Maternal caregiving moderates the impact of antenatal maternal cortisol on infant stress regulation

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ABSTRACT

Background. Emerging evidence suggests that antenatal exposure to maternal stress signals affects the development of the infant stress response systems. Animal studies indicate that maternal sensitive caregiving can reverse some of these effects. However, the generalizability of these findings to humans is unknown. This study investigated the role of maternal caregiving in the association between multiple markers of maternal antenatal stress and infant stress regulation.

Methods. The sample consisted of 94 mother-infant (N=47 males, mean postnatal weeks=12; SD=1.84) dyads. Maternal levels of Interleukin-6, C-Reactive Protein, diurnal cortisol and alpha amylase, depressive and anxiety symptoms were assessed in late pregnancy (mean gestational age=34.76; SD=1.12), whereas postnatal symptomatology, caregiving and infant cortisol response to the inoculation were evaluated at 3 months.

Results. Hierarchical linear models showed a significant interaction between maternal antenatal cortisol, caregiving and time on infant cortisol reactivity, while controlling for gender, maternal age and postnatal depression. Specifically, higher levels of maternal antenatal cortisol were associated with greater cortisol response only among infants of less emotionally available mothers. All other markers of antenatal stress were not significantly associated with infant cortisol reactivity either independently or in interaction with maternal caregiving.

Conclusions. Albeit preliminary, results provide the first evidence in humans that maternal sensitive caregiving may eliminate the association between antenatal maternal cortisol and infant cortisol regulation.

KEYWORDS: Cortisol, Caregiving, Pregnancy, Stress, Inflammation, Alpha-Amylase

MAIN TEXT

1. Introduction

Dysregulation of the stress response systems is increasingly regarded as one mechanism through which early adversity 'gets under the skin' to influence lifetime physical and mental health (Kuhlman, Chiang, Horn, & Bower, 2017). Antenatal exposure to maternal stress signals is an emerging factor that may affect the development of the infant stress response systems (Glover, O'Connor, & O'Donnell, 2010). Disruptions in early caregiving have also been associated with impaired children stress regulation (McLaughlin et al., 2015) and animal evidence indicates that early caregiving can moderate the impact of antenatal stress on offspring's stress regulation (e.g. Del Cerro et al., 2010). However, how antenatal and postnatal environmental exposures act together to shape infant stress response in humans is still unknown. The current study investigates, for the first time, the interplay of maternal antenatal stress signals and postnatal caregiving in influencing infant stress regulation.

Depressive and anxiety symptoms have been the most widely studied measures of maternal antenatal stress and mounting evidence indicates that they might have a detectable impact on fetal development even at subclinical levels (reviewed in Van den Bergh et al., 2017). However, evidence for an association between antenatal maternal depressive or anxiety symptoms and stress reactivity in the offspring is inconsistent (Bleker et al., 2018). As compared to self-reported measures, biological stress measures might reflect different aspects of maternal stress experience and allow to directly examine the potential mechanisms that underline the programming of fetal stress response systems. To date, scholars have focused primarily on maternal cortisol, whereas alternative mediators of antenatal stress, involving for example the Sympathetic Nervous System (SNS) or inflammatory pathways, have been poorly explored. Although mechanisms are likely to be complex, stress-induced releases of maternal glucocorticoids, inflammatory markers or catecholamines are hypothesized to affect, either directly or indirectly, the set-point of the fetal stress response systems, leading to long-term altered behavioral and physiological outcomes (Rakers et al., 2017).

While several studies investigated the association between maternal antenatal cortisol and infant cortisol reactivity (e.g. Davis, Glynn, Waffarn, & Sandman, 2011; Irwin et al., 2021), only four reports assessed cortisol daily output through multiple diurnal samples. A higher maternal cortisol increase after awakening in late gestation was associated with a flatter cortisol response at birth (Nazzari et al., 2019), a blunted cortisol reactivity in 3-month-old boys (Giesbrecht, Letourneau, Campbell, & Team, 2017) and impaired cortisol habituation at 9 months (de Weerth, Buitelaar, & Beijers, 2013). Furthermore, higher maternal antenatal evening cortisol was associated with 12-month-olds' greater cortisol response (Osborne et al., 2018).

Only two studies have examined the association between antenatal levels of maternal salivary alpha-amylase (sAA), a non-invasive SNS marker, and infant stress reactivity. While maternal diurnal sAA in early pregnancy distinguished among 6-month-olds' stress reactivity patterns (Rash et al., 2015), no significant association were found in late pregnancy (Nazzari et al., 2019; Rash et al., 2015). Likewise, the association between maternal antenatal inflammation and infant stress regulation is largely unexplored (Nazzari and Frigerio, 2020). Osborne and colleagues (2018) reported a positive association between several maternal inflammatory markers and 12-month-olds' cortisol reactivity in a sample of depressed mothers, but this result was not replicated in a community sample soon after birth (Nazzari et al., 2019).

A failure to account for the role of early caregiving in shaping the development of the stress response systems might explain literature inconsistencies. Indeed, while several studies have accounted for the effects of postnatal symptomatology (e.g. Irwin et al., 2021), the potential role of maternal parenting in moderating antenatal maternal influences on child development, has been largely neglected (with few exceptions Endendijk et al., 2005; Bergman et al., 2010; Frigerio & Nazzari, 2021). Long-standing evidence suggests that a sensitive caregiver can buffer child's stress reactivity (Gunnar & Hostinar, 2015), while disturbances in early care may disrupt stress regulation (e.g. Albers, Riksen-Walraven, Sweep, & Weerth, 2008). Furthermore, animal data indicates that maternal adequate caregiving can reverse offspring's antenatal stress-related morphological and hormonal alterations (Del Cerro et al., 2010; Maccari et al., 1995). Only two human studies investigated whether maternal caregiving moderated the association between

antenatal stress and infant cortisol regulation and none included maternal stress biomarkers. Kaplan and colleagues (2008) found that maternal sensitivity protected from higher resting cortisol levels among 4-month-olds of antenatally depressed/anxious mothers. In contrast, Grant and colleagues (2009) reported that antenatal maternal anxiety and postnatal sensitivity contributed independently to 7-month-olds' cortisol response.

The current study aims to extend available evidence on the effects of maternal antenatal stress on infant stress regulation by 1) combining the assessment of multiple biological and psychological markers of maternal antenatal stress (i.e. cortisol, sAA, C-Reactive Protein (CRP), Interleukin-6 (IL-6), depression and anxiety) and 2) investigating the joint contribution of maternal caregiving. We previously reported a significant association between maternal antenatal diurnal cortisol measures and newborn stress reactivity soon after birth, thus largely independent from postnatal experiences (Nazzari et al., 2019). We now followed-up the same sample of mother-infant dyads to explore the potential moderating role of maternal caregiving in the association between maternal antenatal stress and 3-month-olds' stress regulation. While biological and psychological stress measures are assumed to be markers of the same underlying construct, weak or null associations have been reported between these measures across pregnancy (e.g. Harville et al., 2009), suggesting that the effects of these factors on offspring neurodevelopment might occur through different pathways (O'Donnell & Meaney, 2017). For this reason, markers of maternal antenatal stress were analyzed separately in order to shed light on the prenatal risk phenotype that might be more implicated in the development of infant stress regulatory systems. Due to limited available literature, we made only a broad a-priori hypothesis. We predicted that maternal insensitive caregiving would exacerbate the negative association between antenatal maternal stress and infant cortisol reactivity, while higher maternal sensitivity would protect infants from the antenatal risk exposure.

2. Methods and Materials

2.1 Participants

Mothers and infants (N=94) were part of an ongoing longitudinal study investigating the effects of antenatal maternal stress on infant development (Nazzari et al., 2019; Nazzari et al., 2020a). Antenatal eligibility criteria were: aged 18-45, with singleton uncomplicated pregnancy, non-smoker, not afflicted by any disease or taking any medications and with no known substance/alcohol abuse or psychiatric disorders (except for depression and anxiety). From the sample of 110 pregnant women, 94 mother-infant dyads had data available at the 12-weeks postnatal phase. Sample attrition was due to 1) intrauterine death (N=1), 2) newborns' serious health problems (N=2), 3) parents not consenting to the inoculation (N=2), 4) infants being vaccinated in a district outside those involved in the project (N=11). Dyads who withdrawn from the postnatal phase did not differ from participants on any socio-demographic variables, depression or anxiety scores (all ps>.10). Women (mean age=33.04, SD=3.83) were mostly Italian (96.8%), middle-high class (94.3%) and primiparous (90.4%). Infants (50% males) were born full-term, mostly by vaginal delivery (68.1%), 14.9% by vacuum-assisted delivery and 16% by cesarean-section (N=11 elective, N=5 operative).

Maternal biological samples were collected between 34–36 gestational weeks (mean gestational age=34.76; SD=1.12). Furthermore, women reported on anxiety and depressive symptoms during pregnancy and 12 weeks postpartum. Infants' cortisol reactivity was evaluated during the first routine inoculation visit at 12 weeks (SD=1.84) whereas maternal caregiving was assessed in a session at the Medea Institute.

The study protocol was reviewed and approved by the Ethics Committees of the Medea Institute, of University College London, and of all hospitals involved and both parents gave written informed consent.

2.2 Maternal assessment

Depressive and anxiety symptoms. Depressive symptoms were assessed through the Italian version of the Edinburgh Postnatal Depression Scale (EPDS; Benvenuti et al., 1999), a 10-item scale extensively employed to screen for perinatal depression. Anxiety symptoms were evaluated through the Italian version of the 20-items state anxiety subscale of the State-Trait

Anxiety Inventory (STAI; Pedrabissi & Santinello, 1989) a well-validated questionnaire that assesses anxiety symptoms experienced in the last few days.

Inflammatory markers. Eighty-two pregnant women out of 94 (87.2%) provided antecubital venous blood samples. Women who consented to blood draw did not differ from women who did not on any socio-demographic variables, anxiety or depression scores (all ps>.10). Serum IL-6 and CRP levels were assayed in duplicate as described in Appendix S1.

Salivary cortisol and alpha amylase. Unstimulated saliva samples were collected on two consecutive days immediately upon awakening, 30 min post-waking and at bedtime and assayed for salivary cortisol and for sAA as detailed in Appendix S1. To capture the overall daily secretion of cortisol and sAA, the area under the curve (AUCg) was calculated using the trapezoid method with respect to the ground (Pruessner et al., 2003). Since the daily values were highly correlated ($r=0.58$, $p<.001$ for cortisol, $r=0.74$, $p<.001$ for sAA), the mean of the two days was used. In order to examine the robustness of the associations found for AUCg, averaged cortisol and sAA values at awakening, rather than AUCg, were employed in supplementary analyses.

Caregiving behaviors. Maternal caregiving was evaluated through the four parental dimensions (i.e. Sensitivity, Structuring, Non-intrusiveness and Non-hostility) of the Emotional Availability (EA) Scales, Infancy/Early Childhood Version (4th edition, Biringen, 2008) during a 15 min-videotaped free-play session. The four EA scales were moderately inter-correlated ($rs= 0.50$-0.73), thus they were standardized and summed to create an overall index of maternal EA (Cronbach's $\alpha= .85$), as previously done (Taylor-Colls & Fearon, 2015). Two certified reliable blind coders rated the videos independently. The inter-rater agreement was calculated on 20 (21.3%) randomly chosen dyads. Intra-class correlation (ICC) coefficients ranged from .75 to .91 with a mean ICC of .84 ($p<.001$).

2.3 Infant assessment

Salivary cortisol. Three saliva samples were collected immediately before (baseline) and 20 and 40 minutes after the inoculation in the morning (except for 4 infants who were examined at 2 pm). Time of the day was examined as a covariate in the analyses. For the inoculation procedure,

the infant was undressed, laid down and two injections respectively, for the first dose of the hexavalent and pneumococcal vaccine, were administered in the infants' thigh, as routinely done in Italy at 3 months of age. Seventy-eight infants had complete cortisol data, whereas one (N=14) or two (N=2) samples were missing for 16 infants due to insufficient saliva volume. Infants with complete or partial data did not differ on any maternal, infants or situational variables (all ps>.10). Samples were assayed following the same procedure described for maternal cortisol. More details about the inoculation procedure are reported in Appendix S1.

2.4 Statistical analyses

2.4.1 Data screening and covariates

The distributions of all biomarkers were positively skewed, thus variables were natural log (ln) transformed to approximate normal distributions. Samples greater than 3 SD from the mean were removed (n=7 for maternal cortisol, n=4 for sAA, n=3 for IL-6, n=4 for infants cortisol). Pearson bivariate correlations and univariate analysis of variance were preliminarily performed to investigate potential confounders (i.e. gender, age, maternal age, education, socio-economic status, postnatal anxiety and depression, mode of delivery, gestational age, birth weight, actual weight, postnatal smoke exposure, breastfeeding, use of nutritional supplements, time of the day, time from last feeding/sleeping, feeding before the salivary collection) of the association between maternal antenatal influences and infant cortisol highlighted in prior works (e.g. Miller et al., 2005). Maternal age and postnatal EPDS scores were significantly associated with infants' cortisol levels 20-min post-stressor (respectively, r=.24, p=.03 and r=-.22, p=.04). Furthermore, females showed marginally higher levels of cortisol at the 40-min post-stressor collection as compared to males ($F(1, 83)=3.70$, p=.06). Thus, maternal age, postnatal EPDS and infant gender were included as covariates in the main analyses.

2.4.2 Analytic Models

Hierarchical Linear Models (HLMs) were estimated to model trajectories of infant cortisol response to the inoculation as a function of antenatal maternal stress and EA, while accounting for

the hierarchical structure of the data. HLMs allow to obtain reliable estimates of effects despite missing values for one or more time-points and to compare individual trajectories of response, rather than absolute levels, while controlling for initial values effects (Hruschka, Kohrt, & Worthman, 2005). HLMs were specified at two levels where infants were level 2 and time was level 1. Time was centered at baseline so that the model intercept represents the mean cortisol baseline level. The baseline model of cortisol response included a linear and a quadratic slope for time that allow curvilinear trajectories in infant cortisol and individual differences in peak cortisol. A random intercept and a random linear slope were included to allow between-person variability. Maternal antenatal stress levels and EA scores were entered in the model as continuous variables and centered around the grand mean; gender was centered at males. Model fit was tested with likelihood deviance difference test for nested models. Statistical analyses were performed using SPSS 24 and MLwiN 3.05.

3. Results

Descriptive statistics and unadjusted bivariate correlations among study variables are displayed, respectively, in Table 1 and 2.

-- Table 1 and 2 about here --

Infants showed the expected cortisol response to the inoculation, as indicated by the significant linear and quadratic time slopes (ps<.001). Also, the random linear slope term was significant (p<.001), thus suggesting between-infant variability in the cortisol linear increase. As shown in Table 3, maternal antenatal stress markers were not significantly associated with infant cortisol reactivity. However, there was a significant three-way interaction among maternal antenatal cortisol, EA and the linear and quadratic time slopes (p<.05) on infant cortisol levels, while adjusting for gender, maternal age and postnatal depression. In particular, as illustrated in Figure 1, at higher levels of maternal antenatal cortisol (+1SD), there was a significant association between maternal EA and the linear (Estimate=-0.002 SE=0.001 p=.007) and quadratic (Estimate=0.000 SE=0.000 p=.005) slopes of infant cortisol response, with lower levels of maternal

EA being associated with greater cortisol reactivity as indicated by a steeper increase in cortisol levels 20 minutes after the inoculation and a steeper decrease 40 minutes after. In addition, at higher level of maternal EA (+1SD), there were no differences in infants' cortisol response to the inoculation depending on levels of antenatal maternal cortisol (ps=.64-76), while at lower levels of maternal EA (-1SD), infants antenatally exposed to higher maternal cortisol levels showed a significantly steeper linear and quadratic slopes (ps=.02). The overall improvement of the model fit over the baseline model was significant (deviance difference (9)=121.32, p<.001). Although maternal antenatal cortisol and EA scores were modeled as continuous predictors, for illustrative purposes, cortisol reactivity profiles are displayed in Figure 1 for infants of women with higher (+1 SD) and lower (-1SD) cortisol levels and higher (+1SD) and lower (-1SD) EA scores.

-- Table 3 and Figure 1 about here –

Supplementary analyses including cortisol/sAA waking measures, rather than AUCg levels, yielded comparable findings and are reported in Appendix S1.

4. Discussion

The current study sought to extend available literature on the programming effect of maternal antenatal stress on infant stress regulation by combining several psychological and biological indices of maternal stress and taking into account the moderating role of postnatal caregiving. To the best of our knowledge, findings provide the first human evidence for a buffering role of maternal caregiving behaviors, as assessed through the EA scales, in the association between maternal antenatal cortisol levels and infant cortisol reactivity in a low-risk sample of healthy women and 3-month-old infants. Specifically, in line with our broad hypothesis, higher levels of maternal antenatal diurnal cortisol were associated with greater cortisol response to the inoculation only among infants of less emotionally available mothers, while the association became non-significant among infants of highly sensitive mothers. Furthermore, the magnitude of the association between maternal EA and infants' cortisol reactivity depended upon maternal cortisol levels. Importantly, the reported associations were independent of maternal postnatal depressive symptoms, age and infant gender. In contrast, maternal antenatal sAA, IL-6 or CRP levels, anxiety

and depressive symptoms were not significantly associated with infants' cortisol response to the inoculation either independently or in interaction with maternal EA.

We previously reported that variations in maternal diurnal cortisol in late gestation were associated with altered patterns of stress reactivity in the offspring few hours after birth (Nazzari et al., 2019). The current findings suggest that these effects are likely to persist until 3 months of age if the infant is postnatally exposed to a less sensitive maternal caregiving. These results are consistent with a growing literature on the association between antenatal maternal cortisol and infant cortisol regulation (e.g. Davis et al., 2011; Irwin et al., 2021) and support the hypothesis that the effects of antenatal stress exposure arise from an alteration of the foetal stress response systems that might carry possible long-lasting effects on how the brain will respond to stress (Glover et al., 2010). However, it is essential to emphasize that the correlational nature of the study does not allow us to draw causal conclusions and that the pathways possibly underlying the current findings are still to be clarified. On the one hand, hereditary transmission might explain the association between maternal and infant cortisol (e.g. Van Hulle et al., 2012) that was found in infants of less emotionally available women. Alternatively, higher diurnal cortisol levels in late pregnancy might result in fetal cortisol over-exposure and affect the set-point of the stress response systems (Glover et al., 2010). Importantly, our results extend evidence from animal models suggesting that the associations between maternal antenatal cortisol and offspring's stress reactivity, either mediated by genetic factors or by in utero mechanisms, might be over-ridden by a postnatal sensitive rearing environment with important conceptual, methodological and clinical implications.

First, while maternal antenatal cortisol diurnal levels alone were not associated with 3-month-olds' stress reactivity, a different picture appeared when the interactive effects between maternal antenatal cortisol levels and postnatal EA were examined. This is consistent with mounting evidence showing that the combination of multiple factors, rather than a single one, is more likely to be involved in determining psychobiological developmental trajectories (e.g., Appleyard et al., 2005). It could be speculated that antenatal exposure to higher levels of maternal cortisol would make the individual more susceptible to the effects of adversities, such as

insensitive parenting, later in life, in line with a “multiple hit” (Bayer et al., 1999) or “cumulative stress” hypothesis (Nederhof & Schmidt, 2012). This may occur through a sensitization of the HPA axis so that antenatal stress exposure set the developing HPA-axis to be more reactive to stress, thus increasing the impact of stressors that came upon later in life (Koss and Gunnar 2018). While this is quite well-characterized in animal models where exposure to prenatal stress and altered maternal behavior disrupts the activity of the HPA axis (Vallée et al., 1999), to the best of our knowledge, it is novel in humans. Noteworthy, from a methodological perspective, this result emphasizes the need for research into the effects of antenatal stress to account for the influence of postnatal care, as overlooking this aspect might lead to misguiding findings and explain inconsistencies in the literature.

Furthermore, the magnitude of the link between maternal EA and infants’ cortisol reactivity depended upon the levels of maternal diurnal cortisol during pregnancy, so that maternal EA was significantly related to infants’ cortisol response only at higher levels of maternal antenatal cortisol. Albeit preliminary, this finding is in line with the emerging notion that antenatal experiences could program individual postnatal plasticity (Pluess & Belsky, 2011). We might speculate that antenatal exposure to higher levels of maternal cortisol could increase infants’ sensitivity to environmental influences and make them more susceptible to the effects of variations in maternal care.

The mechanisms underlying the moderating effects of maternal care in the link between antenatal cortisol exposure and infant stress reactivity are still to be uncovered. Early in development, a sensitive caregiver is hypothesized to buffer infants’ neuroendocrine stress responses by suppressing the HPA-axis hormonal cascade and inducing greater prefrontal cortex regulation of the amygdala or by stimulating oxytocin release which has inhibitory effects on the HPA-axis (Gunnar & Hostinar, 2015). We might speculate that in infants of less emotionally available mothers, the parental stress buffering is less effective, thus exacerbating the association between antenatal cortisol exposure and stress reactivity and leading to enhanced cortisol response to the inoculation. Epigenetic mechanisms are an additional pathway through which caregiving experiences can shape stress regulation and interact with antenatal exposures (Weaver et al., 2004).

Some non-significant results are noteworthy. First, consistent with prior work (e.g. Davis et al., 2011) maternal antenatal cortisol and depressive/anxiety symptoms were not significantly associated, thus calling into question the role of cortisol as a mediator of maternal distress on fetal development. It is possible that the HPA axis-resetting occurring during pregnancy might overcome our ability to identify significant associations between endocrine and self-reported stress measures. Or, it is plausible that mild levels of distress are associated with subtle alterations in cortisol diurnal pattern, rather than with averaged daily cortisol, as shown elsewhere (Nazzari et al., 2020b). Secondly, maternal depressive/anxiety symptoms were not related to infant stress regulation in the final adjusted models. This finding is in line with most studies included in a recent review which failed to detect an association between antenatal depressive symptoms and children cortisol reactivity both in clinical and non-clinical samples (Bleker et al., 2018). Interestingly, Osborne and colleagues (2018) showed a larger cortisol stress response at 12, but not at 2 months, in infants prenatally exposed to maternal depression, thus suggesting that the effects of antenatal maternal distress might be delayed and appear later in development. However, as little evidence of an association between maternal antenatal mood and stress biomarkers during pregnancy exists (e.g. Harville et al., 2009), it is also possible that self-reported measures does not fully capture maternal stress experience throughout pregnancy and that it is the complex interplay between maternal psychological distress, stress-related physiological changes and postnatal environment, rather than self-reported symptoms alone, to affect the development of infants' stress regulation. Likewise, in line with our previous report (Nazzari et al., 2019), we did not detect any significant association between maternal antenatal sAA, CRP and IL-6 levels and infant stress regulation. Two previous studies reported an association between infant cortisol and, respectively, maternal antenatal sAA (Rash et al., 2016) or inflammation (Osborne et al., 2018). However, substantial methodological variations limit comparisons with the current findings. Lastly, maternal EA was not significantly associated with either antenatal or postnatal depressive/anxiety symptoms. This is in line with previous reports in low-risk (e.g. Endendijk et al., 2005), but not high risk (e.g. Frigerio, Porreca, Simonelli, & Nazzari, 2019), samples and possibly suggests that the nature of the sample might have constrained the range in maternal EA.

Some study limitations warrant attention. First, results are based on a relatively small community sample and markers of maternal antenatal stress were assessed only in late pregnancy, thus limiting generalizability of findings to high-risk populations and different gestational windows. Further, given the exploratory nature of the study and the need to limit the risk of Type II error, Bonferroni corrections for multiple testing were not applied (Perneger, 1998). Instead, we reported all the comparisons made and exact p values to enable the reader to evaluate the relative weight of the results. Replication of these findings in different and larger cohorts is needed. Second, maternal salivary samples in late pregnancy were collected at home and compliance with the protocol was not objectively measured. Third, besides maternal EA, additional postnatal environmental factors, such as paternal caregiving, may moderate the association between maternal antenatal stress and infant stress regulation and were not addressed in the current study. Similarly, the observational nature of the study does not allow to disentangle the role of antenatal factors from the effects of other related factors, including maternal diet or shared genetic effect, that are likely to play a role in the "risk" transmission from mother to infant and causal inferences cannot be drawn.

5. Conclusions

Despite the limitations, the current study provides the first evidence that sensitive maternal caregiving may erase the effect of an antenatal risk exposure, as indexed by high diurnal maternal cortisol, on infant stress regulation. These results are strikingly similar to those reported in animal work and emphasize the joint contribution of antenatal and postnatal environments in a comprehensive model of early-life programming of later outcomes. Furthermore, findings highlight the need to account for the role of variations in caregiving in future research, as they are likely to alter the unfolding of antenatal processes. From a clinical perspective, findings suggest that enhancing maternal EA, especially in situations of high stress in pregnancy, should be a key target of postnatal interventions in order to attenuate the long-term consequences of antenatal adversity on child development.

KEY POINTS

- Exposure to maternal stress signals during pregnancy is an emerging risk factor for the development of the infant stress response systems.
- Animal models indicates that early caregiving may buffer these effects, but the generalizability of these findings to human is unknown.
- We provided the first evidence for a buffering role of maternal emotional availability in the association between maternal antenatal cortisol levels and infant cortisol reactivity.
- Findings suggest that enhancing maternal sensitive caregiving, especially in situations of high stress in pregnancy, should be a key target of postnatal interventions. Further, they highlight the need to account for the role of variations in caregiving in future research, as they are likely to alter the unfolding of antenatal processes.

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DATA STATEMENT: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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TABLES

Table 1 – Means, Standard Deviations (SD) and Ranges for all study variables

Study Variable	Mean	SD	Range
Maternal cortisol (µg/dl)			
Waking	0.38	0.14	0.13-0.83
Waking +30’	0.50	0.15	0.10-0.91
Bedtime	0.18	0.06	0.01-0.41
AUCg	263.76	59.87	83.76-442.71
Maternal sAA (U/ml)			
Waking	70.51	65.47	3.00-463.84
Waking +30’	48.16	37.31	2.80-171.37
Bedtime	95.73	67.03	3.28-310.96
AUCg	3631.20	674.94	1777.74-5102.50
Maternal CRP (ng/ml)	3697.74	2633.86	480.04-11179.80
Maternal IL-6 (pg/ml)	1.73	1.09	0.48-6.47
Maternal prenatal EPDS	5.72	4.56	0-19
Maternal prenatal STAI-S	35.52	9.24	20-64
Maternal postnatal EPDS	4.87	3.91	0-22
Maternal postnatal STAI-S	32.91	8.61	20-66
Maternal sensitivity	22.16	3.36	15-29
Maternal structuring	22.99	2.82	14-29
Maternal non-intrusiveness	22.02	4.01	13-29
Maternal non-hostility	26.26	1.98	20-29
Infant cortisol (µg/dl)			
Baseline	0.31	0.23	0.03-1.10
20-min post-stressor	0.78	0.35	0.01-1.86
40-min post-stressor	0.47	0.25	0.11-1.37

Note: AUCg, Area Under the Curve with respect to the ground; sAA, salivary Alpha Amylase; CRP, C-Reactive Protein; IL-6, Interleukin-6; EPDS, Edinburgh Postnatal Depression Scale score; STAI-S, State-Trait Anxiety Inventory state score.

Table 2 – Bivariate correlations among study variables

	1	2	3	4	5	6	7	8	9	10	11
1. Maternal Cortisol AUCg											
2. Maternal sAA AUCg	**.31****										
3. Maternal IL-6	.10	.21									
4. Maternal CRP	.01	.03	**.31****								
5. Prenatal EPDS	-.18	.02	.18	.12							
6. Prenatal STAI-S	-.01	.14	.21	.12	**.64*****						
7. Postnatal EPDS	.09	.01	.05	-.05	**.59****	**.38****					
8. Postnatal STAI-S	-.05	-.09	.19	.07	**.42****	**.43****	**.67****				
9. Maternal EA	.07	-.07	-.21	-.17	.12	.04	.16	.07			
10. Cortisol baseline	-.03	-.18	-.20	-.15	-.01	-.01	-.04	-.04	.01		
11. Cortisol post 20’	.15	.03	-.06	.08	-.07	.03	**-.22***	-.17	-.20	.04	
12. Cortisol post 40’	.07	.12	.07	.02	**.22***	.07	.09	-.02	-.06	-.06	**.60****

Table 3 - Full prediction model for the interactive effects of maternal antenatal stress indexes and postnatal EA on infants' cortisol response

	Model 1 Cortisol		Model 2 sAA		Model 3 IL-6		Model 4 CRP		Model 5 EPDS		Model 6 STAI-S	
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p
Fixed effects												
Intercept	**0.265 (0.023)**	**<.001**	**0.265 (0.022)**	**<.001**	**0.281 (0.023)**	**<.001**	**0.287 (0.023)**	**<.001**	**0.257 (0.022)**	**<.001**	**0.258 (0.022)**	**<.001**
Infant gender	-0.007 (0.026)	0.79	-0.008 (0.026)	0.76	-0.020 (0.027)	0.47	-0.022 (0.027)	0.42	-0.008 (0.025)	0.75	-0.002 (0.025)	0.93
Maternal age	0.004 (0.004)	0.22	0.005 (0.003)	0.12	0.005 (0.003)	0.13	0.004 (0.004)	0.24	0.004 (0.003)	0.21	0.004 (0.003)	0.22
Postnatal EPDS	-0.014 (0.017)	0.43	-0.009 (0.017)	0.59	0.010 (0.018)	0.58	0.010 (0.019)	0.60	-0.025 (0.020)	0.22	-0.016 (0.018)	0.39
Antenatal stress index	-0.000 (0.000)	0.55	**-0.000 (0.000)**	**0.02**	**-0.153 (0.072)**	**0.03**	-0.017 (0.023)	0.46	0.031 (0.027)	0.25	0.007 (0.073)	0.92
EA	0.001 (0.005)	0.90	0.011 (0.033)	0.73	0.000 (0.006)	0.94	-0.079 (0.052)	0.13	-0.017 (0.013)	0.20	-0.127 (0.077)	0.10
Antenatal stress index X EA	0.000 (0.000)	0.73	-0.000 (0.000)	0.74	-0.020 (0.023)	0.38	0.010 (0.007)	0.12	0.012 (0.008)	0.13	0.037 (0.022)	0.09
Linear	0.012 (0.007)	0.07	0.011 (0.009)	0.22	**0.021 (0.001)**	**<.001**	-0.003 (0.015)	0.86	**0.026 (0.004)**	**<.001**	0.011 (0.023)	0.64
EA	0.003 (0.002)	0.15	0.001 (0.003)	0.81	**-0.001 (0.000)**	**0.01**	0.001 (0.004)	0.89	0.000 (0.001)	0.86	0.007 (0.007)	0.30
Antenatal stress index	0.000 (0.000)	0.18	0.000 (0.000)	0.21	0.003 (0.006)	0.56	0.003 (0.002)	0.11	-0.003 (0.002)	0.22	0.003 (0.006)	0.64
Antenatal stress index X EA	**-0.000 (0.000)**	**0.04**	0.000 (0.000)	0.99	0.000 (0.002)	0.86	-0.000 (0.001)	0.68	-0.001 (0.001)	0.34	-0.002 (0.002)	0.23
Quadratic	-0.000 (0.000)	0.07	-0.000 (0.000)	0.10	**-0.000 (0.000)**	**<.001**	-0.000 (0.000)	0.89	**-0.001 (0.000)**	**<.001**	-0.000 (0.000)	0.51
Maternal EA	-0.000 (0.000)	0.16	-0.000 (0.000)	0.64	**0.000 (0.000)**	**0.01**	0.000 (0.000)	0.62	-0.000 (0.000)	0.85	-0.000 (0.000)	0.45
Antenatal stress index	-0.000 (0.000)	0.27	-0.000 (0.000)	0.54	0.000 (0.000)	0.85	-0.000 (0.000)	0.24	0.000 (0.000)	0.10	-0.000 (0.000)	0.86
Antenatal stress index X EA	**0.000 (0.000)**	**0.05**	-0.000 (0.000)	0.99	0.000 (0.000)	0.84	-0.000 (0.000)	0.84	0.000 (0.000)	0.34	0.000 (0.000)	0.36
Random effects												
Level 2 (individual)												
Intercept variance	**0.010(0.004)**	**0.03**	0.008(0.004)	0.08	**0.010 (0.004)**	**0.01**	**0.011 (0.004)**	**0.01**	0.008(0.004)	0.06	0.008(0.004)	0.06
Linear slope variance	**0.000(0.000)**	**0.01**	**0.000(0.000)**	**0.02**	**0.000(0.000)**	**0.002**	**0.000(0.000)**	**0.002**	**0.000(0.000)**	**0.02**	**0.000(0.000)**	**0.02**
Intercept/Linear slope covariance	-0.000(0.000)	0.15	-0.000(0.000)	0.31	-0.000(0.000)	0.10	-0.000(0.000)	0.08	-0.000(0.000)	0.19	-0.000(0.000)	0.22
Level 1 (occasions)												
Intercept variance	**0.016(0.003)**	**<.001**	**0.017(0.003)**	**<.001**	**0.012(0.002)**	**<.001**	**0.012(0.002)**	**<.001**	**0.016(0.003)**	**<.001**	**0.017(0.003)**	**<.001**

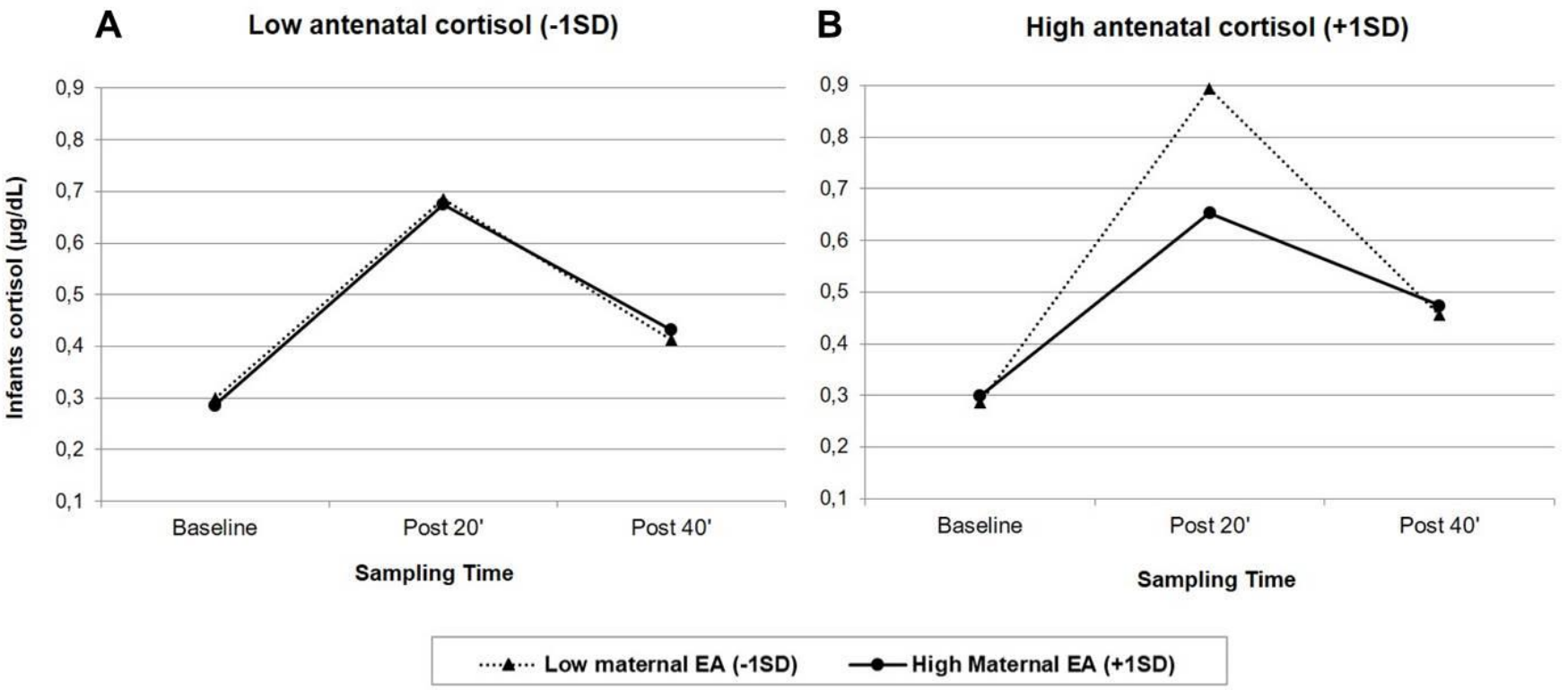

Figure 1 - Averaged infant cortisol values before and after the inoculation for infants of higher (+1 SD) and lower (-1SD) emotionally available mothers jointly with higher (+1 SD) and lower (-1SD) maternal antenatal cortisol, after adjusting for covariates. A, B. The association between maternal antenatal cortisol and infant cortisol reactivity is significant at lower levels of maternal EA (ps=.02), whereas non-significant at higher levels of maternal EA (ps=.64-76). B. The association between maternal EA and infant cortisol response is significant at higher levels of maternal antenatal cortisol (+1SD) (ps=.005-.007).