Prenatal SSRI exposure alters neonatal corticosteroid binding globulin, infant cortisol levels, and emerging HPA function

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KEYWORDS
Depression; Anxiety; Serotonin; Development; Transcortin; Antidepressants; Development; Pregnancy; Birth; Motherhood

Summary
Background: Serotonin influences the development of the hypothalamic-pituitary-adrenal (HPA) system; therefore prenatal exposure to selective serotonin reuptake inhibitor antidepressants (SSRIs) may alter HPA axis development and function. To address this, prenatal exposure to SSRIs and maternal mood were examined in relation to neonatal and infant levels of cortisol and its binding protein, corticosteroid-binding globulin (CBG).

Methods: Serum cortisol and CBG levels were assayed from SSRI-exposed and non-exposed mothers and their neonates at delivery. Maternal mood symptoms were documented at 36 weeks gestation. To determine the long-term implications of changes in CBG, levels of salivary cortisol were assessed in infants at 3 months of age.

Results: Prenatal SSRI exposure significantly increased serum CBG levels in neonates after vaginal delivery \((p \leq 0.038)\), even when controlling for maternal depression. Neonatal serum cortisol levels did not vary with SSRI exposure or antenatal maternal mood, but were significantly higher following vaginal delivery \((p \leq 0.003)\). Neonatal serum CBG levels were associated with infant salivary levels of evening cortisol \((p \leq 0.051)\). In SSRI-exposed infants, increased levels of neonatal CBG predicted a smaller diurnal change in infant salivary cortisol \((p \leq 0.028)\), regardless of maternal depression.

Conclusions: Prenatal SSRI exposure affects the developing HPA system by altering serum CBG levels in neonates and infant salivary cortisol levels. Further research is warranted on the long-term functional implications of the effect of prenatal SSRI exposure on fetal hepatic CBG gene expression and the developing HPA system.

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1. Introduction

The development and function of the hypothalamic—pituitary—adrenal (HPA) axis, a fundamental component of stress regulatory and homeostatic control systems, is shaped by fetal and early life experience which may set pathways that contribute to mental, metabolic and cardiovascular health across the life span (McEwen, 2008; Lupien et al., 2009; Tamashiro and Moran, 2010). Fetal exposure to antenatal maternal mood disturbances has been recognized as a key early life adverse experience that ‘programs’ the developing HPA axis in human neonates (Kapoor et al., 2006; Gloster et al., 2009). For example, maternal cortisol levels and psychosocial stress during pregnancy are associated with delayed neonatal stress regulation in response to a painful stimulus (Davis et al., 2011). Methyltransferase status on the human glucocorticoid receptor gene (NR3C1) is predicted by prenatal maternal depressed mood and is related to infant serum cortisol stress response (Oberlander et al., 2008b). There are also consistent, though limited, reports that perinatal maternal mood, either anxiety or depression, is associated with elevated basal levels of salivary or urinary cortisol in infants (Field et al., 2004, 2006; Brennan et al., 2008). In turn, maternal mood effects can endure into adolescence, with reports that prenatal exposure to maternal anxiety results in blunted diurnal cortisol levels and increased depressive symptoms in adolescent girls (Van der Bergh et al., 2008).

Selective serotonin reuptake inhibitor (SSRI) medications are being used increasingly to manage maternal mood disorders during pregnancy (Oberlander et al., 2006; Ververs et al., 2006; Cooper et al., 2007) and are thought to alleviate depressive symptoms in adults by normalizing the function of the HPA axis (Barden et al., 1995). However, serotonin (5HT) plays an integral part in neurodevelopment as well as in the development and function of the HPA axis (Meaney et al., 1994; Laplante et al., 2002; Andrews and Matthews, 2004). Therefore, questions have been raised about whether placental transfer of SSRIs, and the effects of a blockade of reuptake of presynaptic serotonin, impact fetal programming of the developing HPA axis. Recent research demonstrates that prenatal SSRI-exposed infants have attenuated basal salivary cortisol levels compared to non-SSRI exposed infants (Brennan et al., 2008; Oberlander et al., 2008a) and reduced cardiac autonomic stress responses to an acute painful event (Oberlander et al., 2002, 2005). In addition, exposure to perinatal maternal depression and prenatal SSRI medication is associated with increased internalizing behaviors at 3 years of age (Oberlander et al., 2010).

While salivary or serum cortisol levels are readily available, they are, nevertheless, distal indices of central stress regulation and HPA function in general. In particular, cortisol levels may be of limited value if the levels and functionality of corticosteroid binding globulin (CBG) are not taken into account (Pergamvros et al., 2011). CBG plays a key role in transporting cortisol in the blood and regulating the amounts of non-protein bound or “free” cortisol in plasma that are able to enter target tissues (Sitteri et al., 1982). To date there is no research on whether prenatal maternal mood disturbances or SSRI exposure affects neonatal CBG levels or its function in the neonate. Although the ontogeny of serum CBG levels in humans is not known, hepatic production of plasma CBG in the fetus peaks during late pregnancy in several animal models (Seralini et al., 1989; Smith and Hammond, 1991; Scroccchi et al., 1993; Challis et al., 1995), and prenatal exposure to maternal mood disorders or SSRI medications may act to alter cortisol levels via alterations in CBG production by the fetal liver.

In adults, CBG has been linked to mood disorders and the efficacy of antidepressant medications. Serum CBG levels are decreased in depressed patients (King, 1973; Ktouet et al., 1984) and men suffering from bipolar disorder (Vieta et al., 1997). A recent study in rodents has also demonstrated that CBG-deficient mice display increased depressive-like behavior in response to stressors, further supporting a key role for CBG in depression (Richard et al., 2010). Furthermore, anti-depressant medications, such as amitriptyline, may act by increasing CBG levels in depressed patients, thus decreasing the circulating levels of free cortisol (Deuschle et al., 2003).

The present study was carried out to investigate the effects of prenatal exposure to maternal depressed mood and SSRI medications on maternal and neonatal serum CBG and cortisol levels. To assess any long-term effects of maternal mood or SSRI exposure on CBG levels and the developing HPA axis, salivary cortisol levels were also assessed in infants at 3 months of age.

2. Methods

2.1. Subjects

Neonatal and maternal serum samples were obtained for analysis of cortisol and corticosteroid-binding globulin (CBG) levels from a cohort (n = 86) of mothers recruited in their early second trimester to participate in a study of psychotropic medication use during and following pregnancy. This study was carried out with approval from the University of British Columbia Research Ethics Board, Children’s and Women’s Health Centre of British Columbia Research Review Committee, and informed consent. Serum samples were collected, whenever possible, from the same mothers at 36 weeks gestation and after delivery, and from their neonates, via the umbilical cord after delivery. Samples were missing from 10 mothers at 36 weeks gestation, 21 mothers at delivery and 21 neonates at delivery. This resulted in 47 maternal samples in the non-medicated (non-exposed) group and 29 samples in the SSRI-exposed group at 36 weeks gestation, 40 maternal and 40 neonatal serum samples at delivery in the non-exposed group, and 25 maternal and 25 neonatal serum samples at delivery in the prenatal SSRI-exposed group. None of the mothers took other serotonergic medications during their pregnancies. Mothers in the exposed group were on SSRI medications for an average of 261.07 ± 44.19 days. Mothers in the SSRI treated group were already on medication at the time of recruitment and all continued SSRI medication up to the time of delivery. Three mothers were also taking other antidepressant medication in combination with their SSRI medication during their entire pregnancy, and 2 mothers were also taking antipsychotic medications in combination with their SSRI medications during pregnancy. Outcomes were compared with mothers not using any medication and who were not suffering from anxiety or depression at the time of entry into the study. For ethical and medical reasons randomization of SSRI
exposure was not possible. Therefore, we used measure of mood as a covariate.

2.2. Neonatal health and maternal mood

Neonatal outcomes were tabulated and from the immediate newborn period Apgar scores at 1 and 5 min, sex, birth weight, head circumference, body length and gestational age were obtained. During pregnancy, maternal mood was assessed twice, once at the time of study enrollment (approximately 26–28 weeks) and again at 36 weeks gestation (36.2 ± 0.7 weeks). Maternal mood was assessed using three instruments: The Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960), The Hamilton Rating Scale for Anxiety (HAM-A) (Hamilton, 1959), and the Edinburgh Prenatal Depression Scale (EPDS) (Cox et al., 1987). The HAM-D is a 21-item clinician administered scale that measures the severity of depression in adults. The HAM-A is a 14-item clinician administered scale that measures the severity of anxiety. The EPDS is a 10-item patient-rated instrument used to assess symptoms of depressed mood in both pre- and postnatal settings. Three months after delivery, maternal depressive symptoms were assessed with the EPDS.

2.3. Serum collection

Maternal and cord serum samples were collected in Vacutainer tubes for serum (Sarstedt Inc.). At 36 weeks gestation, maternal serum samples were taken at 8 am, 2.5 h before SSRI medication was taken. Delivery and cord samples were taken at some point beginning immediately after delivery of the neonate and continuing up to 2 h after delivery of the placenta. The timing of SSRI medication intake prior to delivery was also recorded. Samples were centrifuged at 2350 x g for 8 min at 4 °C. Serum was collected and stored at −80 °C until analysis.

2.4. Serum CBG assay

The cortisol-binding capacity of CBG was determined as described previously (Hammond and Lahteenmaki, 1983). Briefly, serum samples were diluted, 1/500 for maternal samples or 1/200 for cord samples, and incubated for 30 min at room temperature with dextran-coated charcoal (DCC) suspension to remove endogenous steroids. The DCC was then precipitated by centrifugation and aliquots of the supernatants were added into duplicate tubes containing 1 pmol of [1,2-3H] cortisol (specific activity: 70Ci/mmole; ARC, St. Louis, MO, USA) and to 1 tube containing 0.5 µg of cold cortisol for the evaluation of the non-specific binding. After incubation for 1 h at room temperature, tubes were placed into an ice-water bath for 30 min. Ice-cold DCC was then added for 10 min to remove unbound steroids after centrifugation (1800 x g at 4 °C) for 10 min. Supernatants were transferred into scintillation vials and 4 ml of Aqueous Counting Scintillant (Amersham Bioscience, UK) was added. The samples were counted in a scintillation spectrophotometer Beckman Coulter LS6000K. Specifically bound counts were obtained by subtracting the non-specific background counts to the average of the total bound counts and converted in nmol/ml of serum. Interassay variability of less than 10%.

2.5. Serum cortisol analysis

Maternal and cord serum samples were analyzed in duplicate using a commercially available RIA kit for total cortisol from MP Biomedicals (Orangburg, NY). The assay has a sensitivity of 0.17 ng/ml, with average intra-assay and inter-assay coefficients of variation less than 10%.

2.6. Salivary cortisol collection and analysis

At 3 months of age, basal unbound cortisol levels were assessed in SSRI-exposed (n = 19) and non-exposed (n = 44) infants via two salivary samples, one in the morning and one in the evening. Saliva was collected by placing a sorbette (Sorbette: Salimeters) in the infant’s mouth for 3 min. Sorbettes were refrigerated at 4–8 °C until they were centrifuged for 6 min at 1550 × g. Cortisol was measured with a commercially available chemiluminescent technique (IBL-Hamburg) at the Technical University of Dresden (Dresden, Germany). The assay has a sensitivity of 0.16 ng/ml, with intra-assay and inter-assay coefficients of variation less than 12%. A diurnal change in cortisol was calculated for each infant.

2.7. Statistical analysis

Because, previous work has demonstrated that neonatal cortisol levels are elevated after vaginal delivery compared to after c-section (Goldrand et al., 1976; Talbert et al., 1977; Isherwood et al., 1981), we included delivery type, vaginal versus c-section, in analyses. Therefore, two-way analysis of variance tests (ANOVA) were calculated with maternal or neonatal serum cortisol and CBG levels as a within-subjects factor and exposure (SSRI-exposed, non-exposed) and delivery type (vaginal, c-section) as between-subjects factors. Log-transformed serum cortisol levels were used for analytical purposes. An analysis of covariance (ANCOVA) was performed on serum cortisol (log) and CBG levels using measures of maternal mood (HAM-D) and neonatal characteristics (Apgar scores at 5 min, gestational age, etc.) as covariates. In addition, ANOVA tests were performed on morning and evening salivary cortisol levels and the diurnal change in salivary cortisol (squared) at 3 months of age. T-tests were also performed on maternal and neonatal demographic characteristics. To examine the relationships between neonatal and maternal CBG and cortisol levels, and infant salivary cortisol levels, linear regression models were used with key maternal mood and infant covariates. Post hoc tests utilized Fisher LSD. Significance was set at p ≤ 0.05.

3. Results

Maternal and neonatal demographic characteristics are presented in Table 1. SSRI-treated women had higher depression scores at 36 weeks gestation compared to non-treated women, but this effect did not reach significance (HAM-D, p ≤ 0.057). Prenatally SSRI-exposed neonates were a significantly lower gestational age at birth (F(1,63) = 8.44, p ≤ 0.005) and had significantly lower Apgar scores at 1 min (F(1,63) = 25.26, p < 0.000001). Antenatal SSRI-treated women had significantly higher depressive symptoms, as measured by the EPDS, at 3 months postpartum compared to
non-treated women ($F(1,61) = 4.489$, $p \leq 0.038$; Table 1). There were no other significant differences in demographic characteristics between groups ($0.07 \leq p \leq 0.88$).

### 3.1. Maternal serum cortisol and CBG levels at 36 weeks gestation

Maternal CBG levels at 36 weeks gestation were associated with maternal morning cortisol levels at 36 weeks gestation ($p \leq 0.017$; Fig. 1A), and this relationship was not markedly influenced by SSRI treatment ($p \leq 0.058$, adjusted $R^2 = 0.052$, $\beta = 0.276$, $t = 2.395$). Maternal depression scores were associated with maternal CBG and cortisol levels during late gestation, such that increased reports of depressed mood (HAM-D 36 weeks) were associated with lower levels of both maternal CBG and cortisol levels at 36 weeks gestation, regardless of SSRI treatment (CBG: $p \leq 0.034$, adjusted $R^2 = 0.063$; cortisol: $p \leq 0.051$, adjusted $R^2 = 0.054$; Fig. 1B and C). HAM-D scores at 36 weeks, and not SSRI treatment, were significant predictors of these relationships (CBG: $\beta = -0.285$, $t = -2.409$, $p \leq 0.019$; cortisol: $\beta = -0.294$, $t = -2.415$, $p \leq 0.018$).

There were no significant differences between non-exposed and SSRI-exposed maternal levels of CBG or cortisol at 36 weeks of gestation, controlling for maternal mood scores ($0.08 \leq p \leq 0.12$; Table 2).

### 3.2. Maternal serum cortisol and CBG levels at delivery

Maternal CBG levels at delivery did not differ between SSRI-treated and non-treated women, even when controlling for mode of delivery and maternal mood scores at 36 weeks gestation ($0.30 \leq p \leq 0.50$; Table 2). Maternal cortisol levels at delivery were significantly elevated in women after vaginal delivery, compared to after c-section delivery, regardless of SSRI treatment or maternal depression scores at 36 weeks gestation ($F(1,46) = 14.55$, $p \leq 0.0004$, partial $\eta^2 = 0.24$; Fig. 2A). Maternal CBG levels at delivery and delivery type, particularly vaginal delivery, significantly predicted maternal cortisol levels at delivery, regardless of maternal SSRI treatment ($p \leq 0.0001$, adjusted $R^2 = 0.354$, CBG: $\beta = 0.398$, $t = 3.271$, $p \leq 0.002$, delivery type: $\beta = -0.428$, $t = -3.697$, $p \leq 0.001$; Fig. 2B). Maternal serum CBG levels were positively associated at 36 weeks gestation and at delivery, regardless of maternal SSRI treatment or prenatal maternal mood ($p \leq 0.011$, adjusted $R^2 = 0.131$; Fig. 2C). Similarly, maternal serum cortisol levels at 36 weeks gestation were positively associated with maternal cortisol levels at delivery regardless of maternal SSRI treatment or maternal depression scores ($p \leq 0.008$, adjusted $R^2 = 0.183$; Fig. 2D).

### 3.3. Neonatal serum cortisol and CBG levels at birth

Neonatal cortisol levels were significantly elevated after vaginal delivery regardless of SSRI exposure, maternal mood, or duration of labor ($F(1,58) = 9.36$, $p \leq 0.003$, partial $\eta^2 = 0.14$; Fig. 3A). However, neonatal cord serum cortisol levels did not significantly differ between SSRI-exposed and non-exposed neonates ($p \leq 0.450$). Neonatal CBG levels did not vary with SSRI exposure alone ($p \leq 0.422$) or in relation to when SSRI medication was taken prior to delivery ($p \leq 0.311$), but there was a significant interaction between SSRI exposure and delivery type ($F(1,60) = 4.51$, $p \leq 0.038$, partial $\eta^2 = 0.07$, controlling for maternal HAMD scores are 36 weeks gestation; Fig. 3B). Namely, SSRI-exposed neonates,

### Table 1 Maternal and neonatal characteristics.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>No exposure mean (s.d.) $(n = 40)$</th>
<th>SSRI exposure mean (s.d.) $(n = 25)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at birth</td>
<td>34.25 (5.15)</td>
<td>33.28 (4.97)</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>17.97 (2.90)</td>
<td>17.84 (4.22)</td>
</tr>
<tr>
<td>Delivery type (vaginal/c-section)</td>
<td>30/10</td>
<td>19/6</td>
</tr>
<tr>
<td>Maternal mood third trimester EPDS</td>
<td>5.13 (5.05)</td>
<td>6.08 (4.07)</td>
</tr>
<tr>
<td>HAM-A total score</td>
<td>9.53 (6.60)</td>
<td>11.60 (4.99)</td>
</tr>
<tr>
<td>HAM-D total score</td>
<td>7.68 (6.06)</td>
<td>10.44 (4.74)</td>
</tr>
<tr>
<td>Neonate characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal exposure (d)</td>
<td>Not applicable</td>
<td>259.9 (46.5)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3538.98 (457.08)</td>
<td>3308.24 (551.63)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>35.02 (1.25)</td>
<td>34.21 (1.31)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>52.29 (4.99)</td>
<td>50.80 (2.30)</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>39.84 (1.56)</td>
<td>38.69 (1.50)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>20/20</td>
<td>10/15</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>8.73 (0.64)</td>
<td>7.08 (1.91)</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>9.00 (0.32)</td>
<td>8.88 (0.44)</td>
</tr>
<tr>
<td>Maternal mood 3 mo postpartum EPDS</td>
<td>3.98 (3.90)</td>
<td>6.53 (5.36)</td>
</tr>
<tr>
<td>Infant characteristics at 3 mo Age (weeks)</td>
<td>14.14 (2.20)</td>
<td>13.59 (0.89)</td>
</tr>
</tbody>
</table>

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\(a p \leq 0.07. \\
\(b p \leq 0.05.\)
Prenatal SSRI exposure, neonatal corticosteroid binding globulin and infant cortisol

born by vaginal delivery, had significantly higher serum CBG levels than non-exposed vaginally delivered neonates ($p < 0.009$). Among neonates born by c-section, no significant difference between prenatal SSRI-exposed and non-exposed neonates was present ($p < 0.55$).

Neonatal cord cortisol and cord CBG levels were not associated ($p < 0.313$), regardless of SSRI exposure, maternal depression symptoms at 36 weeks gestation and delivery type. Maternal cortisol levels at delivery and delivery type both predicted neonatal cortisol levels, regardless of SSRI exposure and maternal depression symptoms at 36 weeks gestation ($p < 0.0001$, adjusted $R^2 = 0.388$, maternal cortisol; $\beta = 0.371$, $t = 2.793$, $p < 0.008$; delivery type; $\beta = -0.350$, $t = -2.519$, $p < 0.016$; Fig. 3C). At delivery, maternal serum CBG levels did not predict neonatal serum CBG levels, regardless of SSRI exposure, prenatal maternal mood or delivery type ($p < 0.11$). Similarly, at delivery there was no association between maternal cortisol levels and neonatal CBG levels ($p < 0.348$), or maternal CBG levels and neonatal cortisol levels ($p < 0.763$).

3.4. Infant salivary cortisol levels at 3 months of age

At 3 months of age, morning and evening basilar cortisol did not differ between SSRI-exposed and non-exposed infants ($p < 0.90$; Table 3), controlling for maternal mood symptoms at 36 weeks gestation and at 3 months postpartum.

The magnitude of the change in salivary cortisol from morning to evening was smaller in SSRI-exposed infants, however this did not reach significance ($p < 0.065$), controlling for prenatal and postnatal maternal depression scores and time saliva sample was taken. Cord serum cortisol levels did not predict morning or evening cortisol at 3 months nor did they predict the change in salivary cortisol levels across the day at 3 months ($0.331 < p < 0.948$). Neonatal serum CBG levels were associated with salivary levels of evening cortisol at 3 months of age ($p < 0.051$, adjusted $R^2 = 0.07$; Fig. 4A). Neonatal CBG did not predict morning salivary cortisol levels at 3 months of age ($p < 0.735$) nor the diurnal change in 3-month salivary cortisol levels ($p < 0.433$), controlling for SSRI exposure and pre- and post-natal maternal depression symptoms. However, in SSRI-exposed infants, diurnal change in salivary cortisol at 3 months was predicted by neonatal serum CBG levels ($p < 0.028$, adjusted $R^2 = 0.309$, $\beta = -0.605$; Fig. 4B). In particular, increased levels of neonatal CBG predicted a smaller diurnal change in 3-month salivary cortisol levels in SSRI-exposed infants.

4. Discussion

In this study prenatal SSRI exposure and delivery type influenced neonatal serum CBG levels and early emerging HPA patterns of function. However, the persistence of these effects appeared to be due to prenatal SSRI exposure. Importantly, SSRI exposure resulted in significantly elevated neonatal CBG levels after vaginal delivery and SSRI-exposed infants tended to have a smaller diurnal change in salivary cortisol at 3 months of age. In SSRI-exposed infants, there was an association between neonatal CBG and infant salivary cortisol levels with high levels of neonatal CBG predicting a
smaller diurnal change in cortisol at 3 months of age. Serum cortisol levels were significantly elevated in mothers and neonates after vaginal delivery, regardless of SSRI exposure and maternal mood scores at 36 weeks gestation. In summary, our findings point to a link between prenatal SSRI exposure, neonatal levels of CBG and the diurnal change in salivary cortisol levels at 3 months of age.

4.1. Prenatal SSRI exposure, neonatal CBG and cortisol

In the present study, neonatal serum cortisol levels were elevated after vaginal delivery, regardless of SSRI exposure or maternal depression scores. This is in agreement with previous work demonstrating that neonatal cortisol levels are elevated after vaginal delivery compared to after c-section (Goldkrand et al., 1976; Talbert et al., 1977; Isherwood et al., 1981). Therefore, our findings indicate that SSRI-exposed neonates have the capacity to mount a regular serum cortisol response to labor and delivery. Previous work has documented that prior to vaginal delivery, cord serum levels of cortisol are significantly lower in prenatally SSRI-exposed neonates (Davidson et al., 2009). However, we report no difference in serum cortisol levels in prenatally SSRI-exposed neonates immediately after delivery. This discrepancy may be due to the timing of sample collection, i.e., whether it occurred before or after delivery.

Table 2  Cortisol levels (ng/ml) in maternal serum at 36 weeks gestation and CBG levels (nM) in maternal serum at 36 weeks gestation and delivery. There were no significant differences between non-exposed and SSRI-exposed maternal levels of cortisol or CBG at 36 weeks of gestation, regardless of maternal mood scores (0.08 ≤ p ≤ 0.12). There were no significant differences between maternal CBG levels at delivery, regardless of delivery type, SSRI exposure or maternal mood at 36 weeks gestation (0.30 ≤ p ≤ 0.50).

<table>
<thead>
<tr>
<th>36 weeks gestation</th>
<th>Maternal cortisol levels (ng/ml)</th>
<th>Maternal CBG levels (nM)</th>
<th>Delivery</th>
<th>Maternal CBG levels (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exposure mean (s.d.) (n = 40–47)</td>
<td>79.92 (21.05)</td>
<td>650.21 (205.99)</td>
<td>687.04 (333.93)</td>
<td>84.46 (25.87)</td>
</tr>
<tr>
<td>SSRI exposure mean (s.d.) (n = 25–29)</td>
<td>84.46 (25.87)</td>
<td>708.55 (292.48)</td>
<td>809.01 (307.81)</td>
<td>708.55 (292.48)</td>
</tr>
</tbody>
</table>

Figure 2  Mean (±SEM) serum levels of cortisol in mothers at delivery and associations between maternal serum cortisol and CBG levels. (A) Maternal cortisol levels at delivery were significantly elevated in women after vaginal delivery, compared to after c-section delivery, regardless of SSRI treatment or maternal depression scores at 36 weeks gestation (p ≤ 0.0004). (B) Maternal CBG levels at delivery and delivery type, particularly vaginal delivery, significantly predicted maternal cortisol levels at delivery, regardless of maternal SSRI treatment (R² = 0.354, p ≤ 0.0001). (C) Maternal serum CBG levels were positively associated at 36 weeks gestation and at delivery, regardless of maternal SSRI treatment or prenatal maternal mood (R² = 0.131, p ≤ 0.011). (D) Similarly, maternal serum cortisol levels at 36 weeks gestation were positively associated with maternal cortisol levels at delivery regardless of maternal SSRI treatment or maternal depression scores (R² = 0.183, p ≤ 0.008).
vaginal delivery. In addition, we found that maternal serum cortisol levels after delivery followed the same pattern as the levels in neonates, with elevated levels of cortisol after vaginal delivery, regardless of SSRI exposure or maternal depression. The levels of neonatal and maternal cortisol at delivery were also positively correlated. Therefore, it can be concluded that serum total cortisol response to delivery is ‘normal’ in the mother and neonate, regardless of SSRI exposure or maternal depression symptoms.

We found that serum CBG levels were significantly elevated in SSRI-exposed neonates after vaginal delivery. Maternal depression scores and the length of labor did not significantly impact the difference in CBG levels between SSRI-exposed and non-exposed neonates. We also did not find an overall effect of vaginal delivery or c-section delivery on neonatal CBG levels. It is possible that CBG levels in SSRI-exposed neonates are more rapidly increased in response to increased levels of cortisol (Challis et al., 1995) and may act to ‘buffer’ the developing HPA system. For example, an increase in CBG levels was evident in SSRI-exposed neonates after vaginal delivery, when total serum cortisol levels were greater, compared to after c-section delivery. Thus, free, unbound, cortisol levels may be lower in vaginally delivered SSRI-exposed neonates than in non-exposed vaginally delivered neonates. Our data also demonstrate that the increase in neonatal CBG after SSRI exposure may have long-term implications and act to decrease basal levels of free cortisol in the infant. Indeed, at 3 months of age, we report that infants prenatally exposed to SSRI medications have a smaller diurnal change in free cortisol. Previous work has shown that 3-month-old prenatally SSRI-exposed infants have lower evening basal free cortisol levels, and a more regulated stress response compared to non-exposed infants, controlling for maternal mood (Oberlander et al., 2008a). This attenuation of free cortisol in the infant prenatally exposed to SSRI medication may be due to an increase in the levels of CBG. In support of this idea, our data link neonatal CBG levels in prenatally SSRI-exposed infants to the diurnal change in free cortisol reported in these same infants at 3 months of age. This relationship between neonatal CBG levels and the 3 month cortisol levels was not evident in non-exposed neonates. These data provide evidence for a fetal ‘programming’ effect of prenatal SSRI exposure on the regulation and function of the HPA system, via alterations in neonatal CBG levels.

The increased serum CBG levels in prenatally SSRI-exposed neonates, and a potential decrease in free cortisol, may result in a “buffered” HPA response to stressors. In support of this idea, infants prenatally exposed to SSRI medications have a blunted facial response to pain (Oberlander et al., 2005). However, as previously suggested (Oberlander et al., 2008a), increased CBG levels and decreased free cortisol levels may be a result of a more efficiently regulated HPA axis. For example, evidence from animal models demonstrates that serotonin can act to increase the density of hippocampal glucocorticoid receptors (Erdeljan et al., 2001, 2005) and decrease the glucocorticoid response to stress (Meaney et al., 1994). Therefore, increased serotonin levels in the fetus, as a result of SSRI medications, may increase the density of hippocampal glucocorticoid receptors and thus more tightly ‘regulate’ the physiological changes in the HPA system that appear later in life.

4.2. SSRI treatment, maternal CBG and cortisol

In the present study, SSRI treatment did not have a significant impact on maternal CBG levels during late gestation or at delivery, and there was no association between neonatal and
maternal levels of CBG at delivery. A lack of association between maternal and neonatal CBG levels indicates that hepatic synthesis of CBG in mother and neonate occurs independently, and is under different regulation, as observed in several animal models (Seralini et al., 1989; Challis et al., 1995). However, we found positive correlations between maternal cortisol and CBG levels at delivery and 36 weeks gestation, suggesting a continuum of the relationship between cortisol and CBG from late pregnancy to delivery in the mother. In line with this, others have reported that the cortisol to CBG ratio pre- and post-partum remains unchanged in women (Landgraf-Leurs et al., 1983).

Mood disorders are often coupled with altered HPA system function in adults and SSRI medications have been shown to regulate this dysfunction (Barden et al., 1995). In support of the role of SSRI medications regulating the HPA system, we found no differences between SSRI-exposed and non-exposed women in morning serum levels of cortisol or CBG at 36 weeks gestation. We also found that maternal serum levels of cortisol and CBG at 36 weeks gestation were negatively correlated with measures of depression; suggesting that the higher levels of CBG or total cortisol were associated with lower levels of depression. Recent work has reported no relationship between maternal depression scores and maternal plasma cortisol levels during gestation (Davis et al., 2011). The absence of a positive relationship between maternal antepartum depression and gestational cortisol levels appears counterintuitive but previous research documents that a hypo-activation of cortisol in response to depression can occur and may occur more frequently in women (Bremner et al., 2007). In addition, increased severity of depression or perceived stress may result in greater alterations in the maternal HPA system during gestation.

### 4.3. Summary

Our findings suggest there is a fetal 'programming' effect of prenatal SSRI medication that involves enhanced 'regulation' of the HPA system via increased CBG levels in the neonate. The exact mechanisms and outcomes of these alterations in CBG and the HPA system remain to be investigated. Given the key role that the HPA system plays in many areas of development, from physiology to behavior, further research is warranted on the long-term functional implications of the effect of prenatal SSRI exposure on fetal hepatic CBG gene expression and the developing HPA system.

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### Conflict of interest

The authors have no biomedical financial interests or potential conflicts of interest to report.
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