65.2–121)	1.0
Cortisone to cortisol ratio	0.54 (0.40–1.11)	0.56 (0.39–0.76)	0.8

N.c.: not calculated, Valid percentages are reported in cases of missing data.
[a] According to independent t-tests, Mann-Whitney U test, Pearson χ^2, or Fisher's exact test.

Table 2
Characteristics of participating mothers (cohort 1) along with each continuous variable's non-parametric correlation coefficients (Spearman's *rho*, r_s) with maternal serum cortisol, cortisone, and cortisone to cortisol ratio.

Variable (n)	Serum cortisol (r_s)	Serum cortisone (r_s)	Cortisone to cortisol ratio (r_s)
Age, years (216)	−0.01	−0.04	−0.03
Height, cm (216)	0.04	0.04	0.04
First trimester BMI, kg/m^2 (210)	−0.01	−0.01	−0.02
Gestational age at sampling, days (216)	−0.02	−0.05	−0.04
Days until parturition at sampling (216)	0.04	0.05	0.06
Gestational age at delivery, days (216)	0.02	−0.01	0.02
Infant birth weight, g (213)	0.05	0.13[a]	**0.15**[b]
Infant birth length, cm (208)	0.07	0.12[c]	0.08
Mean arterial blood pressure, mmHg (215)	0.00	0.07	0.06

[a] $p = 0.050$.
[b] $p = 0.032$.
[c] $p = 0.076$.
All other p-values > 0.1.

Table 3
Maternal cortisol, cortisone, and their ratio, in relation to potential confounders (cohort 1).

	n (%)	Serum cortisol nmol/L median (IQR)	Serum cortisone nmol/L median (IQR)	Cortisone to cortisol ratio median (IQR)
All	216	163 (119–240)	87.7 (64.4–158)	0.55 (0.39–0.82)
Infant sex				
Female	96 (45.1%)	157 (119–231)	87.8 (63.1–163)	0.55 (0.41–0.77)
Male	117 (54.9%)	166 (118–248)	87.7 (65.1–179)	0.56 (0.40–0.85)
Parity				
Primipara	98 (45.4%)	151 (121–236)	90.7 (67.1–194)	0.58 (0.41–0.88)
Multipara	118 (54.6%)	173 (115–241)	86.7 (62.3–143)	0.53 (0.37–0.76)
Education				
≤12 years	40 (18.8%)	155 (118–213)	84.0 (63.6–114)	0.54 (0.40–0.75)
>12 years	173 (81.2%)	163 (118–244)	87.7 (64.4–196)	0.56 (0.40–0.85)
Blood sampling time				
9–12 AM	119 (55.1%)	177 (118–251)	89.3 (65.3–158)	0.57 (0.38–0.83)
12.30–16 PM	97 (44.9%)	152 (119–205)	86.1 (62.7–172)	0.54 (0.41–0.77)
SSRI treatment at visit	20 (9.3%)	135 (115–213)	90.0 (66.0–109)	0.60 (0.49–0.72)
Smoking during 1st trimester	8 (3.7%)	172 (128–245)	98.6 (52.5–271)	0.49 (0.27–1.75)
Pregnancy induced hypertension	8 (3.7%)	140 (110–151)[a]	85.3 (58.2–102)	0.64 (0.52–0.80)
Postmature delivery	14 (6.5%)	176 (138–333)	93.0 (59.9–194)	0.51 (0.26–0.84)

N.c.: not calculated.
[a] $p = 0.071$.
All other p-values > 0.1.

Table 4
Linear regression analysis of birth weight, stratified for group, and adjusted for known confounders (cohort 1)

	Covariate	Unstandardized β (95% CI)	Standardized β	*p*	Adjusted R^2 (*model*)
Healthy controls (*n* = 118)	ln [cortisone to cortisol ratio]	149 (−253–551)	0.06	0.5	*0.362*
	Maternal height	12.5 (1.3–23.7)	0.17	**0.029**	
	Parity	294 (155–434)	0.32	**<0.001**	
	Infant sex	215 (79–350)	0.23	**0.002**	
	Gestational age at delivery	31.7 (21.8 41.6)	0.49	**<0.001**	
	Smoking during 1st trimester	−69 (−610–471)	−0.02	0.8	
	Gestational age at sampling	−0.3 (−14.6–14.1)	0.00	1.0	
	Hour of day at sampling	0.0 (0.0–0.0)	−0.02	0.8	
Psychiatric morbidity (*n* = 92)	ln [cortisone to cortisol ratio]	1162 (580–1744)	0.35	**<0.001**	*0.333*
	Maternal height	20.2 (6.1–34.3)	0.25	**0.006**	
	Parity	161 (−19–341)	0.17	0.1	
	Infant sex	−65 (−239–108)	−0.07	0.5	
	Gestational age at delivery	19.4 (9.0–29.9)	0.34	**<0.001**	
	Smoking during 1st trimester	−52 (−436–332)	−0.02	0.8	
	SSRI treatment at visit	−113 (−322–97)	−0.10	0.3	
	Gestational age at sampling	9.3 (−3.7–22.3)	0.13	0.2	
	Hour of day at sampling	0.0 (0.0–0.0)	0.07	0.4	

4. Discussion

The major finding of the present study was that, contrary to our hypothesis, third trimester maternal serum cortisol, cortisone, and cortisone to cortisol ratio were similar between women with an antenatal depressive episode or other psychiatric morbidities and healthy controls. This finding is in line with a number of studies suggesting no difference in gestational cortisol levels between women with antenatal depression, anxiety, or stress and healthy controls (Hellgren et al., 2013; Iliadis et al., 2015)

A non-pregnant individual's serum cortisone to cortisol ratio has been proposed to be set by the total 11β-HSD type 1 and type 2 activity combined (Heilmann et al., 1999; Homma et al., 2001) and a low cortisol to cortisone conversion has been found to be associated with renal disease and hypertension (Homma et al., 2001). We found a mean cortisone to cortisol quotient that was at least three times higher than what has been previously reported in healthy non-pregnant controls (Homma et al., 2001; Simunkova et al., 2008). During pregnancy, the placental 11β-HSD activity is likely to affect the maternal serum cortisone to cortisol ratio (Li et al., 2013), but it is also possible that the 11β-HSD2 activity in the maternal kidney and 11β-HSD1 activity in the maternal liver and adipose tissue are influenced by the endocrinology of pregnancy, as the enzymes may be inhibited by progesterone and other steroid hormones (Brown et al., 1996; Ricketts et al., 1998), as well as induced by adrenocorticotropic hormone (ACTH) (Vogeser et al., 2001), all of which serum concentrations are high during late pregnancy.

The apparent bimodal distribution of cortisone to cortisol ratio in our sample was an unexpected result which could not be explained by known demographic variables. Previously, normal distribution of cortisone to cortisol ratio has been demonstrated in healthy (non-pregnant) controls, and a bimodal distribution in hypertensive diabetes mellitus patients (Homma et al., 2001). As the subpopulations in that study could be discerned by liver and kidney function correlates, we speculate that the bimodal distribution found in our comparatively healthy population could represent different physiological strategies for handling the increased demands on the liver and the kidneys during late pregnancy.

Of course, measurements of 11β-HSD activity in placental tissue or amniotic cortisol concentration are better indicators of fetal cortisol exposure, which also take fetal cortisol production into account. However, unlike placental and amniotic samples, maternal serum ratio is possible to obtain repeatedly during pregnancy and from a larger number of women, and could therefore prove to be useful when targeting effects of maternal stress, or metabolic state, on fetal development

We found a weak but significant positive association between cortisone to cortisol ratio and infant birth weight. Goedhart et al. have reported on the largest study to date on maternal serum cortisol and birth weight (Goedhart et al., 2010). According to their findings, the initial negative association between cortisol and birth weight disappeared following adjustment for gestational age at delivery, infant gender, ethnicity, maternal age, parity, BMI, and smoking (Goedhart et al., 2010). Even though the negative impact of fetal cortisol exposure on birth weight has been established in experimental animal studies (Edwards et al., 1993), and corroborated by human correlation studies (Aufdenblatten et al., 2009; Stewart et al., 1995), the cortisol level in the maternal circulation is an unreliable measure of fetal exposure, mainly due to the placental 11β-HSD activity and fetal cortisol synthesis. Thus, the fact that we could detect an association between the cortisone to cortisol ratio and birth weight with less than a tenth of the sample-size of Goedhart et al. (2010), but not their initial correlation between serum cortisol and birth weight, could support the notion that the cortisone to cortisol ratio is a better indicator of the impact of maternal cortisol on fetal growth.

In line with Goedhart et al., 2010 and our present results, Li et al. (2012) did not find birth weight to be associated with maternal serum cortisol, sampled on the day of the delivery. However they did observe a negative correlation between maternal cortisol and ultrasound estimates of fetal head circumference, in the group of women who were in labour at the time of sampling (Li et al., 2012).

In the present study, the association between the cortisone to cortisol ratio and birth weight was in significant interaction with maternal psychiatric status, as it was not present in the healthy controls, but was driven by the women who experienced psychiatric morbidity during pregnancy. This finding is potentially explained by previous findings of lower placental 11β-HSD2 activity in women with prenatal anxiety (O'Donnell et al., 2012). Considering that there seems to be little correlation between placental 11β-HSD gene expression and the protein level, or the enzymatic activity (Novembri et al., 2013; Wachter et al., 2009), the lack of group difference in gene expression suggested by our results does not exclude the possibility that the activities of placental 11β-HSD is affected by psychiatric morbidity (O'Donnell et al., 2012). There is also some evidence that other parts of the glucocorticoid signaling pathway in placenta are altered in women with psychiatric morbidity during pregnancy (Reynolds et al., 2015). Yet another possible explanation to the interaction between psychiatric morbidity, cortisone to cortisol ratio and birth weight is that the maternal level of corticosteroid binding protein has been shown to be negatively

correlated with depressive symptoms (Pawluski et al., 2012), which would allow a larger portion of the total serum cortisol to cross the placenta in women with psychiatric morbidity.

However, with only the maternal serum ratio and placental 11β-HSD expression levels at hand, we are not in a position to infer how much of the interindividual variation in cortisone to cortisol ratio that is dependent on placental 11β-HSD activity. Clearly, the major limitation of the study is that no functional measure of placental 11β-HSD activity was obtained.

Measurements of 11β-HSD activity in placental tissue or amniotic cortisol concentration are better indicators of fetal cortisol exposure, which also take fetal cortisol production into account. However, unlike placental and amniotic samples, the maternal serum ratio is possible to obtain repeatedly during pregnancy and from a larger number of women, and could therefore prove to be useful when targeting effects of maternal stress, or metabolic state, on fetal development.

Apart from a negative impact on birth weight, cortisol has also been implied to shorten gestational length (Field et al., 2006). We found no association between cortisol (or cortisone to cortisol ratio) and gestational length in our population. It should be pointed out that the gestational length was restricted to 35 weeks or more by inclusion criteria. Also, the gestational age at serum sampling was later in our study than in previous studies reporting an association between cortisol and prematurity (Field et al., 2006). It has been suggested that the impact of maternal glucocorticoids on fetal growth is less prominent in late gestation, when the fetus has its own cortisol production (Reynolds, 2013). To assess whether the cortisone to cortisol ratio during late pregnancy is associated with fetal growth during the same time period, or if our finding is due to relative intraindividual stability of the ratio over the course of pregnancy, a longitudinal design would be needed.

Contrary to the findings of O'Donnell et al. (2012), we noted no difference in placental expression levels of *HSD11B1* or *2* according to psychiatric status (cohort 2). In the smaller group of women who were part of cohort 1 and 2, we saw a weak negative correlation between relative *HSD11B2* expression and maternal cortisone levels only. Except for the small sample size (n = 33), also the difference in time between the week 38 blood draw and the postpartum placental sampling makes this analysis highly exploratory. The placental *HSD11B2* expression was less pronounced with increasing gestational age at delivery, which is in line with an earlier study pointing to a decrease in placental 11β-HSD2 protein content and enzymatic activity toward term pregnancy (Murphy and Clifton, 2003). Lower placental *HSD11B2* expression levels have also been reported to be associated with spontaneous start of labour (Novembri et al., 2013). However, as mentioned before, their enzymatic activities are reported not to be in close correlation with the gene expression, perhaps due to the rapid changes in expression levels near term (Novembri et al., 2013; Wachter et al., 2009).

A major strength with our study is that the cortisol and cortisone concentrations were measured with tandem mass spectrometry, a method which has been shown to be superior to an immunological assay for determining cortisol during late pregnancy (Jung et al., 2011).

One limitation to the study is that the number of participants was too low to perform robust subgroup analyses of diagnose and co-morbidity groups. Although our tentative subgroup analyses did not indicate differences between women with depression or anxiety and controls, the small size of the anxiety group and the high level of co-morbidity prevent us from drawing firm conclusions. The observed lack of difference between healthy women and those with psychiatric morbidity may thus be an oversimplification that conceals different patterns of cortisol metabolism during pregnancy in different patient groups. Limitations also include the significant group differences in the demographic variables (gestational age at sampling, maternal age, and educational level). While none of these variables were found to be significantly associated with the cortisone to cortisol ratio, it cannot be ruled out that these differences may have confounded an existing group difference. Also, as cortisol and cortisone are both subject to diurnal rhythms as well as gestational age changes, the timing of sampling is crucial for the interpretation of the results. The increasing serum level of cortisol during pregnancy is accompanied by a blunted diurnal rhythm in cortisol concentration (Buss et al., 2009), but cortisol and cortisone still display a diurnal rhythm at the very end of term pregnancy (Seron-Ferre et al., 2001). Although the sampling in the current study was not standardized to a single time-point, which would be the ideal design, a majority of the samples were taken at either 10:30 AM or 2:30 PM, and the cortisone to cortisol ratio did not differ significantly between AM and PM samples. Furthermore, final regression analyses were adjusted for sampling time-point, and gestational length.

In conclusion, we found that late pregnancy maternal serum cortisone, cortisol, and their ratio, did not differ between women with psychiatric morbidity during pregnancy and healthy pregnant controls. However, we found a positive correlation between maternal cortisone to cortisol ratio and infant birth weight, which was only present in the psychiatric morbidity group, suggesting that in healthy women the effects of a low cortisol to cortisone conversion is buffered by a factor that women with psychiatric morbidity lack. Further studies assessing functional 11β-HSD1 and 2 activity in women with antenatal psychiatric morbidity are needed to clarify this finding.

Conflicts of interest

None.

Contributors

The conception of the study was made by ISP with the assistance of AS and CH. ISP, JO, ÅE, and ESV have designed the lay out for the gene expression analyses. CH has included participants and conducted participant visits, performed the statistical analyses, and drafted the first version of the manuscript. ÅE has prepared the placental samples for analysis and RF has run the placental expression analysis and calculated the results. JB and SJKAU have developed and performed the mass-spectrometry analyses. All authors have participated in the critical revision of the manuscript and approved of the final version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2016.04.006.

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