Cerebrospinal fluid CRH levels in late pregnancy are not associated with new onset postpartum depressive symptoms

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Context: Corticotropin releasing hormone (CRH) participates in the hypothalamic-pituitary-adrenal (HPA) axis and in neural circuits involved in the pathophysiology of depression. During pregnancy, the placenta produces large amounts of CRH, and production ceases abruptly after delivery. The relationship between CRH in the cerebrospinal fluid (CSF) during pregnancy and peripartum mood disorders has not been investigated.

Objectives: To determine whether there are differences in CSF CRH concentrations of pregnant and non-pregnant women and whether CSF CRH concentrations in late pregnancy are associated with the presence of depressive symptoms during pregnancy and in the early postpartum period.

Design: This was a prospective cohort study conducted from January to April, 2011.

Setting: The study was conducted in one public and two private hospitals in Brasilia, Brazil.

Patients: Patients included 107 healthy pregnant women who underwent elective cesarean delivery and 22 non-pregnant healthy women who underwent spinal anesthesia for elective surgical sterilization.

Intervention: CRH in CSF was measured in pregnant and non-pregnant women by ELISA.

Main outcome measure: The association between CSF CRH concentration at delivery and maternal depression assessed before cesarean section and postpartum (4 to 8 wk) with the Edinburgh Postnatal Depression Scale (EPDS), with a cutoff of $\geq$ 13.

Results: CRH concentration in the CSF was significantly higher in pregnant (4.1 $\pm$ 0.51 Log CRH) than in non-pregnant women (3.6 $\pm$ 0.26 Log CRH) ($p < 0.001$). Depressive symptoms starting after delivery occurred in 5.6% of women. CRH concentration in CSF was not different between women without depressive symptoms and women showing such symptoms during pregnancy or in the postpartum period.

Conclusion: CRH concentration in the CSF was higher in pregnant than in non-pregnant women. However, in this sample, CSF CRH in late pregnancy was not associated with new onset depressive symptoms in the early postpartum period.

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Abbreviations:

Depression following childbirth has an uncertain etiopathogenesis, but is likely to result from the interaction of biological, psychological, and social factors (1). In the biological sphere, special importance has been given to the endocrine system (2), particularly to the hypothalamic-pituitary-adrenal (HPA) axis (3–8), because of its dramatic changes in pregnancy and in the peripartum period (2).

Outside pregnancy, dysregulation of the HPA axis is strongly implicated in the pathophysiology of depression (9), with data indicating its hyperactivity in melancholic depression and hypoactivity in atypical depression (10). HPA axis function undergoes significant changes during pregnancy and after childbirth. Corticotropin releasing hormone (CRH), which is undetectable in the plasma of nonpregnant women, is produced by the placenta throughout pregnancy, increasing exponentially up to the time of delivery, followed by a sharp drop after placental delivery (11). This condition, exclusive to the transition between pregnancy and the postpartum period, led Kamerer et al to propose that depressive symptoms arising during pregnancy and after childbirth could be different and that these differences could be related to the distinct features of HPA axis functioning in the antenatal and postnatal periods. According to these authors, depression arising during pregnancy could be predominantly melancholic and triggered by hyperactivity of the HPA axis, whereas depression beginning in the postpartum period would be predominantly atypical due to hypoactivity of HPA axis (12). Moreover, the authors suggest that these differences should be accounted for when investigating the biological basis of depressive symptoms during pregnancy and in the postpartum period (12).

Several studies have addressed the association between plasma CRH concentrations and perinatal depression, but yielding different results. Some suggest increased plasma CRH levels during pregnancy are associated with increased risk of depression both during pregnancy (3) and after delivery (4, 7, 8), whereas others have not found this association (5).

Because changes in neural circuits involving CRH might have a key role in the development of depression (9, 10, 13–15), we believe that analysis of CRH in the cerebrospinal fluid (CSF) can give more information than analysis of CRH in plasma. Currently, it is not known whether the concentration of CRH in CSF is different between pregnant and nonpregnant women, and the relationship between CRH levels in CSF during pregnancy and the risk of perinatal depression remains largely unexplored. We hence investigated whether there are differences in CSF CRH concentrations between pregnant and nonpregnant women and whether CSF CRH concentrations in late pregnancy are associated with depressive symptoms during pregnancy and early postpartum period.

Materials and Methods

Study Population

From January to February, 2011, all women admitted for elective cesarean delivery with gestational age between 37 and 42 weeks were invited to participate in the study. Exclusion criteria were labor or regular uterine contractions, membrane rupture, twin pregnancy, hypertension, diabetes, use of antidepressant medication in the past six months, use of corticosteroids during pregnancy, and the diagnosis of any obstetric or fetal diseases.

The control group comprised a convenience sample of 22 nonpregnant women recruited from a public hospital in Brasília. From January to February, 2011, women who were subjected to spinal anesthesia for elective surgical sterilization were invited to participate. Similarly to the group of pregnant women, the exclusion criteria for women in the control group were the use of antidepressant medication or corticosteroids in the last six months. This study was approved by the Research Ethics Committee of the Brasilia Catholic University, Brazil.

One hundred and eleven pregnant women met the inclusion criteria. After delivery, four women were not evaluated for the presence of depressive symptoms (three moved to a different city and one expressed the desire to abandon the study); the remaining 107 were analyzed.

Evaluation of depressive symptoms

The Edinburgh postnatal depression scale (EPDS) was used (16), with a cutoff of 13 or more points. The Portuguese version was validated for Brasilia mothers, with a positive predictive value (PPV) of 76% for the EPDS cutoff of 13 or greater (17). The women included in the latter work had social and educational levels similar to the ones in the present sample. EPDS scores were obtained at two different moments, in the hospital immediately before elective cesarean section, and at home four to eight weeks after delivery.

Hormone measures

CSF was not collected at a specific time of the day, as placental CRH secretion is continuous (18). CRH concentration was measured using the enzyme-linked immunosorbent assay (ELISA) (EK-019–06 kit, Phoenix Pharmaceuticals, USA), which is licensed for use with CSF samples. Because CRH binding protein has not been identified in CSF (19), no extraction method for this peptide was employed. The values were converted to a logarithmic scale to induce normality and reported as log-CRH.

Statistical analysis

Differences in age between the pregnant and nonpregnant women were analyzed using the χ^2 test. Analysis of variance of CSF CRH concentrations according to biological and sociodemographic variables was performed using Levene's test. Differences in CSF CRH concentration between pregnant women with and without depressive symptoms and between pregnant and nonpregnant women were analyzed using Student's t test. The program SPSS version 19 was used. $P < .05$ was considered significant.

Results

In pregnant women, mean CSF CRH levels were 66.64 (± 37.39) pg/mL, ranging from 11.72 to 270.61 mg/mL. Mean Log CSF CRH was 4.08 (± 0.51) log pg/mL, ranging from 2.54 to 5.60 log pg/mL. CSF CRH concentrations were significantly higher in pregnant than in nonpregnant women (4.08 ± 0.51 Log CRH and 3.63 ± 0.26 Log CRH, respectively; $P < .0001$; Figure 1).

In pregnant women, there was no significant difference in CSF CRH concentrations according to sociodemographic variables such as parity, education, or marital status. Likewise, there was no difference according to age, body mass index (BMI), or gestational age, as indicated in Table 1.

EPDS scores, obtained during pregnancy and in the postpartum period (6.14 ± 1.29 weeks after childbirth), indicated depressive symptoms in 17 women, with the following distribution: five (4.7%) had depressive symptoms only before delivery, six (5.6%) only after delivery, and six (5.6%) both before and after delivery. CRH concentrations in CSF did not differ between women without depression and women with depression at any time point ($P = .54$), or women with depression only before delivery ($P = .67$), only after delivery ($P = .42$), or both before and after delivery ($P = .87$) (Table 2).

Discussion

This study revealed higher CSF concentrations of CRH in pregnant women when compared to nonpregnant women. This finding differs from the results of the only previously published study addressing this question, in which no difference was found between twelve pregnant and ten nonpregnant women (20). Although both studies examined women at term, this study excluded factors that might interfere with CSF CRH concentrations, such as labor and medications. Another explanation for the differing findings is the remarkable difference in sample sizes. It is plausible to assume that smaller differences in CRH concentration would be revealed only by larger samples.

A strength of this study is the large number of CSF

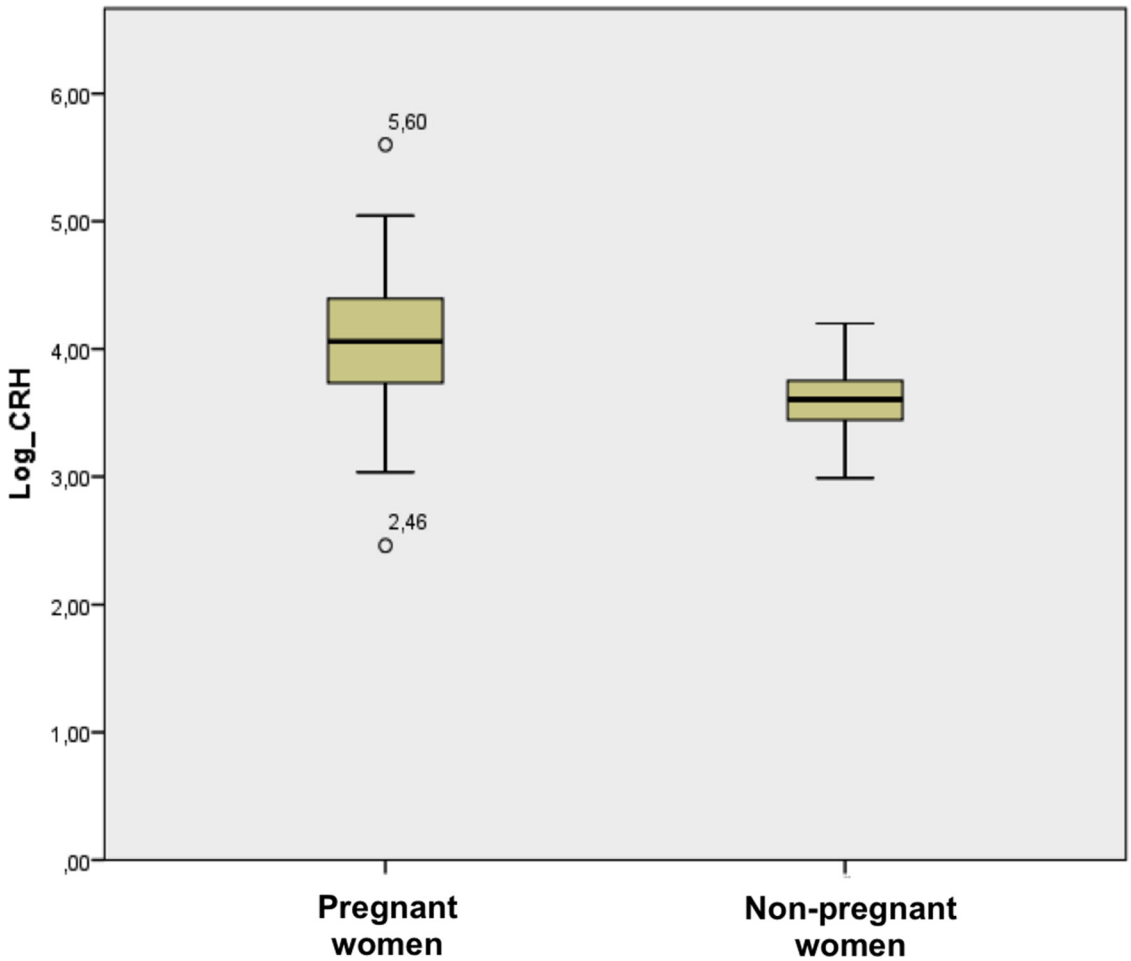

Figure 1. Mean CSF CRH concentration of pregnant (4.1 SD 0.51) and nonpregnant women (3.6 SD 0.26) (p = <0.001). Student's t test. The circles represent outliers.

Table 1. Maternal characteristics and late pregnancy mean CSF logCRH.

	N (%)	Mean (SD) log CRH	F[a]
Maternal age, yr			0.93
< 25	9 (8)	3.96 (0.44)	
25–30	40 (37)	4.05 (0.48)	
31–35	33 (31)	4.10 (0.55)	
36–40	19 (18)	4.13 (0.54)	
> 40	6 (6)	4.16 (0.43)	
Marital status			0.20
Married	84 (79)	4.09 (0.53)	
Cohabiting	9 (8)	4.2 (0.39)	
Single, divorced, other	14 (13)	3.92 (0.31)	
Maternal education			0.63
Less than high school	2 (2)	4.04 (0.5)	
High school completed	13 (12)	3.94 (0.41)	
Some college	10 (9)	4.25 (0.44)	
College degree	57 (53)	4.04 (0.48)	
Graduate degree	25 (23)	4.18 (0.6)	
Parity			0.92
Para 0	60 (56)	4.09 (0.44)	
Para 1 +	47 (44)	4.07 (0.58)	
Maternal body mass index, kg/m^2			0.53
< 25	79 (74)	4.11 (0.55)	
25 – 29.9	17 (16)	3.96 (0.36)	
≥ 30	11 (10)	4.09 (0.32)	
Gestational age at delivery, wk			0.83
< 38	16 (14)	4.02 (0.47)	
38 – 39.9	86 (77)	4.07 (0.52)	
≥ 40	9 (8)	4.14 (0.55)	

[a] Levene's test.

Table 2. Gestational age at delivery, mean EPDS score and mean log CRH (95% confidence interval) by maternal depressive symptoms.

	Age, y (m, SD)	GA at delivery, wk (mean, SD)	EPDS, pregnancy (mean, SD)	EPDS, pp (mean, SD)	Mean log CRH (95% CI)
Control (n = 22)	30.9 (3.275)	NA	NA	NA	3.63 (3.52–3.74)
Pregnant women (n = 107)	31.6 (5.13)	39.0 (0.8)	6.0 (4.7)	6.6 (5.1)	4.08 (3.99–4.18) [a]
DS only during pregnancy (n = 5)	32.2 (4.08)	38.7 (0.71)	14.6 (2.3)	10.4 (1.9)	4.17 (3.66–4.68)
DS only after childbirth (n = 6)	31.3 (5.16)	39.0 (0.68)	7.5 (3.95)	15.2 (1.77)	4.25 (3.77–4.73)
DS during pregnancy and after childbirth (n = 6)	31.8 (3.43)	39.5 (0.94)	17.0 (2.16)	18.5 (2.93)	4.04 (3.68–4.38)
DS during pregnancy or after childbirth (n = 17)	31.7 (4.03)	39.1 (0.86)	12.9 (5.1)	14.9 (4.0)	4.15 (3.95–4.35)
No DS (n = 90)	31.6 (5.33)	38.9 (0.76)	4.7 (3.22)	5.0 (3.56)	4.07 (3.96–4.18)

DS: depressive symptoms (EPDS score of 13 or greater); GA: gestational age; pp: postpartum.

[a] $P < 0.05$ *vs.* control group.

samples analyzed. Studies with CSF are typically composed of small samples in all populations, and especially in pregnant women. The fact that Brazil has culturally a high rate of elective cesarean section in private hospitals enabled the analysis of CSF samples of over one hundred pregnant women without any medical or obstetric condition.

We believe that the CRH found in the CSF of pregnant women is of placental origin, since plasma CRH concentrations increase one thousand-fold at the end of pregnancy, reaching the amount typically found in the pituitary portal system (21). Furthermore, it has been shown that during the early postnatal weeks the response of the HPA axis to stimulation with exogenous CRH is blunted

(22), suggesting reduced CRH hypothalamic secretion. An important question is whether CRH of placental origin crosses the blood-brain barrier. Although this issue has been addressed in few studies, there is data indicating that CRH can cross this membrane (23) and it is therefore possible that placental CRH could mediate central CRH actions.

To date, studies that have addressed the relationship between CRH and perinatal depression have been based on plasma measurement of this hormone and have yielded conflicting results (3–8). Some suggest that elevated plasma CRH levels during pregnancy indicate increased risk of depression in pregnancy but not after delivery (3), whereas others have shown that high circulating CRH levels are associated with increased risk of depression in the postpartum period but not during pregnancy (4, 7, 8). It is important to point that the study with a largest sample size addressing this question, which included over one thousand women during pregnancy and approximately five hundred three months after delivery, found no such association (5), a result that agrees with our findings in the CSF.

Prior studies evaluating the role of CRH in perinatal depression have two major differences when compared with this study: measurement of the hormone in plasma and the time criterion used to define "postpartum depression". Regarding the first point, it should be considered that most of the placental CRH circulates bound to its carrier protein, and therefore it is not possible to assume that peripheral circulating levels completely reflect its level and activity in the central nervous system (CNS). An increase in CSF CRH levels has been demonstrated in depression occurring outside the pregnancy-puerperal cycle, and these higher levels return to normal after antidepressant treatment (24). It is therefore reasonable to imagine that changes in CSF CRH concentrations in late pregnancy are related to the occurrence of depressive symptoms before or after delivery. From this viewpoint, this study adds to the body of knowledge by pioneering the analysis of this relationship by measuring CRH concentrations in the CSF of pregnant women.

The second vulnerable point of studies that have addressed the association of plasma CRH concentrations and perinatal depression heretofore has been that all women with depressive symptoms after delivery (3–8) have been considered to be sufferers of "postpartum depression". These studies have not distinguished women whose symptoms began after delivery from those who were already depressed during pregnancy and persisted in that state after delivery. That, as we discussed elsewhere, may represent a potential bias (25). As it is possible that depression with onset before or after delivery might follow opposite courses regarding the activity of the hypothalamic-pituitary-adrenal axis (12), it is possible that CRH concentrations in these two groups are different. Our major focus in this observational study was to obtain some insights into the role of CRH in the development of depressive symptoms first arising in the postpartum period. Therefore, in this study, we have taken care to distinguish women with depressive symptoms before childbirth from those with new onset symptoms in the postpartum period, but found no association between CSF CRH concentrations and the presence of depressive symptoms at either time point analyzed, ie, before or after delivery.

We excluded women who were using or had used antidepressants recently because these treatment affects the CRH levels in CSF (24). This might have excluded a number of women at risk for postpartum depression, but enabled us to analyze a group of women that would be more representative of patients with a subtype of depressive symptoms related to a greater susceptibility of the CNS to hormonal changes in the postpartum period. The strict definition of PPD used here (beginning after childbirth) may have been the reason for the low frequency of women with depressive symptoms in our sample (5.6%). It is important to point that a previous study that similarly distinguished depressive symptoms according to the time point of their first occurrence found a similar rate of new onset depression in the postpartum period (5.7%) (26). We believe that the inclusion of women at risk for depression in the postpartum period is of great clinical importance in investigations addressing potential markers of depression after childbirth that can eventually be used to predict the occurrence of depression at this time point. Since our study focused on the biological basis of depression arising in the postpartum period, we analyzed a less heterogeneous group, comprised by women who presented with depressive symptoms exclusively after delivery.

It is not at all surprising that in this study, and in the largest one evaluating the relationship between plasma CRH concentration and perinatal depressive disorders (5), no association between CRH and perinatal depression was found. A broad approach to the etiology of depression should include factors such as genetic vulnerability, the individual's personality, and biological and social influences. The attempt to find a unique biological factor that explains the disorder is not in keeping with current concepts regarding the genesis of mood disorders.

This study has some limitations. The instrument used to define depression was the EPDS rather than a structured interview, which is the gold standard for diagnosis of this disorder. Additionally, although the women were asked to describe their feelings over the previous seven days and not

only on the day itself, the time point when depressive symptoms were first assessed by using the EPDS, immediately before the cesarean, may have been inappropriate.

Measurement of CSF CRH concentration at late pregnancy may also be viewed as a potential pitfall in detecting differences between women with and without depressive symptoms, due to its high levels at this time point. In a previous study addressing the association between plasma CRH levels and postpartum depression, plasma CRH concentrations were determined at the 15th, 19th, 25th, 31st and 37th weeks, and it was shown that women who presented depressive symptoms in the postpartum period had higher plasma CRH concentrations after the 19th week. Moreover, at late pregnancy, the difference in plasma CRH concentrations between women who presented depressive symptoms after childbirth and those who did not was even higher (4). It is therefore possible that differences in CSF CRH concentrations determined at late pregnancy would have been detected.

In conclusion, mean CSF CRH concentration in pregnant women was significantly higher than in nonpregnant women. CRH concentration in CSF measured in late pregnancy was no different between women with and without depressive symptoms during pregnancy nor between women with and without depressive symptoms after delivery. These results suggest that CRH concentration in the CSF in late pregnancy may not play a significant rol in the pathophysiology of depression arising in the postpartum period.

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