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Maternal stress during pregnancy is associated with decreased cortisol and cortisone levels in neonatal hair

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ABSTRACT

Background

Hair glucocorticoids (GCs) offer a retrospective view on chronic GC exposure. We assessed whether maternal pre- and postnatal stress was associated with neonatal and maternal hair GCs postpartum (pp).

Methods

On the first day pp 172 mother-infant pairs donated hair, of whom 67 had consulted a center of expertise for psychiatric disorders during pregnancy. Maternal stress was scored on the Hospital Anxiety and Depression Scale during the first/second (n = 46), third trimester (n = 57), and pp (n = 172). Hair cortisol and cortisone levels were determined by liquid chromatography-tandem mass spectrometry, and associations with maternal hospital anxiety subscale (HAS) and hospital depression subscale (HDS) scores, and antidepressant use were analyzed with linear regression.

Results

Neonatal hair GCs were negatively associated with elevated HAS-scores during the first/second trimester, log 10 (β [95% CI]) cortisol −0.19 (–0.39 to 0.02) p = 0.07, cortisone −0.10 (–0.25 to 0.05) p = 0.17; third trimester, cortisol −0.17 (–0.33 to 0.00) p = 0.05, cortisone −0.17 (–0.28 to –0.05) p = 0.01; and pp, cortisol −0.14 (–0.25 to –0.02) p = 0.02, cortisone −0.07 (–0.16 to 0.02) p = 0.10. A similar pattern was observed for elevated HDS-scores. Maternal hair GCs were positively associated with elevated HAS-scores pp (cortisol 0.17 [0.01 to 0.32] p = 0.04, cortisone 0.18 [0.06 to 0.31] p = 0.01), but not prenatally or with elevated HDS-scores. Antidepressant use was associated with elevated maternal hair GCs ($p \leq 0.05$), but not with neonatal hair GCs.

Conclusion

Exposure to excessive pre- and perinatal maternal stress was associated with a decrease in neonatal hair GCs, while elevated stress-scores around birth were associated with increased maternal hair GCs and elevated stress-scores earlier in gestation were not associated with maternal hair GCs pp. Further studies are needed to test associations with infant neurodevelopment.

INTRODUCTION

Anxiety or depressive disorders are associated with alterations in hypothalamic-pituitary-adrenal (HPA) axis activity and reactivity, although the evidence is not unequivocal.[1-4] Anxiety and depressive disorders are common in pregnancy, with numbers ranging from 1 in 10 to 1 in 5 pregnant women.[5-7] Although many observational studies described associations between prenatal exposure to maternal stress and neurodevelopmental problems,[8,9] caution must be exercised in the interpretation of some of these findings due to the use of subjective measures of stress, while quantitative indices of HPA axis activity are lacking.[10]

As part of the physiological changes during pregnancy, both maternal and fetal glucocorticoids (GCs) exert a positive feedback effect on the placenta by stimulating the synthesis of placental corticotropin-releasing hormone (CRH). Due to this physiological feed-forward response, maternal cortisol increases during gestation.[11] At the same time, increasing estrogen levels augment the synthesis of corticosteroid-binding globulin,[12] resulting in only a modest increase in free cortisol.[13] Moreover, placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts maternal cortisol to inert cortisone. Accordingly, the fetus is partially protected from overexposure to maternal cortisol.[14] Lower placental 11β-HSD2 activity and, consequently, increased delivery of maternal cortisol to the fetus has been associated with decreased fetal growth.[15] Longer-term consequences of increased fetal exposure to maternal cortisol may include increased HPA axis reactivity and susceptibility to neurodevelopmental problems.[8]

Hair cortisol and cortisone levels represent long-term GC exposure in adults and children above the age of 4 years.[16-18] Accordingly, GC levels in newborn hair might offer a retrospective view on the GC regulation during the last part of pregnancy.[19,20] Kapoor et al. studied hair GC levels in the offspring of rhesus monkeys that were randomized to receive exposure to a startle paradigm for 10 minutes per day, 5 days a week for one-fifth the duration of pregnancy, and found decreased hair cortisol in the exposed offspring, but no difference in hair cortisone.[21] In humans, cortisol in neonatal hair obtained directly postpartum (pp) was higher with advancing gestational age and birth weight.[22] Unfortunately, in this study the impact of maternal stress was not studied. A recent study in humans,[20] testing neonatal hair GC levels in association with maternal hair GC levels and perceived stress, found similar results as Kapoor et al [21] did. However, hair cortisone levels were not taken into account, and hair cortisol levels were measured with an immunoassay technique. Moreover, this study had only included physically and mentally healthy mothers, presumably with low amounts of prenatal stress.

Therefore, in the present study we assessed whether pre- and perinatal exposure to maternal stress is associated with neonatal hair cortisol and cortisone levels directly pp. In addition, we tested associations between maternal stress and GCs in maternal hair

obtained at the same time. To this end, we used data from a cohort in which women with severe distress during pregnancy were overrepresented.

METHODS

Study design and participants

The present study was part of a prospective cohort study that aimed to explore biomarkers, including neonatal hair GCs and 5-hydroxyindoleacetic acid level in urine, for poor neonatal adaptation after prenatal exposure to selective antidepressants (SADs) and maternal stress.[23,24]

A total of 172 mother-infant pairs were recruited at the maternity department, as well as at the psychiatric-obstetric-pediatric (POP) clinic of the OLVG-West Hospital, Amsterdam, The Netherlands, which offers consultation to women with psychiatric disorders before, during, and after pregnancy on an outpatient basis. The reasons for which pregnant women sought advice at the POP clinic were (1) a history of psychiatric disease, and/or (2) symptoms of distress, and/or (3) current or past use of antidepressants. Approximately one-third (n =65) of our sample consisted of women who visited the POP clinic. The other part of the sample (n =107) consisted of mothers admitted pp to the maternity ward for medical reasons in themselves and/or in their infants. Therefore, in this cohort women who experienced severe distress during pregnancy were overrepresented. Among participants, 66 (38%) used SADs, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), noradrenergic or specific serotonin antidepressants (NaSSAs), or a combination of these. Sixty-four of these women sought advise at the POP clinic.

Inclusion and exclusion criteria were similar for both groups. Inclusion criteria were: an expected hospital stay of ≥72 hours after delivery, and willingness to donate hair from themselves and their infants, and to complete the Hospital Anxiety and Depression Scale (HADS) questionnaire directly pp. Exclusion criteria were: use of psychotropic medication other than SADs, use of systemic corticosteroids, non-pharmacologic drugs, or alcohol, smoking during the third trimester of pregnancy, insufficient knowledge of the Dutch or English language, mental impairment of one or both parents, and multiple pregnancies. Parents were informed and written informed consent was obtained within 24 hours after delivery. The study was approved by the Medical Ethics Committees of the OLVG-West Hospital and the VU University Medical Center.

Assessment of maternal stress

As part of standard care at the POP clinic, the HADS [25] was administered as an index of stress experienced in the previous week. The HADS contains 14 items, namely 7 for

anxiety and 7 for depression. From these items, Hospital Anxiety Subscale (HAS) and Hospital Depression Subscale (HDS) scores are derived. A score ≥8 (out of 21 points for each subscale separately) is considered the cut-off for relevant stress.[25,26] The retest reliability of the HADS was found to correlate well with the previous 6 weeks.[26] Furthermore, among pregnant women, the anxiety subscales of the HADS and the Edinburgh Postnatal Depression Scale showed strong correlation.[27]

During the first or second trimester, and/or the third trimester, the HADS was administered only in the women who visited the POP clinic. Directly pp, that is, within 12 to 36 hours, the HADS was administered in all women. At the maternity ward, mothers were asked whether they had used SADs at least during the last two weeks of pregnancy.

Hair glucocorticoid levels

Mother-infant pairs donated hair on the first day pp. A lock of hair was cut from the posterior vertex as close as possible to the scalp. A minimum of 1.25 mg hair is needed for a reliable measurement. Fetal hair growth velocity and the timing of transition from lanugo via vellus into terminal hair strands varies significantly between infants.[28] Therefore, the total length of neonatal hair was analyzed. In mothers, the centimeter of hair closest to the scalp was analyzed, representing the mean levels of cortisol and cortisone during the last month of gestation, as adult hair grows approximately 1 (range: 0.6 – 1.4) cm per month.[29] There is evidence of 11β-HSD2 expression in human eccrine sweat glands and vascular endothelium,[30] raising the possibility of local conversion of blood-borne cortisol to cortisone within skin and/or hair follicles. Therefore, it is unknown which analyte is the best representative of serum cortisol. The sum of hair GCs might be indicative of chronic circulating cortisol and was therefore calculated.

Hair cortisol and cortisone levels were measured as described previously by Noppe et al.[17] In short, hair was washed with isopropanol, and hair GCs were extracted using methanol and solid-phase extraction. Subsequently, cortisol and cortisone concentrations were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Waters XEVO-TQ-S system, Waters Corporation, Milford, MA, USA) with positive electrospray ionization, and reported in pg/mg hair. The Lower Limit of Quantitation (LLoQ) of our assay is dependent on the amount of hair extracted. An intra-assay CV of 8.9% was measured at a hair cortisol concentration of 1.8 pg/mg. The intra-assay CV for hair cortisone was 4.4% at a level of 12.5 pg/mg.

Data analysis

Maternal and neonatal characteristics were compared between the pairs whose mothers visited the POP clinic and the pairs admitted for medical reasons, using independent t-tests, Chi Square, or Fisher exact tests (Table 1).

Table 1: Characteristics of mother-infant pairs

			Total group (n =172)		POP mothers (n=67)		Other mothers (n=105)	
Neonatal	Males		92	(53%)	34	(51%)	58	(55%)
	Gestational age	wks	39.4	± 1.7	39.2	± 1.6	39.5	± 1.7
	Birth weight	g	3,445.5	± 582.6	3358.5	± 511.4	3500.2	± 619.3
		percentile	53.6	± 26.4	49.3	± 24.0	56.3	± 27.6
	Hair cortisol pp	pg/mg hair	162.8	(102.8 – 232.2)	155.3	(111.4 – 202.9)	171.3	(96.8 – 291.0)
	Hair cortisone pp	pg/mg hair	83.2	(63.1 – 109.8)	79.3	(63.9 – 105.1)	87.2	(61.4 – 128.9)
Maternal	Primiparous		85	(49%)	29	(43%)	56	(53%)
	Age	yr	33.8	± 4.7	33.7	± 4.5	34.0	± 4.8
	Ethnicity	Dutch	97	(56%)	41	(61%)	56	(53%)
		Caucasian, non-Dutch	16	(9%)	4	(6%)	12	(11%)
		non- Caucasian	59	(35%)	22	(33%)	37	(35%)
	Antidepressants	SSRI	45	(26%)	44	(66%)	1†	(1%)
		SNRI	7	(4%)	7	(10%)	-†	
		NaSSA	9	(5%)	8	(12%)	1†	(1%)
		Combination*	5	(3%)	5	(8%)	-†	
	HADS score pp	HAS score ≥8	30	(17%)	19	(28%)	11†	(11%)
		HDS score ≥8	19	(11%)	11	(16%)	8	(8%)
	Hair cortisol pp	pg/mg hair	5.4	(3.6 – 10.6)	6.9	(4.4 – 12.0)	4.8†	(3.4 – 9.9)
	Hair cortisone pp	pg/mg hair	19.5	(14.5 – 31.2)	21.7	(15.4 – 46.9)	18.2†	(12.9 – 26.8)

Data are presented as mean ± SD, median (interquartile range), or n (%). Abbreviations: pp = postpartum; HADS = Hospital Anxiety and Depression Scale; HAS = Hospital Anxiety Scale; HDS = Hospital Depression Scale; SSRI = selective serotonin reuptake inhibitors; SNRI = serotonin-norepinephrine reuptake inhibitors, NaSSA = noradrenergic or specific serotonin antidepressants.

* These women were treated with a combination of SSRI with NaSSA (n =4), or NaSSA with SNRI (n =1) † Different from POP mothers, $P < 0.05$

Hair cortisol and cortisone levels were skewed to the right and therefore logarithmically transformed prior to analysis. Linear regression was used to assess associations between HADS scores and hair GC levels. Associations with maternal stress were assessed with hair GC level as dependent factor, and HAS or HDS score as continuous or dichotomous (with a score of ≥8 points as cut-off for elevated stress) independent factor.[25] Among infants whose mothers visited the POP clinic, the relative contributions of pre- and perinatal stress exposure were tested by using combinations of (1) low prenatal and low perinatal (reference), (2) low prenatal and high perinatal, (3) high prenatal and low perinatal, and (4) high prenatal and high perinatal levels of stress exposure. Low prenatal stress exposure was defined as low HAS and HDS scores in both the first/second and the third trimester, while high prenatal stress exposure was defined as a score ≥8 on one or both subscales in the first/second and/or the third trimester. Likewise, low peri-

natal stress exposure was defined as low HAS and HDS scores pp, while high perinatal stress exposure was defined as a score ≥8 on one or both subscales pp. Associations with maternal SAD use were analyzed with hair GC level as dependent factor, and SAD use as dichotomous independent factor.

Confounders were selected a priori, based on the literature.[21-23,31] Sex, birth weight percentile, gestational age, and primiparity were added to the multivariable model, one by one. Subsequently, based on statistical impact (i.e., a >10% change in beta) the final model was created. When a confounder was found to have a statistical impact on more than 50% of the associations being analyzed, we also explored the univariate association with the outcome. In addition, similar to Kapoor et al.,[21] interaction between perinatal stress (HADS scores pp) and sex on neonatal hair GC levels was tested.

RESULTS

The characteristics of participants are shown in Table 1. A total of 67 women visited the POP clinic, of whom 98% reported SAD use, 28% had an elevated HAS score, and 16% had an elevated HDS score. For mothers admitted pp for medical reasons in themselves and/or in their infants (n =105), these numbers (2%, 11%, and 8%, respectively) were similar to previously reported prevalence rates in the normal population.[5-7,32] Sex distribution, gestational age, birth weight, parity, maternal age, and ethnicity did not differ between the groups. Neonatal hair cortisone levels were significantly lower in female neonates (median [IQR]: 75.1 [59.8 – 99.7] pg/mg for females and 92.1 [65.4 – 129.2] pg/mg for males, p=0.049). Neonatal hair cortisol levels did not differ significantly between the sexes.

The characteristics of mother-infant pairs by time point are shown in Supplementary Table 1. The great majority of women who visited the POP clinic during the first or second, and/or the third trimester, used SADs: 44 out of 46 (96%) and 54 out of 57 (95%) respectively. Those who visited the POP clinic during the first or second trimester, and/or third trimester, more often used SADs during the entire pregnancy: 41 out of 46 (89%) and 43 out of 57 (75%) respectively.

The association between maternal stress and neonatal hair GCs

HADS scores during pregnancy were only known for the mothers who visited the POP clinic (n=65), namely, 46 during the first or second trimester, and 57 during the third trimester. As part of the routine follow-up, 38 of them were seen on both occasions – 8 only during the first or second trimester, and 19 only during the third trimester (Supplementary Figure 1).

Table 2: Maternal stress in association with neonatal hair cortisol and cortisone levels

			Postpartum (n =166)		**3rd trimester** (n =57)		**1st – 2nd trimester** (n =45)	
			crude	adjusted	crude	adjusted	crude	adjusted
Cortisol	**Anxiety**	HAS score	-0.02 (-0.03; 0) *	-0.01 (-0.02; 0)	-0.02 (-0.03; 0) *	-0.01 (-0.03; 0)	-0.02 (-0.04; 0) *	-0.03 (-0.04; -0.01) *
		Elevated HAS	-0.14 (-0.26; -0.03) *	-0.09 (-0.20; 0.01)	-0.17 (-0.33; 0) *	-0.10 (-0.26; 0.07)	-0.19 (-0.39; 0.02)	-0.22 (-0.40; -0.03) *
	Depression	HDS score	-0.01 (-0.03; 0)	-0.01 (-0.02; 0)	-0.01 (-0.03; 0)	-0.01 (-0.03; 0.01)	-0.03 (-0.05; -0.01) *	-0.03 (-0.05; -0.01) *
		Elevated HDS	-0.12 (-0.26; 0.02)	-0.10 (-0.23; 0.02)	-0.19 (-0.38; -0.01) *	-0.12 (-0.31; 0.07)	-0.35 (-0.57; -0.12) *	-0.32 (-0.53; -0. 11) *
Cortisone	**Anxiety**	HAS score	-0.01 (-0.02; 0) *	-0.01 (-0.02; 0)	-0.01 (-0.03; 0) *	-0.01 (-0.02; 0.01)	-0.01 (-0.03; 0.01)	-0.01 (-0.03; 0)
		Elevated HAS	-0.09 (-0.18; 0.00) *	-0.07 (-0.16; 0.02)	-0.17 (-0.28; -0.05) *	-0.12 (-0.23; 0.00)	-0.10 (-0.25; 0.05)	-0.12 (-0.26; 0.01)
	Depression	HDS score	-0.01 (-0.02; 0) *	-0.01 (-0.02; 0) *	-0.01 (-0.02; 0)	-0.01 (-0.02; 0.01)	-0.01 (-0.02; 0.01)	-0.01 (-0.02; 0.01)
		Elevated HDS	-0.10 (-0.21; 0.01)	-0.09 (-0.19; 0.02)	-0.17 (-0.31; -0.03) *	-0.11 (-0.25; 0.03)	-0.09 (-0.27; 0.09)	-0.07 (-0.24; 0.10)

Data are presented as Log10-transformed β (95% CI). The adjusted model is corrected for primiparity.

Abbreviations: HAS = Hospital Anxiety Scale; Elevated HAS = score ≥8; HDS = Hospital Depression Scale; Elevated HDS = score ≥8

* $p < 0.05$

We were able to collect enough hair (median [IQR]: 5.1 [3.2 – 8.9] mg, while at least 1.25 mg is required) in 166 newborns (97%). In crude analyses, maternal anxiety and depression experienced during pregnancy or pp were negatively associated with the neonatal hair cortisol level (Table 2). Similar, but weaker, associations were found for the neonatal hair cortisone level. Associations with the sum of neonatal hair GCs resembled those with neonatal hair cortisol alone (data not shown). Correction for sex, gestational age, or birth weight percentile did not change these associations. Correction for primiparity strengthened the associations with first or second trimester stress scores. Associations with maternal stress during the third trimester or pp became non-significant or disappeared when parity was factored in (Table 2). When tested univariately, primiparity was associated with higher neonatal hair cortisol and cortisone levels (log10-transformed β [95% CI]: 0.24 [0.16; 0.32] p <0.001, and 0.10 [0.04; 0.17] p <0.001, respectively). There was no evidence for interaction between dichotomous HADS, HAS, or HDS scores and sex on neonatal hair GC levels.

Among the infants whose mothers visited the POP clinic, persistent exposure to elevated maternal stress was associated with the largest decrease in neonatal hair GC levels (Table 3).

Table 3: Maternal stress over time among the women who visited the POP clinic, in association with neonatal hair cortisol and cortisone levels.

	Exposure category	*n*	**Cortisol**		**Cortisone**	
			Beta	(95% CI)	Beta	(95% CI)
HAS	Low prenatally & low perinatally	36	ref		ref	
	High prenatally & low perinatally	11	-0.09	(-0.30; 0.12)	-0.14	(-0.29; 0.02)
	Low prenatally & high perinatally	6	-0.03	(-0.29; 0.23)	0.01	(-0.18; 0.20)
	High prenatally & high perinatally	12	-0.19	(-0.39; 0.01)	-0.15	(-0.29;- 0.01) *
HDS	Low prenatally & low perinatally	50	ref		ref	
	High prenatally & low perinatally	2	-0.09	(-0.36; 0.19)	-0.18	(-0.38; 0.03)
	Low prenatally & high perinatally	5	-0.13	(-0.55; 0.29)	-0.14	(-0.45; 0.17)
	High prenatally & high perinatally	8	-0.27	(-0.49;- 0.05) *	-0.17	(-0.33; 0.00) *

Data are presented as Log10-transformed β (95% CI). Abbreviations: HAS = Hospital Anxiety Scale; HDS = Hospital Depression Scale score * p <0.05

The association between maternal stress and maternal hair GCs

Maternal anxiety experienced directly pp, but not in gestation, was positively associated with maternal hair cortisol and cortisone levels pp (Table 4). Similar findings were obtained with the sum of maternal hair GCs (data not shown). Correction for sex, gestational age, birth weight percentile, or primiparity did not change these associations.

Table 4: Maternal stress in association with maternal hair cortisol and cortisone levels

			Postpartum (n= 169)		**3rd trimester** (n= 56)		**1st – 2nd trimester** (n= 45)	
			crude	adjusted	crude	adjusted	crude	adjusted
Cortisol	**Anxiety**	HAS score	0.01 (-0.01; 0.03)	0.01 (0; 0.03)	-0.01 (-0.04; 0.01)	-0.01 (-0.03; 0.01)	0 (-0.03; 0.03)	0 (-0.03; 0.03)
		Elevated HAS	0.15 (0; 0.30)	0.18 (0.03; 0.33) *	-0.14 (-0.36; 0.08)	-0.08 (-0.30; 0.15)	-0.03 (-0.30; 0.25)	-0.05 (-0.33; 0.22)
	Depression	HDS score	0 (-0.02; 0.02)	0 (-0.02; 0.02)	0 (-0.03; 0.02)	0 (-0.02; 0.02)	0.01 (-0.03; 0.04)	0.01 (-0.02; 0.04)
		Elevated HDS	0.02 (-0.17; 0.20)	0.03 (-0.15; 0.21)	-0.08 (-0.33; 0.17)	-0.01 (-0.26; 0.25)	0.18 (-0.14; 0.50)	0.21 (-0.11; 0.53)
Cortisone	**Anxiety**	HAS score	0.02 (0; 0.03) *	0.02 (0.01; 0.03) *	-0.01 (-0.03; 0.01)	-0.01 (-0.03; 0.02)	0 (-0.02; 0.03)	0 (-0.02; 0.02)
		Elevated HAS	0.17 (0.05; 0.29) *	0.19 (0.07; 0.31) *	-0.15 (-0.35; 0.04)	-0.11 (-0.31; 0.10)	-0.04 (-0.27; 0.18)	-0.06 (-0.29; 0.17)
	Depression	HDS score	0.01 (-0.01; 0.02)	0.01 (-0.01; 0.02)	0 (-0.02; 0.02)	0 (-0.02; 0.02)	0 (-0.03; 0.03)	0 (-0.02; 0.03)
		Elevated HDS	0.10 (-0.05; 0.25)	0.11 (-0.04; 0.25)	-0.01 (-0.23; 0.21)	0.06 (-0.17; 0.29)	0.11 (-0.16; 0.39)	0.13 (-0.15; 0.41)

Data are presented as Log10-transformed β (95% CI). The adjusted model is corrected for primiparity.

Abbreviations: HAS = Hospital Anxiety Scale; Elevated HAS = score ≥8; HDS = Hospital Depression Scale; Elevated HDS = score ≥8

* $p<0.05$

The association between SAD use and neonatal and maternal hair glucocorticoid levels

Sixty-six women (38%) used SADs. Forty-five women were on SSRIs, 7 on SNRIs, 9 on NaSSAs, and 5 on a combination of these, including SSRIs with NaSSAs (in 4) and NaSSAs with SNRIs (n=1).

The use of SADs was not associated with neonatal hair GC levels, but it was positively associated with maternal hair cortisol and cortisone levels pp (log-10 transformed β [95% CI]: 0.14 [0.02, 0.26] p =0.02, and 0.18 [0.09; 0.27] p <0.001 respectively).

DISCUSSION

In this study, maternal stress during pregnancy and pp was associated with decreased neonatal hair GC levels, with the lowest values seen in the infants of mothers with persistent stress. In addition, maternal distress pp, but not in gestation, was associated with increased maternal hair GC levels pp. SAD use during pregnancy was unlikely to explain these associations.

We recently published data suggesting that neonatal hair GC levels are influenced by the third-trimester increase in HPA axis activity.[19] This phenomenon might offer an explanation for the high neonatal hair GC levels observed in our study, although evidence is lacking concerning which part of intrauterine GC regulation is reflected in neonatal hair. It has been hypothesized that neonatal hair GC levels might reflect amniotic-fluid GC levels.[21] However, this must be balanced against evidence from studies in adults suggesting that hair GCs reflect the body's HPA axis activity, with hair GCs being associated with long-term integrated salivary cortisol.[33] It is conceivable that neonatal hair GCs reflect a combination of maternal and fetal GCs, determined by placental factors like the third-trimester increase in placental CRH and 11β-HSD2 activity and fetal adrenal maturation. The balance between cortisol and cortisone in hair may be different than in blood, as 11β-HSD2 is widely expressed in epithelial tissues.[30]

It is unclear why the HAS score, but not the HDS score pp, was positively associated with maternal hair GCs pp. In general, in patients with psychiatric symptomatology anxiety and depressive symptoms are highly overlapping. Findings from studies investigating associations between HADS scores and indices of HPA axis activity in matrices other than hair, like saliva, are highly contradictory. One study found that the HAS score, but not the HDS score, correlated positively with the cortisol awakening response among patients with coronary artery disease.[34] Another study among patients with coronary artery disease found that the HDS score correlated positively with salivary cortisol in men, but not in women, while in women, but not in men, the HAS score correlated negatively with salivary cortisol.[35] In patients with low back pain, HADS scores were unrelated to

the diurnal cortisol rhythm measured in saliva,[36] while in the normal population, HADS scores were inversely associated with peak salivary cortisol during a psychological stress protocol.[37]

Previous studies have shown that maternal stress is associated with reduced placental 11β-HSD2 activity,[38-41] thereby allowing a larger proportion of maternal cortisol to reach the fetus. In contrast, we found that higher maternal stress scores were associated with lower neonatal hair GCs levels, in spite of evidence of increased maternal HPA axis activity. There are several explanations possible for these discrepant findings. First, maternal HPA axis activity may be lower in those pregnant women with chronic stress, so that, despite reduced placental 11β-HSD2 activity, a smaller amount of cortisol is able to cross the placenta. Some studies have shown blunted HPA axis activity in chronic stress,[4] but it is unclear whether their findings could be extrapolated to pregnancy, when HPA activity is regulated to a large extent by placental CRH. However, the stressed women in our cohort had elevated hair GC levels, representing increased HPA axis activity in the last trimester. Second, the third-trimester surge in the production of GCs by the fetal adrenal probably contributes much more to the fetal GC pool than maternal HPA axis activity, at least in the last part of gestation. Still, the decrease in placental 11β-HSD2 activity as observed in chronic distress could contribute significantly to the fetal GC pool at earlier stages of gestation, when placental 11β-HSD2 activity might be lower.[42] We speculate that increased exposure to maternal cortisol earlier in gestation might lead to a long-lasting suppression of fetal HPA axis activity. Analysis of the entire neonatal hair, which we did in our study, probably reflects the endocrine milieu during the last trimester, as it may take 20 weeks until the scalp is fully covered with anagen phase hair, turning into the catagen phase between week 24 and 28.[43]

In rat pups, GC receptor density in the forebrain was found to develop in a sex-specific manner, with females having more GC receptors than males.[44] It could therefore be hypothesized that prenatal maternal stress might have sex-specific effects on neonatal HPA axis activity. However, we as well as others did not find evidence for sex differences in, or sex-specificity of the effects of prenatal maternal stress on, neonatal hair GC levels.[20-22]

Fetal exposure to maternal stress or to excessive maternal GCs has been associated with neurodevelopmental problems as well as alterations in HPA axis settings.[8,9] The mechanisms behind these associations are not yet fully elucidated. Therefore, long-term follow-up of our cohort is warranted to explore associations with HPA axis development, including aspects like stress reactivity and development of diurnal rhythmicity, in addition to neurodevelopment.[12]

The major strength of this study is the unique sample of women experiencing a wide range of stress levels during pregnancy and pp, including an overrepresentation of severely distressed women. In addition, to the best of our knowledge, this is the first translational study that measured hair GC levels in both infants and their mothers who

experienced a reliably quantified amount of stress during pregnancy and pp.[45] A limitation of our study is that HADS scores during pregnancy were known only for the women who sought consultation at the POP clinic. Other limitations are the lack of information on neonatal hair growth (and, thereby, the lack of standardization on newborn hair length), and the self-report of SAD use. In addition, the small sample size did not allow us to test associations by type of SAD. Lastly, ideally other tests of HPA axis activity, relating to HPA axis reactivity and rhythmicity, should have been included too.[46]

In conclusion, we found that maternal stress was associated with increased maternal hair GC levels and decreased neonatal hair GC levels, with the lowest values seen in children of mothers with persistent distress throughout pregnancy. It is suggested that maternal stress during pregnancy increased intra-uterine GC exposure, thereby suppressing fetal HPA axis activity.

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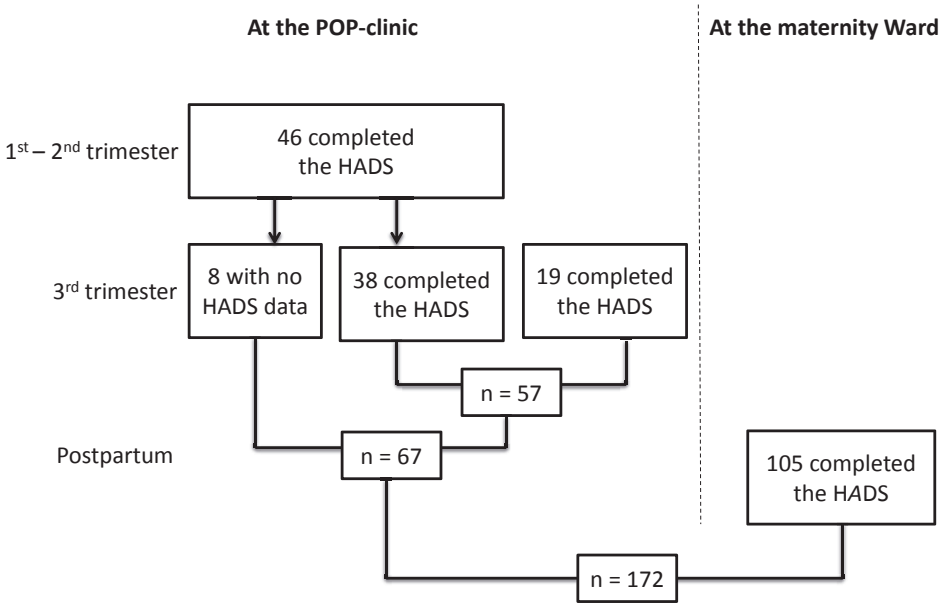

Supplementary Figure 1: Flowchart of pre- and postnatal assessments.

Supplementary Table 1: Characteristics of mother-infant pairs by time point

			Pairs with available HADS data in 1st-2nd trimester		Pairs with available HADS data in 3rd trimester		Pairs with available HADS data pp	
		N =	**46**		**57**		**172**	
Neonatal	Gestational age	wks	39.1	± 1.5	39.3	± 1.6	39.4	± 1.7
	Birth weight	g	3,328	± 540	3,370	± 497	3,446	± 583
		percentile	48.7	± 24.7	48.4	± 23.6	53.6	± 26.4
	Hair cortisol pp	pg/mg hair	156.3	(114.0 – 222.9)	154.3	(110.3 – 204.6)	162.8	(102.8 – 232.2)
	Hair cortisone pp	pg/mg hair	84.7	(64.5 – 109.1)	79.7	(63.9 – 106.3)	83.2	(63.1 – 109.8)
Maternal	Type of SAD	SSRI	33	(72%)	37	(65%)	45	(26%)
		SNRI	6	(13%)	5	(9%)	7	(4%)
		NaSSA	2	(4%)	8	(14%)	9	(5%)
		Combination*	3	(7%)	4	(7%)	5	(3%)
	Timing of SAD use	Entire pregnancy	41	(89%)	43	(75%)	54	(31%)
		≥3rd trimester	2	(4%)	3	(5%)	3	(2%)
		<3rd trimester	1	(2%)	8	(14%)	9	(5%)
	HADS scores 1st-2nd trimester	HAS score ≥8	17	(37%)	15	(26%)	17	(10%)
		HDS score ≥8	9	(20%)	8	(14%)	9	(5%)
	HADS scores 3rd trimester	HAS score ≥8	11	(24%)	23	(40%)	23	(13%)
		HDS score ≥8	7	(15%)	14	(25%)	14	(8%)
	HADS scores pp	HAS score ≥8	13	(28%)	17	(30%)	30	(17%)
		HDS score ≥8	7	(15%)	9	(16%)	19	(11%)
	Hair cortisol pp	pg/mg hair	6.9	(3.9 – 13.9)	7.1	(4.6 – 13.9)	5.4	(3.6 – 10.6)
	Hair cortisone pp	pg/mg hair	23.1	(16.9 – 62.1)	25.3	(17.5 – 54.8)	19.5	(14.5 – 31.2)

Data are expressed as mean ± SD, median (interquartile range), or n (%). Abbreviations: pp = postpartum; SAD = Selective antidepressant; HADS = Hospital Anxiety and Depression Scale; HAS = Hospital Anxiety Scale; HDS = Hospital Depression Scale; SSRI = selective serotonin reuptake inhibitors; SNRI = serotonin-norepinephrine reuptake inhibitors, NaSSA = noradrenergic or specific serotonin antidepressants.
*These subjects were treated with SSRI and NaSSA (n =4), or with NaSSA and SNRI (n =1).