

Maternal cortisol in late pregnancy and hypothalamic–pituitary–adrenal reactivity to psychosocial stress postpartum in women

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Abstract

Hypothalamic–pituitary–adrenal (HPA) activity is altered postpartum and has been associated with several puerperal disorders. However, little is known about the association of maternal HPA activity during pregnancy with maternal HPA responsiveness to stress after parturition. Within a longitudinal study with an experimental component, we assessed in 22 women the salivary cortisol awakening response (CAR) at the 36th week of gestation and 6 weeks postpartum, as well as pituitary–adrenal and emotional responses to a psychosocial laboratory stressor at 8 weeks postpartum. CAR in late pregnancy negatively predicted maternal adrenocorticotropic (ACTH; $\beta = -0.60$; $P = 0.003$), plasma cortisol ($\beta = -0.69$, $P < 0.001$), and salivary cortisol ($\beta = -0.66$; $P = 0.001$) but not emotional stress reactivity (all $P > 0.05$) at 8 weeks postpartum, whereas CAR at 6 weeks postpartum failed to predict hormonal (ACTH: $\beta = 0.02$; $P = 0.933$, plasma cortisol: $\beta = -0.23$; $P = 0.407$, salivary cortisol: $\beta = -0.15$; $P = 0.597$) or emotional (all $P > 0.05$) stress responses at 8 weeks postpartum. The activity of the HPA axis during pregnancy is associated with maternal HPA responsiveness to stress postpartum. Putative biological underpinnings warrant further attention. A better understanding of stress-related processes peripartum may pave the way for the prevention of associated puerperal disorders.

Keywords: cortisol awakening response (CAR), hypothalamic–pituitary–adrenal (HPA) axis, pregnancy, postpartum blues, depression, stress

Introduction

Hypothalamic–pituitary–adrenal (HPA) responses to physical or psychological stress are reduced during the postpartum period (for reviews, see Neumann 2001; Tu et al. 2005a; Slattery and Neumann 2008). This phenomenon may help the mother to conserve energy required for lactation, protect against stress-associated inhibition of lactation, relieve psychological stress, and enhance her immune function (see Altemus et al. 1995; Lightman et al. 2001). However, alterations of HPA activity have also been associated with mood disturbances and several puerperal disorders, including postpartum blues and postpartum depression (see

Brunton and Russell 2008). The factors that may cause these HPA changes during the postpartum period are largely unknown. To identify them, most human studies focused on maternal characteristics postpartum (for a review, see Tu et al. 2005a). However, maternal processes during pregnancy may also play an important role, but have as yet received little or no attention.

The maternal organism undergoes remarkable neuroendocrine changes during pregnancy, optimizing fetal growth and development, protecting the fetus from adverse exposures, and preparing the mother for timely parturition. More specifically, gestation dramatically affects the maternal HPA axis, leading to

increased basal levels of corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH), bound cortisol, unbound cortisol in human plasma (see Lindsay and Nieman 2005), and a more pronounced salivary cortisol awakening response (CAR; de Weerth and Buitelaar 2005). The physiological consequences of this increase in cortisol remain a matter of debate, but most discussions have focused on effects on the fetus (see Mastorakos and Ilias 2003). However, another physiological effect of increased cortisol concentrations at the end of pregnancy may be related to postpartum HPA reactivity. The CAR represents the steep increase of cortisol secretion within the first 30 min after awakening, usually leading to the highest cortisol concentrations throughout the day (Weitzman et al. 1971; Spath-Schwalbe et al. 1992; Van Cauter et al. 1994; Clow et al. 2004; Meinlschmidt and Heim 2005). As basal cortisol levels are already markedly increased during late pregnancy, the cortisol increase after awakening leads to an even stronger exposure to cortisol during the prepartum period. This exposure may profoundly change the regulation of the maternal HPA axis, with lasting effects persisting throughout the postpartum period.

Maladaptations of the mother's neuroendocrine systems during pregnancy have long-term effects on endocrine systems and behavior in the offspring (see Wadhwa 2005; Kapoor et al. 2006). In particular, the maternal HPA axis and associated "fetal, prenatal, or perinatal programming" of the stress response in the offspring have received a great deal of attention during recent years (e.g., Kofman 2002; Meaney et al. 2007; Phillips 2007). Several studies in animals and humans have shown that hormonal adaptations during late pregnancy have effects on endocrine mechanisms associated with physiological functioning of the mother postpartum, including lactation and maternal behavior (for a review, see Brunton and Russell 2008). However, even though numerous studies have separately examined alterations of the HPA axis during pregnancy and during the postpartum period, no human study has as yet investigated whether there is an association between the common hormonal changes during pregnancy and HPA axis reactivity to stress in the mother postpartum. A better knowledge of this association would increase our understanding of the maternal psychobiology during the peripartum period, add a new perspective to the meaning and function of HPA modifications during gestation, and may provide new insights into mechanisms that may underlie the etiopathology of puerperal disorders.

Our primary objectives were to determine, within a longitudinal study with an experimental component, (i) whether the CAR during late pregnancy, as an indicator of the markedly increased cortisol concentrations at the end of pregnancy, predicts maternal HPA responsiveness to a psychosocial stress test postpartum, (ii) whether this prediction is specific to the CAR during

pregnancy or a feature of the CAR independent of pregnancy, and (iii) whether the CAR during late pregnancy also predicts mood changes of the mother in response to a psychosocial stressor postpartum.

Materials and methods

Study design and setting

We conducted a longitudinal study with an experimental component. Pregnant women were recruited by local advertisements between the 20th and the 36th week of gestation (WG) for paid participation in a study of lactation and stress. The assessments took place at three time points during the peripartum period. In the 36th WG and at 6 weeks postpartum, women collected saliva and responded to questionnaires at home. At 8 weeks postpartum, women reported to the laboratory to respond to further questionnaires and were exposed to a standardized psychosocial laboratory stress protocol.

Participants

Twenty-two healthy women participated in this study [mean age (standard deviation, SD): 30.1 (4.2) years], including 10 (45%) primiparous and 12 (55%) multiparous women, which is comparable to the general population of women giving birth. All women were of Caucasian ethnicity. Twenty women were married or had been cohabiting for at least 1 year, and two women were single. Six weeks prior to the expected date of parturition, 16 women were working as employees, four women were studying at university, 1 woman was a housewife, and one woman gave no respective information. All women had uncomplicated pregnancies and gave birth to a healthy full-term (> 37 weeks) singleton infant by the vaginal route. Participants underwent a medical examination and a diagnostic interview before entering the study and were considered eligible if they were free of chronic diseases, mental disorders, medication, smoking, and drug or alcohol abuse. We exclusively included breastfeeding participants in the analyses, with eight of the women additionally feeding with formula supplementation. None of the women had resumed menses before the day on which the assessment at 8 weeks postpartum was completed.

The study was conducted at the University of Trier in accordance with the Declaration of Helsinki. The ethics committee of the University of Trier approved the study protocol and all volunteers provided written informed consent.

Procedures

The salivary CAR was assessed in the 36th WG and at 6 weeks postpartum. We instructed participants to

collect saliva samples at home 0, 30, 45, and 60 min after awakening [first timed wake-up sample at 6.30 am, with use of an alarm clock if necessary, as it has been shown previously that spontaneous awakening versus alarm awakening does not affect the CAR (Pruessner et al. 1997; Wust et al. 2000)]. We asked the participants to choose a weekday to collect samples and to abstain from consuming any food or brushing their teeth prior to the four samples in order to prevent any contamination. Adherence to protocol was assessed by self-report questionnaire.

At 8 weeks postpartum, we assessed HPA axis responses to an established psychosocial stressor in the laboratory using the Trier Social Stress Test (TSST). The TSST consists of an unprepared speech and mental arithmetic task performed in front of an audience (Kirschbaum et al. 1993) and elicits large and robust HPA responses (Dickerson and Kemeny 2004). We instructed the participants to consume their normal food and to abstain from caffeine, alcohol, and running or other strenuous activity during the 24 h before the TSST. To prevent effects of breastfeeding on HPA responses, the time between the last breastfeeding and start of the TSST was at least 100 min. Participants reported to the laboratory at 14h00. On arrival, all mothers had a catheter inserted into a brachial vein 45 min before the TSST (for details, see Heinrichs et al. 2001). To reduce the likelihood of stress from having the catheter inserted influencing and confounding the TSST hormone assessments, especially in more emotional participants, we did not take the baseline blood and saliva sample until 45 min later, immediately before the stressor (−1 min). After this, participants underwent the TSST, which took 15 min including the introduction. We obtained further blood samples at 1, 30, and 60 min after exposure, while collecting further saliva samples 10, 20, 30, 45, and 60 min after the cessation of the stress test. Participants completed questionnaires on mood and state anxiety before and after the TSST (for details, see Heinrichs et al. 2001).

Blood and saliva sampling

Saliva was collected using Salivette (Sarstedt, Nuembrecht, Germany) collection devices in the 36th WG and at 6 and 8 weeks postpartum. Samples were kept in freezers in the subjects' residences until delivered to the laboratory. The Salivette tubes were stored in the laboratory at −20°C until required for biochemical analysis. Before assaying for free cortisol, we thawed and spun samples at 1500 g for 5 min to obtain 0.5–1.0 ml clear saliva with low viscosity.

We collected blood on ice in EDTA-coated tubes and centrifuged them at 4°C at 2500 g for 5 min. Plasma samples were stored at −80°C until required for biochemical analysis.

Biochemical analyses

ACTH and total plasma cortisol concentrations were determined with a commercial chemiluminescence assay (Nichols Institute Diagnostics, Bad Nauheim, Germany). The free cortisol concentration in saliva was analyzed using a time-resolved immunoassay with fluorescence detection, as described previously (Dressendorfer et al. 1992). The limits of detection were 0.5 pg/ml for ACTH, 0.5 nmol/l for saliva cortisol, and 0.8 µg/dl for plasma cortisol. Inter- and intra-assay coefficients of variance were below 12 and 10%, respectively, for all analytes.

Psychological assessment

To measure emotional changes to the TSST, we employed two questionnaires. To assess changes in mood, we applied a mood questionnaire that is especially suited for repeated measures within several minutes or hours. Twelve items are rated on a 5-point scale, ranking from 1 (= not at all) to 5 (= very strongly). Factor analyses revealed three scales, termed elevated versus depressed mood, wakefulness versus sleepiness, and calmness versus restlessness (Steyer et al. 1997). Moreover, to assess changes in state anxiety, we applied the German version of the State-Trait Anxiety Inventory (Spielberger et al. 1970).

Statistical analyses

As a measure for the CAR, we calculated the area under the curve with respect to ground (AUC_G) using the trapezoid formula, aggregating the four saliva cortisol levels assessed in the morning (Pruessner et al. 2003). We used the AUC_G, as it is (i) the most important measure of total hormonal secretion (Fekedulegn et al. 2007), representing the markedly high cortisol concentrations after awakening at the end of pregnancy and (ii) more reliable than the area under the curve with respect to increase (Hellhammer et al. 2007). To describe and verify whether there was a cortisol response to awakening during pregnancy as well as 6 weeks postpartum, we computed the parameter “increase” as the difference between the peak value after the TSST and the pre-stressor baseline value. Paired sample *t*-tests were calculated to detect increases in hormone concentrations in the first 30 min after awakening or during the TSST (baseline to peak), as well as differences between the amplitude of the AUC_G CAR at the 36th WG and at 6 weeks postpartum. Furthermore, we performed a two factor (pre vs. postpartum and time of saliva sampling) repeated measure analysis of variance (ANOVA), followed by Bonferroni post hoc procedures, to compare the CAR at the 36th WG and the CAR at 6 weeks postpartum. The reported results were corrected by the Greenhouse–Geisser procedure

where appropriate (reflected by the degrees of freedom with decimal values). We calculated interrelations between the AUCg CAR at the 36th WG and the AUCg CAR at 6 weeks postpartum using Pearson's correlations.

To predict increases of the endocrine parameters in the TSST by the salivary CAR (AUCg CAR) in late pregnancy (36th WG), we computed linear regression models with the AUCg CAR in late pregnancy as a fixed factor and increase of ACTH, plasma cortisol, and salivary cortisol as dependent variables. To assess the potential influences of age, parity, and the feeding with formula supplementation in addition to breastfeeding as possible confounding factors, we computed Pearson's correlations between the mother's age, parity (primiparous/multiparous), and formula supplementation (yes/no) with increases of ACTH, plasma cortisol, and salivary cortisol in the TSST.

To investigate whether the prediction of the endocrine responses to the TSST 8 weeks postpartum by the CAR at the 36th WG is specific for the CAR during pregnancy, we performed further analyses. Linear regression models were computed using AUCg CAR at 6 weeks postpartum as a fixed factor to examine whether the AUCg CAR at 6 weeks postpartum predicts increase of ACTH, plasma cortisol, and salivary cortisol responses to the TSST. As some participants did not provide saliva samples at 6 weeks postpartum, there were missing data for the AUCg CAR of seven subjects at 6 weeks postpartum. We therefore had to exclude these subjects from all calculations that incorporated the postpartum AUCg CAR. To assess a possible distortion of our results due to these excluded subjects, we carried out a sensitivity analysis and compared the excluded and included cases regarding the AUCg CAR at the 36th WG, HPA responses to the TSST, age, and the associations between the AUCg CAR at the 36th WG and the HPA responses to the TSST. To investigate whether the prediction of the endocrine responses to the TSST 8 weeks postpartum by the CAR at the 36th WG is not merely a consequence of an association between morning cortisol during pregnancy and ACTH or cortisol baseline levels in the afternoon, we computed linear regression models with the AUCg CAR in late pregnancy as a fixed factor and the baseline hormone levels before the TSST as dependent variables. Moreover, we also computed Pearson's correlations between the baseline levels before the TSST and HPA responses to the TSST, for plasma ACTH, plasma cortisol and salivary cortisol, respectively.

To test whether the TSST led to a significant change in mood and state anxiety, paired sample *t*-tests were performed, comparing the values before and after the TSST. We computed "change" parameters for each mood scale and for state anxiety as the difference between the respective value after stress and the prestressor baseline value.

To assess whether the AUCg CAR in the 36th WG predicted mood or anxiety changes in the TSST, we computed linear regression models with the AUCg CAR in late pregnancy as a fixed factor and the change parameters as dependent variables.

Where necessary, we log-transformed variables to fit analysis assumptions, which was verified by goodness-of-fit tests. For the sake of comparability to other studies, non-transformed values are shown when means are reported. The level of significance was determined at $P < 0.05$. Bonferroni correction was applied in the case of multiple comparisons. Statistics were performed using the computer statistical package SPSS (16.0; SPSS, Inc., Chicago, IL, USA).

Results

Comparison and association of the CARs pre and postpartum

The CARs at the 36th WG and at 6 weeks postpartum are depicted in Figure 1. There was a significant increase in salivary cortisol between the time of awakening and 30 min after awakening both at the 36th WG and at 6 weeks postpartum. The AUCg CAR was greater at the 36th WG than at 6 weeks postpartum [$t(14) = 5.48$; $P < 0.001$]. In line with this, the ANOVA revealed significant main effects for the factor pre versus postpartum assessment [$F(1,14) = 25.73$; $P < 0.001$], with post-hoc tests showing significant differences in cortisol concentrations when comparing the samples at a specific time of sampling at the 36th WG with the respective sample at 6 weeks postpartum (all $P < 0.05$), as well as for the factor time of saliva sampling [$F(2.05,28.63) = 8.72$; $P = 0.001$], with post-hoc tests showing a significant increase in cortisol concentrations between the

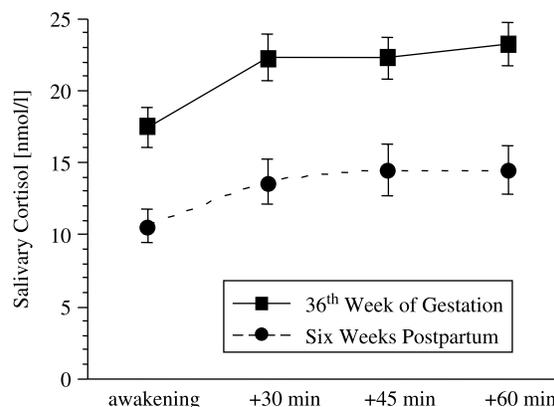


Figure 1. Cortisol awakening responses. Plot of the salivary CAR (mean \pm standard error of mean, SEM) at 36th WG, week of gestation (solid line, squares; $n = 22$) and at 6 weeks postpartum (dotted line, bullets; $n = 15$). *Statistically significant increase in salivary cortisol from awakening to 30 min after awakening [paired one-tailed *t*-tests; 36th WG: $t(21) = 3.48$, $P = 0.001$; 6 weeks postpartum: $t(14) = 2.05$, $P = 0.029$].

first and the three other samples (all $P < 0.05$). The interaction effect was not significant [$F(1.80,25.20) = 0.49$; $P = 0.598$]. There was no correlation between the AUCg CAR at the 36th WG and at 6 weeks postpartum ($r = 0.253$; $P = 0.363$).

ACTH and cortisol responses to stress

There was a significant ACTH, plasma cortisol, and salivary cortisol response to the psychosocial stress test – the TSST – with peak levels at 1 min (ACTH, plasma cortisol) and 20 min (salivary cortisol) after the cessation of the stressor (Table I).

Prediction of the hormonal stress response in lactation by CAR in the 36th WG

There were significant negative correlations between the AUCg CAR in the 36th WG and the plasma ACTH ($\beta = -0.60$; $P = 0.003$), plasma cortisol ($\beta = -0.69$, $P < 0.001$), and salivary cortisol ($\beta = -0.66$; $P = 0.001$) responses to the TSST at 8 weeks postpartum (Figure 2). The comparisons remained significant after Bonferroni correction. Possible associations between the mother's age, parity, or additional formula supplementation with increases in ACTH, plasma cortisol, and salivary cortisol in the TSST did not reach statistical significance. However, to exclude potential confounding, we repeated the analyses, controlling for each factor at a time. As expected, this did not change either the magnitude of the effects or the significance of the results.

No prediction of the hormonal stress response in lactation by CAR at 6 weeks postpartum

The AUCg CAR at 6 weeks postpartum failed to predict any hormonal responses to the TSST at 8 weeks postpartum (ACTH: $\beta = 0.02$, $P = 0.933$; plasma cortisol: $\beta = -0.23$, $P = 0.407$; salivary cortisol: $\beta = -0.15$, $P = 0.597$) (Figure 3). Again, to exclude potential confounding by mother's age, parity, or additional formula supplementation, we repeated the analyses, controlling for each factor at a time. As expected, this did not change the magnitude of the effects or the significance of the results.

The sensitivity analyses compared the included cases with the excluded cases (seven subjects) due to

missing data for the calculations incorporating the AUCg CAR at 6 weeks postpartum. They showed that the excluded cases did not differ from the included cases regarding AUCg CAR at the 36th WG, HPA responses to the TSST, and age, as well as the associations between the AUCg CAR at the 36th WG and the HPA responses to the TSST (all $P > 0.05$). Hence, the obtained results should not be distorted by the exclusion of these cases with missing values.

The AUCg CAR at the 36th WG was not associated with ACTH or cortisol levels at baseline before the TSST 8 weeks postpartum (ACTH: $\beta = 0.20$, $P = 0.382$; plasma cortisol: $\beta = -0.04$, $P = 0.853$; salivary cortisol: $\beta = -0.22$, $P = 0.336$). Moreover, there was no association between the baseline hormone levels before the TSST and HPA responses to the TSST (all $P > 0.05$). Hence, the obtained results should not merely be a consequence of an association between morning cortisol during pregnancy and ACTH or cortisol baseline levels in the afternoon, which may be related to HPA responses to the TSST.

Mood and anxiety changes to stress and their prediction by the AUCg CAR at the 36th WG and at 6 weeks postpartum

Mood and anxiety were assessed before and after the psychosocial stress test. There was a trend toward worsened mood after the TSST [$t(21) = -1.882$; $P = 0.074$]. We observed no changes in the scales calmness versus restlessness and wakefulness versus sleepiness (all $P > 0.10$). State anxiety significantly increased in response to the TSST [$t(21) = 3.313$; $P = 0.003$].

The AUCg CAR at the 36th WG failed to predict any mood or anxiety changes in the TSST at 8 weeks postpartum (elevated vs. depressed mood: $\beta = 0.053$, $P = 0.813$; calmness vs. restlessness: $\beta = 0.162$, $P = 0.470$; wakefulness vs. sleepiness: $\beta = -0.003$, $P = 0.989$; state anxiety: $\beta = 0.255$, $P = 0.252$).

The AUCg CAR at 6 weeks postpartum failed to predict any mood or anxiety changes in the TSST at 8 weeks postpartum (elevated vs. depressed mood: $\beta = 0.472$, $P = 0.076$; calmness vs. restlessness: $\beta = 0.169$, $P = 0.547$; wakefulness vs. sleepiness: $\beta = 0.283$, $P = 0.306$; state anxiety: $\beta = 0.004$, $P = 0.252$).

Table I. Hormone levels before (baseline, between 2 and 3 pm) and at the peak level (within 1 and 60 min) after the TSST.

	Baseline	Peak	$T(DF)$ -value*	P -value
ACTH (pg/ml)	23.94 (7.82)	48.28 (30.89)	$t(21) = 4.06$	< 0.001
Plasma cortisol ($\mu\text{g/dl}$)	6.24 (1.87)	9.93 (3.98)	$t(21) = 5.01$	< 0.001
Salivary cortisol (nmol/l)	6.21 (2.39)	12.24 (6.51)	$t(21) = 5.39$	< 0.001

$N = 22$. Data expressed as mean (SD); ACTH, adrenocorticotropin; DF, degrees of freedom; * Paired one-tailed t -test for change in hormone concentrations from baseline to peak after stress.

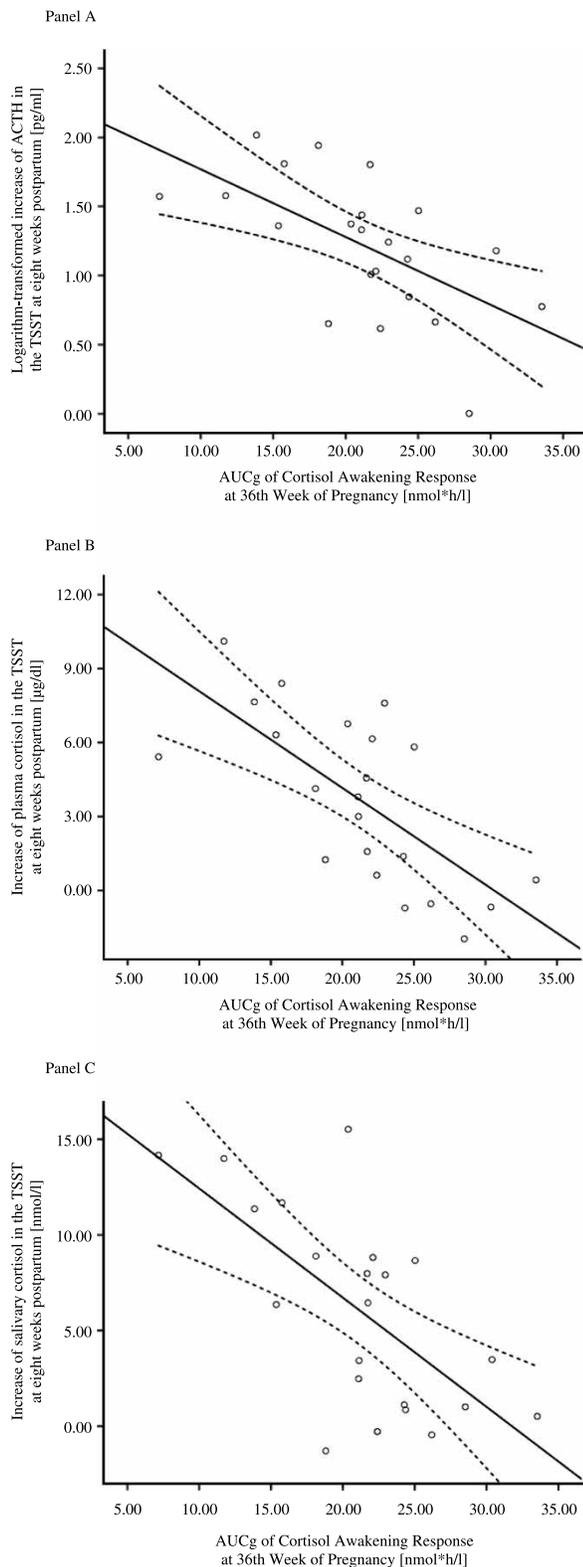


Figure 2. Hormonal stress responses in lactation by CAR at 36th WG. Scatter plots of the linear regression analyses ($n = 22$) of the AUCg of the salivary CAR at 36th WG (predictor) and hormonal responses to the TSST at 8 weeks postpartum (dependant measures), namely ACTH (Panel A), plasma cortisol (Panel B), and salivary cortisol (Panel C). The figures show the observed values, the regression line (solid line), and the 95% confidence interval (dotted lines). ACTH, adrenocorticotropin; AUCg, area under the curve with respect to ground; TSST, Trier Social Stress Test.

Discussion

We found that maternal cortisol responses to awakening during late pregnancy, but not 6 weeks after delivery, negatively predicted maternal ACTH, total plasma cortisol and salivary free cortisol responses to an acute psychosocial stressor 8 weeks postpartum. There was no comparable prediction for mood and anxiety changes to the psychosocial stressor.

Moreover, we found that the maternal CAR at late gestation was significantly heightened compared to the CAR postpartum and that the CARs at both time points were not significantly associated with each other (see de Weerth and Buitelaar 2005).

To the best of our knowledge, a possible link between naturally occurring variations in HPA activity during pregnancy and neuroendocrine parameters in lactation has not yet been assessed in animal studies. However, pregnancy stress significantly increases HPA reactivity in lactating rats bred for high anxiety-related behavior (Neumann et al. 2005), providing evidence that events during pregnancy may influence maternal HPA reactivity postpartum. The lack of association between the postpartum CAR and HPA axis to the TSST is in line with a previous study in non-pregnant subjects (Schmidt-Reinwald et al. 1999). Moreover, Gustafsson et al. (2008) did not detect an association between the cortisol response to a psychological stressor and morning cortisol levels in adolescents with obsessive-compulsive disorder. Notably, Quirin et al. (2008) reported a small but significant negative relationship between maximum cortisol concentration in the morning and cortisol concentration after an acute intervention, which, however, stimulated the HPA axis with a different procedure only in a subset of participants.

Several mechanisms may underlie our primary finding. One may speculate that individuals with high CAR during pregnancy also show high CAR postpartum and our finding is thus merely a reflection of persisting individual characteristics or perturbations of HPA activity. However, several lines of evidence do not support this hypothesis. First, elevated plasma CRH and cortisol levels in the mother generally return to prepregnancy levels within a few days after delivery in humans (Kauppila et al. 1973; Allolio et al. 1990). Second, we found no intraindividual association between the CAR in pregnancy and the CAR postpartum (*cf.* de Weerth and Buitelaar 2005). Third, the CAR after delivery did not predict HPA responses to the TSST. And finally, the CAR in late pregnancy neither predicted baseline HPA hormone levels in the afternoon at week 8 of lactation, nor were the baseline levels in lactation associated with HPA reactivity to the TSST. However, this should be the case if the reported association between the CAR during pregnancy and HPA responses to stress postpartum were simply a

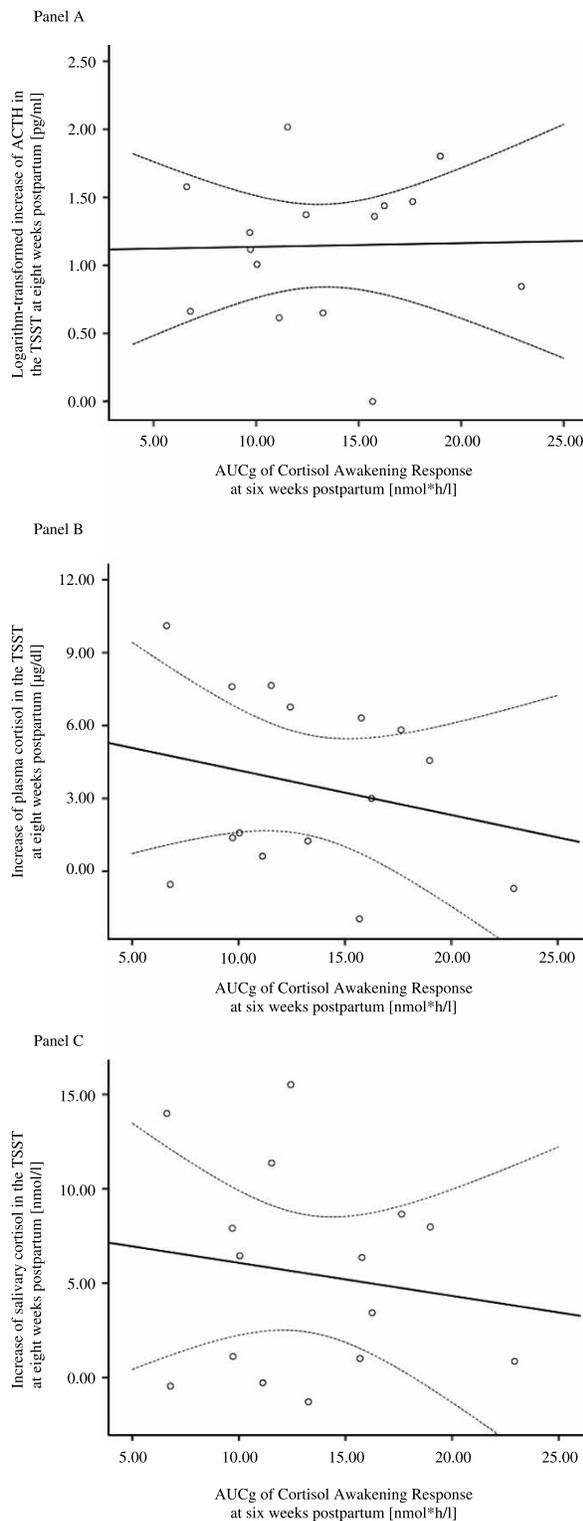


Figure 3. Hormonal stress responses in lactation by CAR at 6 weeks postpartum. Scatter plots of the linear regression analyses ($n = 15$) of the AUCg of the salivary CAR at 6 weeks postpartum (predictor) and hormonal responses to the TSST at 8 weeks postpartum (dependant measures), namely ACTH (Panel A), plasma cortisol (Panel B), and salivary cortisol (Panel C). The figures show the observed values, the regression line (solid line), and the 95% confidence interval (dotted lines). ACTH, adrenocorticotropin; AUCg, area under the curve with respect to ground; TSST, Trier Social Stress Test.

consequence of individual diurnal HPA activity. To determine whether the detected association is exclusive for the peripartum period, future studies should assess individuals with markedly high CAR, comparable to those during late pregnancy.

Possible biological explanations for our primary finding are provided by the evidence for peripartum alterations in various brain systems that control the HPA response (for reviews, see Walker et al. 2001; Slattery and Neumann 2008). Our primary finding consisted of an association in a negative direction, which is in line with the assumption that during pregnancy, hypothalamic maternal CRH secretion is suppressed due to the higher circulating levels of cortisol, and that this suppression extends into the postpartum period (see Mastorakos and Ilias 2003).

We did not find any association between the CAR during late pregnancy and mood and anxiety changes in response to the psychosocial stressor, which is in contrast to the respective association with the hormonal stress responses. One potential reason may be that the psychoendocrine covariance in reactions to acute stress is usually very low or even lacking completely (see Hellhammer and Hellhammer 2008, but *cf.* Schlotz et al. 2008), indicating that the psychological and the endocrine reaction to acute stress represent two rather distinct aspects of the stress response.

The present study has several limitations. First, only breastfeeding mothers were included. Future studies should replicate our findings in non-breastfeeding mothers, given the evidence that breastfeeding influences maternal HPA responses to acute stress in lactation (Heinrichs et al. 2002). Second, we collected saliva samples for CAR assessment on only 1 day at each time point, and assessing salivary cortisol on 2 days increases the reliability of CAR assessments (Hellhammer et al. 2007). However, less accurate assessment of the CAR would only lead to an underestimation of real associations between the CAR and other factors. Furthermore, one may speculate that the postpartum CAR was influenced by disruption in the mothers' sleep cycle due to a possible lack of circadian rhythm in the neonates or by lactation. However, the CAR appears to be unchanged even if sleep is repeatedly interrupted by forced awakenings (Dettenborn et al. 2007). In line with this, we found no association between subjective sleep quality of the mothers in the night preceding the postpartum CAR assessment and the postpartum CAR (data not shown). Notably, no mother reported waking up later than 6.30 am. Moreover, we found a clear CAR in the postpartum period (*cf.* de Weerth and Buitelaar 2005), which makes it unlikely that the CAR postpartum was substantially influenced by a putative disruption in the mother's sleep cycle. Besides this, we did not see higher variation in postpartum CAR or the

postpartum cortisol concentration directly after awakening, compared to the levels during pregnancy, which should be expected if the presence of the newborn introduced noise in the measurement of the mothers' postpartum CAR. Furthermore, it has been shown that breastfeeding as well as bottle-feeding, even upon awakening, does not affect the CAR (Tu et al. 2005b). Finally, at this point, no conclusive statement can be made about other aspects of HPA functioning or the causality of the negative association between CAR during pregnancy and HPA responses to stress postpartum. Even though our design enabled the unraveling of a temporal association between the two parameters, experimental studies are warranted to confirm causality. Moreover, the findings should be replicated in a larger sample.

Our primary finding has several implications. First, it extends the current functional interpretation of HPA modifications during pregnancy as adaptive for the fetus and the mother during pregnancy (Lindsay and Nieman 2005), revealing their relevance for modulating the stress reactivity not only of the pregnant woman, but also of the mother postpartum. Moreover, a better understanding of modulations of the maternal stress reactivity during the peripartum period has clinical relevance. Disturbances of endocrine responses to stress in mothers can have detrimental consequences for the offspring postpartum as, for example, the newborn can be exposed to maternal cortisol through breast milk (Patacchioli et al. 1992). Besides this, knowledge about the association between the neuroendocrine activity in pregnancy and maternal endocrine functioning in the postpartum period may provide new early biological markers and new targets for intervention during pregnancy for later puerperal disorders or symptoms that have been associated with HPA dysregulation, such as postpartum blues (see Magiakou et al. 1996; Brunton and Russell 2008). Finally, future studies should test whether prepartum psychosocial measures (e.g., trait anxiety, stress, and arousal; cf. Thorn et al. 2009) captured in parallel with the CAR samples partially determine the magnitude of the stress response postpartum.

Taken together, we were able to show that a neuroendocrine marker, the salivary CAR during late pregnancy, negatively predicts maternal HPA responses to stress in the subsequent postpartum period. This is the first study to detect such contiguity between specific prenatal and postnatal functioning of a neurobiological stress system in humans. An understanding of this can pave the way for new strategies for prevention and early detection of women at risk for puerperal disorders even during pregnancy.

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