Psychoneuroendocrinology 101 (2019) 253–262

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Beyond the HPA-axis: Exploring maternal prenatal influences on birth outcomes and stress reactivity

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ARTICLE INFO

Keywords:
Pregnancy
Stress
Cortisol
Alpha-amylase
Interleukine-6 (IL-6)
Birth outcomes

ABSTRACT

Accumulating evidence suggests that antenatal maternal stress is associated with altered behavioral and physiological outcomes in the offspring, however, whether this association is causal and the underlying biological mechanisms remain largely unknown. While the most studied mediator of maternal stress influences on the fetus has generally been cortisol, alternative novel markers of stress or inflammation warrant further consideration. The current investigation explored the influence of variations in self-reported symptoms of distress, stress hormones and inflammatory markers on infant birth outcomes and early stress regulation. The sample consisted of 104 pregnant women (mean gestational age = 34.76; SD = 1.12) and their healthy newborns. Maternal self-reported symptoms of depression and anxiety were evaluated through the Edinburgh Postnatal Depression Scale and the State-Trait Anxiety Inventory and levels of serum Interleukine-6 (IL-6), C-Reactive Protein (CRP), salivary cortisol and alpha amylase (sAA) were measured in late pregnancy. Newborns' cortisol and behavioral response to the heel-stick was assessed 48–72 hours after birth. The associations between maternal stress measures and infant birth outcomes and stress reactivity, adjusted for potential confounders, were examined through hierarchical linear regressions and hierarchical linear models. Higher maternal IL-6 levels were associated with smaller head circumference at birth, while diurnal sAA levels were positively associated with birthweight. Maternal diurnal cortisol was related to newborn's stress reactivity: a flatter infant cortisol response to the heel-stick was associated with greater maternal cortisol increases after awakening during pregnancy, while greater infant behavioural reactivity was related to a flatter maternal diurnal cortisol profile. The observational nature of these data does not allow for causal inferences but the current findings illustrate that antenatal factors related to alterations in maternal stress and immune response systems are associated with fetal growth and neonatal stress reactivity. This may have implications for later health and psychological outcomes.

1. Introduction

Mounting evidence indicates that maternal prenatal stress is associated with an increased risk of altered physiological, behavioral, emotional and cognitive outcomes in offspring (Van den Bergh et al., 2017). Maternal depressive or anxiety symptoms have been the most common measures of prenatal stress (e.g. Talge et al., 2007) and findings from many community samples have shown that they might have a detectable impact even at subclinical levels (reviewed in Van den Bergh et al., 2017; Madigan et al., 2018), thus broadening scientific and public health implications.

Notwithstanding the substantial evidence linking antenatal maternal distress to child outcomes, proving that this association is causal is challenging. There is a variety of confounds, including genetic and postnatal environmental factors, that could be related to both maternal distress and offspring outcomes and cause spurious associations (Rice et al., 2018). However, associations between prenatal stress and some indices of child development were found when using a prenatal cross-fostering design, which controls for maternal heritable factors (Rice et al., 2010), and controlling for measured confounding factors, such as smoking or socioeconomic status (e.g. O'Connor et al., 2013), or postnatal effects, such as postnatal symptomatology (e.g. O'Donnell et al., 2013). Thus, it has been proposed that distress-linked alterations in the intrauterine environment might mediate, at least in part, the link between maternal antenatal distress and adverse developmental outcomes, in a process often described as "fetal programming" (e.g. Barker, 2004). Based on animal studies, research has focused primarily on the role of the maternal HPA axis. However, the weight of supporting

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https://doi.org/10.1016/j.psyneuen.2018.11.018
Received 5 April 2018; Received in revised form 10 November 2018; Accepted 12 November 2018

evidence in humans is not yet convincing (Rakers et al., 2017) and it is now clear that complementary or alternative mechanisms of stress, involving, for example, the sympathetic nervous system (SNS) or the inflammatory response system (IRS) warrant further consideration.

1.1. Prenatal HPA axis functioning and offspring's stress reactivity

Excessive fetal exposure to glucocorticoids, both synthetic and endogenous, can have detrimental effects in later life (Seckl and Holmes, 2007). Indeed, while the fetus is largely protected from cortisol exposure through the activity of the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a proportion of maternal cortisol crosses the placenta and influences fetal development (Gitau et al., 1998). Thus, it has been hypothesized that distress-linked elevations in maternal cortisol or impairment of the activity of 11β-HSD2, might lead to more cortisol passing through the placenta and directly affecting fetal development and stress physiology, with long-lasting consequences (O'Donnell and Meaney, 2017).

In humans, only partial support for a link between antenatal maternal cortisol and offspring development has been found (reviewed in Zijlmans et al., 2015). In particular, preliminary findings relate higher maternal cortisol during pregnancy with lower offspring birthweight and/or shorter gestational age (Reynolds, 2013), although results are dependent on the gestational timing and methods of assessment. Additionally, evidence for an association between antenatal maternal cortisol and offspring stress reactivity are scarce and not entirely consistent. In general, higher maternal cortisol levels have been associated with greater cortisol and behavioural stress reactivity in infants (e.g. Davis et al., 2011; Werner et al., 2013), but studies also report inverse associations (O'Connor et al., 2013) or no association (e.g. Tollenaar et al., 2011).

It has been shown that multiple diurnal cortisol measures are more strongly associated with psychosocial stress measures (Pruessner et al., 2003; Harville et al., 2009) and allow to obtain a more reliable estimate of total cortisol output (Adam and Kumari, 2009). However, only few studies have investigated the effect of maternal diurnal cortisol variations on infant birth outcomes (e.g. Kivlighan et al., 2008) or stress reactivity (Giesbrecht et al., 2017; de Weerth et al., 2013).

1.2. Prenatal SNS functioning and offspring's stress reactivity

Although catecholamines, produced by the SNS under stress conditions, do not cross the placental barrier in biologically relevant concentrations (Giannakoulopoulos et al., 1999), stress-related activation of the SNS might affect utero-placental perfusion, indirectly influencing fetal growth and, in turn, activating the fetal HPA axis (Rakers et al., 2017). Furthermore, catecholamines might down-regulate 11β-HSD2 gene expression (Sarkar et al., 2001), thus possibly increasing fetal glucocorticoid exposure.

In the current study, we focused on diurnal variations in maternal salivary alpha amylase (sAA), a non-invasive marker of SNS activity (Nater and Rohleder, 2009) which have shown to relate to previous history of miscarriage (Giesbrecht et al., 2013) and lower birth weight (Giesbrecht et al., 2015). Additionally, Rash et al. (2016) found that maternal prenatal sAA awakening response distinguished among 6-month-olds stress reactivity profiles, while Braithwaite et al. (2017) showed that higher maternal prenatal sAA diurnal levels were associated with lower levels of distress to limits in 2-month-old boys.

1.3. Prenatal IRS functioning and offspring's stress reactivity

Emerging data suggest that psychological distress, and in particular depressive symptoms, are associated with a dysregulation of the IRS as indicated by increased levels of inflammatory markers such as Interleukine-6 (IL-6) and C-reactive protein (CRP) both in non pregnant (Miller and Raison, 2016) and pregnant (Osborne and Monk, 2013) samples. However, knowledge about the role of the IRS in fetal programming is limited. Maternal inflammation during pregnancy has been shown to affect pregnancy and birth outcomes (e.g. Rusterholz et al., 2007; Coussons-Read et al., 2012) and preliminary neuroimaging evidence suggests that higher maternal antenatal IL-6 levels are related to newborns' functional and structural brain alterations (Graham et al., 2017; Rasmussen et al., 2018). Furthermore, Osborne et al., (2018) recently showed that higher maternal pro-inflammatory cytokines levels in late pregnancy were associated with newborns' less optimal neurobehavioral function and 12-month-olds' greater cortisol reactivity.

1.4. Current study

More research is needed to determine the routes by which maternal prenatal stress influences the fetus and its development. While biological and psychological stress measures are assumed to be markers of the same underlying construct, weak or null associations have been reported between self-reported measures and stress biomarkers 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 and Meaney, 2017). Additionally, while it is known that complex interactions occur among the stress and immune systems, it is still unknown whether variations in maternal SNS and IRS functioning might affect fetal development. The current study begins to fill this gap by investigating the influence of variations in maternal self-reported depressive and anxiety symptoms, stress hormones and inflammatory markers in late pregnancy on birth outcomes and infant stress regulation in a sample of healthy women and infants. To our knowledge, the present study is unique in combining the assessment of multiple psychological and biological markers to better characterize the prenatal stress experience and investigate how different indices of prenatal stress relate to infant outcomes. We evaluated infants soon after birth in order to limit the effect of postnatal influences and we focused on late pregnancy as this is a period of rapid infant growth and brain development (Grossman et al., 2003) in which exposure to maternal stress signals is thought to influence the developing fetal stress response system (Davis et al., 2011) and is associated with later risk for emotional problems (Rice et al., 2007). Additionally, we focused on a low risk sample in order to limit possible confounding influences of additional risk factors associated with psychosocial adversity (e.g. teenage motherhood, unemployment, financial problems) and to provide novel data on the role of variations in maternal stress and inflammatory signals on offspring's normative neurodevelopment that could be applied to the investigation of prenatal programming of abnormal development in high risk samples.

2. Material and methods

2.1. Participants and procedure

Study participants included mother-infant dyads from the Effects of Depression on Infants (EDI) Study, an ongoing longitudinal investigation into the effects of maternal depression on infants' bio-behavioral development. Women at 30–33 gestational weeks were consecutively recruited in three Italian hospitals and followed longitudinally. Prenatal inclusion criteria were: aged 18–45 years, normotensive, with singleton uncomplicated pregnancy, non-smoker, not afflicted by any disease or taking any chronic medications, and with no known substance/alcohol abuse problems or chronic psychiatric disorders (except for depression and anxiety). From the initial sample of 110 women, 6 were excluded because of intrauterine death and newborn health problems (N = 2), delivery in a different hospital (N = 1) and lack of behavioral data (N = 3). Most women (mean age = 33.04, SD = 3.83) were Italian (97.1%), well-educated (89.4% had at least high school diploma), middle-high class (94.8%), married (63.5%) or cohabiting (34.6%) and

primiparous (89.4%). Infants (51.9% males) were mostly born by vaginal delivery (82.7%) and full term, except for two born, respectively, at 35 and 36 gestational weeks in good health. Women who were excluded from the postnatal phase did not differ from participants on any demographic variables, depression or anxiety scores.

All pregnant women filled in two questionnaires on anxiety and depression and a demographic and pregnancy information form between 30–33 gestational weeks (mean gestational age = 31.45; SD = 1.40) and provided biological samples between 34–36 gestational weeks (mean gestational age = 34.76; SD = 1.12). Between 48–72 hours after delivery, infants' behavioral and cortisol response to the heel-stick was assessed and women filled in a form on delivery and health. The Ethics Committee of the Scientific Institute Eugenio Medea, of University College London, and of the hospitals involved approved the study protocol.

2.2. Maternal assessment

Psychological assessment. Maternal antenatal depressive symptoms were evaluated through the 10-items Italian version (Benvenuti et al., 1999) of the Edinburgh Postnatal Depression Scale (EPDS; Cox et al.,1987), a self-report questionnaire widely used to screen for perinatal depression. Maternal anxiety symptoms were assessed through the 20-items Italian version (Pedrabissi & Santinello, 1989) of the state-anxiety subscale of the State-Trait Anxiety Inventory (STAI-S; Spielberger et al., 1970) a well-validated self-report measure, which evaluates anxiety symptoms experienced in the last few days.

Health status. Both prenatally and postnatallly, participants were asked to fill in an ad-hoc form to collect health-related data and data related, respectively, to pregnancy (e.g. any complication/risk/medical disorder etc.) and delivery (e.g. length of labor, analgesia etc.).

Biological assessment. Ninety-seven women out of 104 consented to blood draw. Blood was drawn by venepuncture in the morning and kept refrigerated at +4° until it reached the Biological Lab of Medea Institute where serum was centrifuged, aliquoted, and stored at −80°. Biological assays for IL-6 and CRP levels were run in duplicate by using Quantikine High Sensitivity ELISA kits (R&D Systems Europe, LTD) at LaboSpace in Milan according to the manufacturer's instructions. Intra-assay coefficient of variation (CV) was < 6% for IL-6 and < 3% for CRP, inter-assay CV was < 10% for both markers.

Saliva was collected at home on two consecutive days immediately upon awakening, 30 min post-awakening and before going to bed to provide a general index of the diurnal pattern which is thought to better capture individual differences in the HPA axis and SNS activity (O'Donnell et al., 2013). Participants were instructed to collect whole unstimulated saliva samples by passive drool, to record time of collection on a diary and to avoid eating, tooth-brushing and exercising 30 min before collection. Women were reminded of the collection through pre-scheduled phone callings and were instructed to employ a cooler for returning samples. The mean time from the awakening collection and the 30-min post-waking collection was 30.82 min on day 1 (SD = 3.64, range: 20.00–60.00) and 31.44 min on day 2 (SD = 6.81, range: 20.00–90.00). All samples were visually inspected for contamination and diaries were checked for times and potentially interfering behaviors. As 1 pregnant woman collected the second sample more than one hour from awakening, this sample was excluded from analyses (e.g. O'Donnell et al., 2013). All saliva samples were stored frozen at -80° until assayed for salivary cortisol at the Biological Lab of Medea Institute, using a competitive high sensitivity enzyme immunoassay kit (Expanded Range High Sensitivity Cortisol EIA Kit, Salimetrics), and for sAA at the Salimetrics Centre of Excellence testing lab at Anglia Ruskin University, using a kinetic enzyme assay kit (Salimetrics α-Amylase Kinetic Enzyme Assay Kit). All samples for each woman were run in the same assay to minimize method variability. Salivary cortisol assays were run in duplicate, except for 2 samples with minimal volume. Average intra- and inter-assay coefficients were < 6% and < 8%, respectively. A random 10% of the sAA assays were run in duplicate to confirm reliability. The intra-assay coefficient of variation was < 3%.

Cortisol and sAA have the opposite diurnal profile which is maintained during pregnancy: a sharp increase in cortisol concentrations after waking, is followed by a gradual decline across the day (Kivlighan et al., 2008), while sAA levels rapidly decrease after awakening and then gradually increase throughout the day (Giesbrecht et al., 2013). Four summary parameters were calculated to index different aspects of maternal cortisol and sAA diurnal activity, namely, waking levels, response to awakening, diurnal slope and total diurnal output. Specifically, as cortisol and sAA values at each time points across the two days were highly correlated (rs between 0.51 and 0.59 for cortisol and 0.55 to 0.78 for sAA), they were averaged and mean values were used to compute response to awakening (by subtracting waking values from the 30 min post-waking levels) and diurnal slope (by subtracting the bedtime values from the waking values), as done in several prior studies (e.g. de Weerth et al., 2013). Additionally, daily average cortisol and sAA were calculated as the area under the curve (AUCg) using the trapezoid method with respect to the ground (Pruessner et al., 2003) for each day separately and, as the two 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. These composite measures are widely employed (e.g. De Weerth et al., 2013; Giesbrecht et al., 2017) and are thought to reflect different aspects of cortisol and sAA physiology. In particular, the response to awakening and diurnal slope reflect diurnal changes related to the HPA axis or SNS daily functioning, while the AUCg provide unique information about the overall secretion over the day (Adam and Kumari, 2009). As reported in previous studies (e.g. De Weerth et al., 2013), the awakening response and diurnal decline were moderately interrelated suggesting that a greater response to awakening was related to a flatter diurnal slope both for cortisol and sAA (respectively, $r = -0.47$ and $r = -0.55$, $p < .001$).

2.3. Neonatal assessment

Health and birth outcomes. Information on newborns' health (i.e. gestational age, weight, body length and head circumference at birth, Apgar scores, any complication/abnormal sign, any medication/treatment, breast or formula feeding, time of discharge) was extracted from medical records.

Behavioral assessment. Newborns' behaviour was videotaped during 10-minute baseline period, followed by the heel-stick and by 5-minutes recovery between 48 and 72 h after birth (mean = 57.06; SD = 12.15). The average length of the heel stick was 3.99 min (SD = 2.32). Newborns' videotaped behavior was evaluated every 20-seconds on a 5-point scale (i.e. sleep, drowsy, awake and alert, awake and fussy, and crying), according to a modified version of a coding system employed by Davis et al. (2011). The highest state observed during each epoch was coded. As the length of the heel-stick was variable, the first 2 min from the beginning of the blood draw were coded for all infants to ensure complete data for the whole sample. The average state score for each of the three phases (10-minutes baseline, 2-minutes response, 5-minutes recovery) was calculated. For approximately 10% of cases (N = 10), two observers, independently coded newborn's behaviour. Intra-class correlation was, respectively, equal to 1.0 for baseline, 0.99 for response and 0.99 for recovery. Coders were blinded to all prenatal or postnatal data.

Cortisol collection and assay. Salivary cortisol samples were collected after the baseline period before the beginning of the heel-stick, and after 20 and 40 min through specifically designed swab (SalivaBio Infant's Swab, Salimetrics). Infants were not fed in the 30 min before the heel-stick (mean time from last feeding = 68.50 min, SD = 50.05) and were not handled during the study protocol besides that which was strictly required for the examination. Complete cortisol data were available for 49 infants while one or two sample were missing for 30

infants and 25 infants had no data due to insufficient saliva volumes. Infants with complete, partial or missing data did not differ on any sociodemographic/maternal variables, infants-related variables or situational factors. Saliva samples were stored at -80° until assayed for cortisol according to the same procedure described for maternal cortisol. All samples from any individual infant were run on the same assay and in duplicate, excepted for 10 samples with minimal volume. The average intra- and inter-assay coefficients of variance were below 7% and 10%, respectively.

2.4. Statistical analyses

Variables were first examined for outliers and skewness. Distributions of both maternal and infants' biological markers were positively skewed even after removing samples greater than 3 SD from the mean (N = 7 for maternal cortisol, N = 4 for sAA, N = 3 for IL-6, n = 4 for infants' cortisol), thus variables were natural log transformed to approximate normal distributions.

To evaluate the potential effect of variables known to affect stress and immune physiology, preliminary Pearson correlations and univariate analysis of variance (ANOVA) were employed. All variables found to be significantly associated with the outcomes examined where included as covariates in all subsequent analyses. Maternal depression and anxiety were highly correlated ($r = 0.60$, $p < .001$), thus in order to avoid multicollinearity issues and as they are expected to differ in their underlying biology (O'Donnell and Meaney, 2017), they were entered separately in subsequent analyses. Analyses including maternal anxiety rather than depression, yielded comparable results and are reported in Supplementary Tables.

Separate hierarchical regression analyses were performed to evaluate the effects of maternal prenatal psychological and biological stress markers on infant birth outcomes (i.e. birth weight, body length, and head circumference), while adjusting for covariates. Hierarchical Linear Models (HLMs) were estimated to investigate the influence of maternal prenatal stress measures on the trajectories of infants' cortisol and behavioral reactivity, while accounting for the hierarchical structure of the data (three time-points nested within individuals). HLMs were specified at two levels where subjects were level 2 and time was level 1. Time was centered at baseline so that the model intercept represents the mean cortisol/behavioral state at baseline. Before fitting explanatory models including level-2 predictors, a baseline model of cortisol and behavioral response was fitted to describe the trajectory of infants' response, including a linear and quadratic slope for time. A random intercept and a random linear slope were included to allow between-person variability. The explanatory variables were centered around the grand mean and entered in the model one-by-one. Gender was centered at males. Model fit was tested with likelihood deviance difference tests for nested models.

Statistical analyses were performed using SPSS 24 and MLWiN.

3. Results

3.1. Descriptive analyses and confounders

Descriptive characteristics for all study variables are presented in Table 1, whereas correlations between prenatal variables, birth outcomes and stress reactivity are shown in Table 2.

In a series of univariate correlation analyses we evaluated the associations between sociodemographic factors (maternal age, marital status, education and SES), pregnancy- and delivery-related factors (parity, mode of delivery, assisted delivery, length of labor), infant factors (gestational age, birth weight, gender, Apgar scores and postnatal age), situational factors (length of the heel-stick procedure, time of the day and time from last feeding) and newborn outcomes. Gestational age was associated with 20-min post-stressor cortisol levels ($r = -0.31$, $p < .05$) and length of the heel-stick was positively related to both 20-min post-stressor cortisol levels ($r = 0.30$, $p < .05$) and averaged behavioral state during the response period ($r = 0.20$, $p < .05$). Thus, they were included as covariates in HLMs. Additionally, while infant's sex was not related to cortisol or behavior, there was a significant association between fetal sex and maternal waking cortisol levels ($F(101,1) = 5.02$, $p < .05$). As sex-differences in the association between prenatal distress and infant cortisol have also been reported (e.g. Giesbrecth et al., 2017), infant's gender was included as a covariate in subsequent analyses. Furthermore, gestational age, sex and maternal pre-pregnancy BMI were associated with birth outcomes, with infants with higher gestational ages, males and born from mothers with higher BMI, having greater anthropometric measures (ps < .05), and were included as covariates in hierarchical regression analyses.

Table 1
Descriptive statistics for study variables at the prenatal and postnatal assessment.

Study Variable	Mean	SD	Range
Prenatal			
Maternal cortisol (μg/dl)			
Waking	0.38	0.13	0.13-0.83
Waking +30'	0.50	0.15	0.10-0.91
Bedtime	0.18	0.06	0.01-0.41
Response to waking	0.12	0.16	−0.52-0.52
Diurnal slope	0.20	0.13	−0.12-.70
AUCg	262.63	58.59	83.76-442.71
Maternal sAA (U/ml)			
Waking	69.84	64.75	3.00-463.84
Waking +30'	47.74	37.90	2.80-190.10
Bedtime	99.14	80.95	3.28-562.71
Response to waking	−21.07	52.65	−350.94-119.97
Diurnal slope	−28.48	85.67	−405.11-298.32
AUCg	3601.07	690.76	1777.74-5394.20
Maternal CRP (ng/ml)	3730.16	2766.02	480.04-11244.10
Maternal IL-6 (pg/ml)	1.68	1.04	0.48-6.47
Maternal depression (EPDS)	5.41	4.44	0-19
Maternal anxiety (STAI-S)	35.22	8.95	20-64
Postnatal			
Gestational age (weeks)	39.48	1.25	35-42
Birth weight (grams)	3296.83	443.27	2170-4440
Body Length (cm)	50.08	1.88	45-54
Head Circumference (cm)	34.48	1.30	31.50-39.50
Newborns' cortisol (μg/dl)			
Baseline	0.66	0.45	0.06-2.27
20-min post-stressor	0.83	0.63	0.15-3.00
40-min post-stressor	0.82	0.73	0.17-3.04
Newborns' behavior			
Baseline	2.15	1.16	1.00-4.63
Response	3.69	1.10	1.00-5.00
Recovery	2.47	1.17	1.00-4.80

Note: AUCg, area under the curve with respect to the ground; sAA, salivary alpha amylase; CRP, C-Reactive Protein; IL-6, Interleukine-6.

3.2. Association between maternal prenatal variables and birth outcomes

As shown in Table 3, multiple hierarchical regression analyses revealed significant associations between both higher maternal sAA waking and AUCg levels and higher infant birth weight, as well as between higher IL-6 levels and smaller head circumference at birth, after controlling for pre-pregnancy BMI, infant's sex, and gestational age. No significant associations between maternal cortisol or maternal self-reported distress symptoms and any birth outcomes were found.

3.3. Association between maternal prenatal variables and newborns' cortisol reactivity

The unconditional means model for newborns cortisol showed significant variability at the individual level (level-2; $\sigma^2_{u0} = 0.042$, $p < .001$) and between-occasions (level-1; $\sigma^2_{e0} = 0.057$ $p < .001$). Infants displayed the expected cortisol response characterized by a significant linear slope of time ($p < .05$), and a marginally significant

Table 2
Bivariate correlations among prenatal variables, birth outcomes and infant stress reactivity.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Prenatal EPDS														
Prenatal STAI-S	**.60****													
Prenatal Cortisol AUCg	−.17	.00												
Prenatal sAA AUCg	.07	.19	**.24***											
Prenatal IL-6	**.20***	.19	.13	.19										
Prenatal CRP	.13	.15	.11	−.01	**.31****									
Birth weight	.10	.05	−.03	**.18**^{t}**	.03	**.25***								
Body length	.05	.00	−.05	.08	−.03	.14	**.90****							
Head Circumference	.12	−.01	.02	.06	−.18	.09	**.63****	**.59****						
Cortisol baseline	**−.22**^{t}**	−.17	.01	−.00	−.10	.06	.14	.11	.05					
Cortisol post 20'	−.08	−.11	−.03	.04	−.14	−.14	−.02	−.06	.03	.27				
Cortisol post 40'	−.08	−.13	.04	−.02	−.07	.04	−.06	−.08	.00	.01	**.80****			
Behavioral baseline	−.00	−.07	.09	.01	−.01	.02	−.01	.00	−.13	**.31****	.21	.17		
Behavioral response	−.10	−.17	−.05	.11	−.06	−.09	.13	.07	.08	.00	.21	.03	.01	
Behavioral recovery	−.16	−.08	.12	.10	.02	−.07	.11	.13	.08	.12	**.30***	.11	.14	**.51****

Note: AUCg, area under the curve with respect to the ground; sAA, salivary alpha amylase; CRP, C-Reactive Protein; IL-6, Interleukine-6.
*p < .05; **p < .01, ^{t} p = .06, p > 0.05 was considered non-significant.

Table 3
Hierarchical linear regression analyses predicting weight and head circumference at birth.

	Birth weight										**Head circumference**							
	Cortisol AUCg		sAA AUCg		sAA waking		IL-6		CRP		Cortisol AUCg		sAA AUCg		IL-6		CRP	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Step 1:																		
Gender	**−.19**	**.03**	**−.18**	**.04**	**−.18**	**.04**	−.13	.17	−.14	.14	−.17	.08	**−.17**	**.08**	−.10	.34	−.14	.19
Pre-pregnancy BMI	**.23**	**.01**	**.22**	**.01**	**.22**	**.01**	.16	.09	**.19**	**.05**	**.20**	**.04**	**.19**	**.06**	.11	.30	.12	.27
Gestational Age	**.41**	**.00**	**.40**	**.00**	**.40**	**.00**	**.43**	**.00**	**.42**	**.00**	**.24**	**.01**	**.23**	**.02**	**.27**	**.01**	**.25**	**.02**
ΔR^2 for step 1	.28	.00	.26	.00	.26	.00	.25	.00	.24	.00	.15	.00	.14	.00	.11	.03	.10	.03
F_{model}	13.14	.00	12.50	.00	12.50	.00	9.70	.00	9.64	.00	5.28	.00	5.03	.00	3.26	.03	3.25	.03
Step 2:																		
EPDS	.12	.14	.14	.10	.14	.10	.13	.16	.15	.11	.12	.20	.15	.12	.15	.16	.16	.12
ΔR^2 for step 2	.01	.14	.02	.10	.02	.10	.02	.16	.02	.11	.01	.20	.02	.12	.02	.16	.02	.12
F_{model}	10.51	.00	10.21	.00	10.21	.00	7.85	.00	8.01	.00	4.41	.00	4.44	.00	2.97	.02	3.08	.02
Step 3:																		
Biological predictors	−.06	.46	**.18**	**.03**	**.17**	**.05**	−.06	.52	.09	.36	−.09	.35	.02	.82	**−.33**	**.00**	−.01	.96
ΔR^2 for step 3	.00	.46	**.03**	**.03**	**.03**	**.05**	.00	.52	.01	.36	.01	.35	.00	.82	.10	.00	.00	.96
F_{model}	8.48	.00	9.36	.00	9.18	.00	6.32	.00	6.56	.00	3.70	.00	3.53	.01	4.62	.00	2.43	.04

Note: AUCg, area under the curve with respect to the ground; sAA, salivary alpha amylase.

quadratic slope (p = .07). Also the random linear slope term was statistically significant (p < .001), suggesting significant between-person variability in the cortisol linear increase. Overall, this model resulted in a significant improvement in fit over the unconditional means model (deviance difference (4) = 37.50, p < .001).

Fixed independent effects of prenatal variables (i.e. depression, anxiety, cortisol, sAA, CRP and IL-6) on mean cortisol baseline levels and on the linear and quadratic slopes of time were tested separately, while controlling for gestational age, infant's sex and length of the heel-stick. As shown in Table 4, maternal cortisol response to awakening (CAR) was significantly associated with both the linear and quadratic slopes of newborn's cortisol response (p < .01). Specifically, as shown in Fig. 1a, higher maternal CAR during pregnancy was related to a flatter cortisol response to the heel-stick, while lower maternal CAR was associated with greater infants' cortisol reactivity. The inclusion of maternal CAR resulted in a significant improvement in the model fit (deviance difference (3) = 9.173, p < .05). In contrast, there was no significant association between newborns' cortisol response and, respectively, maternal self-reported distress symptoms, sAA, IL-6 or CRP levels.

3.4. Association between maternal prenatal variables and newborns' behavioral reactivity

The unconditional means model for newborns behavioral regulation showed significant variability between-occasions (level-1; σ^2_{e0} = 0.136, p < .001), while variability at the individual level was not significant (level-2; σ^2_{u0} = 0.006, p > .05). There was a significant behavioral change in response to the heel-stick, as indexed by the significant linear and quadratic slopes of time (both ps < .001). The variance of the linear slope term was statistically significant (p < .001), suggesting significant between-person variability in the behavioral linear increase. Additionally, the significant positive association (p < .05) between the random effects of intercept at level-2 and the linear slope indicates that there was a greater increase in the behavioral indices of distress in response to the heel-stick in individuals with higher behavioral states at baseline. Overall, this model results in a significant improvement in fit over the unconditional means model (deviance difference (4) = 106.46, p < .001).

Fixed independent effects of prenatal variables on mean behavioral state at baseline and change over time were tested separately, while controlling for covariates. Maternal diurnal slope was significantly associated with both the linear and quadratic slopes of newborns' behavioral response (p < .05, Table 5). Specifically, as shown in Fig. 1b, a lower maternal cortisol diurnal slope was associated with greater behavioral reactivity in the infant, while a higher maternal diurnal slope was related to a less marked behavioral reactivity. The inclusion of maternal diurnal slope results in a significant improvement of the model fit (deviance difference (3) = 7.8, p = .05). Additionally,

Table 4
Full prediction models for the effects of maternal depression (EPDS) and cortisol diurnal indices on infants' cortisol response.

	Model 1 Waking		Model 2 CAR		Model 3 Diurnal slope		Model 4 AUCg	
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p
Fixed effects								
Intercept	0.508 (0.039)	< .001	0.508 (0.038)	< .001	0.504 (0.038)	< .001	0.505 (0.046)	< .001
Gender	−0.053 (0.052)	0.31	−0.050 (0.051)	0.32	−0.046 (0.050)	0.36	−0.051 (0.051)	0.32
Gestational Age	−0.022 (0.019)	0.25	−0.025 (0.020)	0.21	−0.027 (0.019)	0.16	−0.021 (0.020)	0.29
Heel-stick length	0.001 (0.011)	0.98	0.001 (0.010)	0.93	0.004 (0.011)	0.69	0.001 (0.011)	0.91
EPDS	−0.075 (0.040)	0.06	−0.068 (0.038)	0.08	−0.063 (0.039)	0.11	−0.071 (0.040)	0.08
Cortisol	−0.135 (0.315)	0.66	0.245 (0.253)	0.33	0.337 (0.286)	0.24	0.000 (0.001)	0.94
Linear	−0.005 (0.008)	0.54	0.008 (0.003)	< .001	0.013 (0.005)	< **.01**	0.003 (0.003)	0.27
EPDS	0.002 (0.003)	0.47	0.001 (0.003)	0.59	0.001 (0.003)	0.77	0.001 (0.003)	0.73
Cortisol	0.029 (0.023)	0.20	**−0.042 (0.017)**	< **.01**	0.035 (0.020)	0.08	−0.000 (0.000)	0.48
Quadratic	0.000 (0.000)	0.41	−0.000 (0.000)	< .001	−0.000 (0.000)	< .05	−0.000 (0.000)	0.51
EPDS	−0.000 (0.000)	0.65	−0.000 (0.000)	0.79	−0.000 (0.000)	0.94	−0.000 (0.000)	0.95
Cortisol	−0.001 (0.000)	0.17	**0.001 (0.000)**	< **.01**	0.001 (0.000)	0.09	0.000 (0.000)	0.32
Random effects								
Level 2 (individual)								
Intercept variance	0.034(0.010)	< .001	0.036(0.010)	< .001	0.034(0.010)	< .001	0.034(0.010)	< .001
Linear slope variance	0.000(0.000)	< .001	0.000(0.000)	< .001	0.000(0.000)	< .001	0.000(0.000)	< .001
Intercept/Linear slope covariance	−0.000(0.000)	0.09	−0.001(0.000)	0.07	−0.000(0.000)	0.07	−0.000(0.000)	0.09
Level 1 (occasions)								
Intercept variance	0.022(0.005)	< .001	0.020(0.004)	< .001	0.022(0.005)	< .001	0.022(0.005)	< .001

Note: Cortisol diurnal indices are examined separately in Model 1-4. CAR, cortisol awakening response, AUCg, area under the curve with respect to the ground.

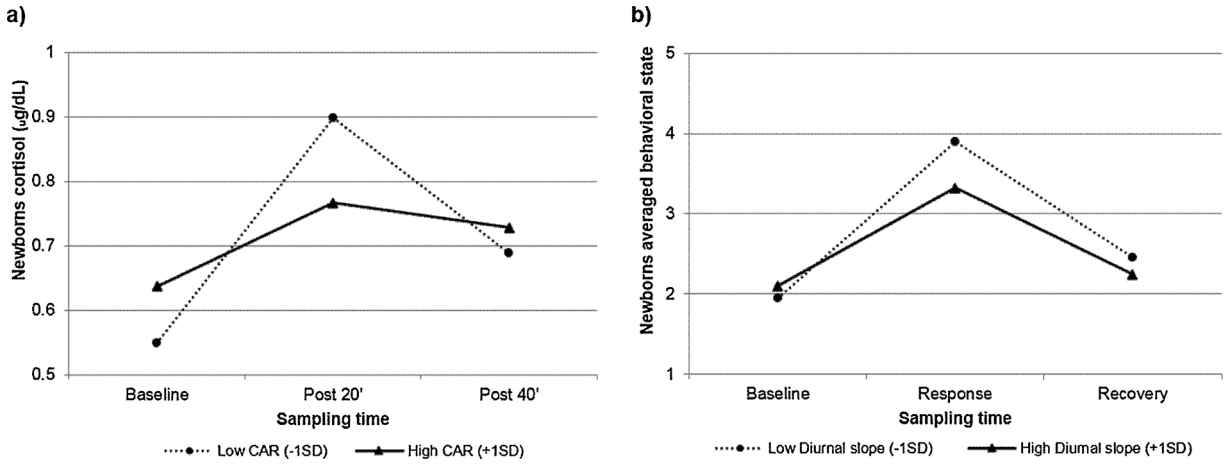

Fig. 1. a) Cortisol values before and after the heel-stick for newborns whose mothers had higher (+1 SD) and lower (-1SD) cortisol awakening response (CAR), after adjusting for covariates; b) Averaged behavioural state before, during and after the heel-stick for newborns exposed prenatally to higher (+1 SD) and lower (-1SD) maternal cortisol diurnal slope, after adjusting for covariates.

maternal cortisol at awakening was significantly associated with the linear slope of newborns' response ($p < .05$), with lower levels of cortisol being associated with a steeper increase in behavioral response to the heel-stick, while higher levels of cortisol were related to a flatter increase. However, the inclusion of maternal cortisol at awakening did not significantly improve the model fit (deviance difference (3) = 6.34, $p = .10$).

Maternal self-reported distress symptoms, sAA, IL-6 and CRP levels were not significantly associated with infants' behavioral regulation.

4. Discussion

The current study was aimed at elucidating the influences of psychological and biological indices of maternal stress in late pregnancy on infants' birth outcomes and stress regulation. Findings provide further support for an association between maternal prenatal diurnal cortisol and infants' stress reactivity. Furthermore, the results add to the limited literature on alternative biological mechanisms involved in fetal programming, by highlighting significant associations between variations in markers of maternal SNS and IRS functioning and birth outcomes in a low-risk sample of mother-infant dyads. In contrast, no significant associations between maternal self-reported distress symptoms and infant outcomes were found.

4.1. Prenatal maternal influences and birth outcomes

Markers of maternal IRS and SNS functioning during pregnancy were significantly associated with newborns' anthropometric measures, such as head circumference and birth weight, whereas cortisol levels and self-reported distress symptoms were not related to any birth outcomes.

The significant association between higher prenatal maternal IL-6 levels and smaller head circumference at birth is a novel finding. While observational epidemiological studies report that maternal inflammatory responses to infections during pregnancy are associated with increased risk of neuropsychiatric disorders, such as

Table 5
Full prediction models for the effects of maternal depression (EPDS) and cortisol diurnal indices on infants' behavioural response.

	Model 1 Waking		Model 2 CAR		Model 3 Diurnal slope		Model 4 AUCg	
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p
Fixed effects								
Intercept	1.086 (0.040)	< .001	1.090 (0.040)	< .001	1.086 (0.040)	< .001	1.086 (0.040)	< .001
Gender	0.001 (0.046)	0.99	0.001 (0.046)	0.99	0.004 (0.046)	0.98	0.008 (0.046)	0.86
Gestational Age	0.020 (0.018)	0.28	0.014 (0.019)	0.46	0.020 (0.018)	0.23	0.022 (0.019)	0.22
Heel-stick length	0.009 (0.010)	0.39	0.011 (0.010)	0.24	0.009 (0.010)	0.36	0.012 (0.010)	0.21
EPDS	0.002 (0.046)	0.96	0.003 (0.046)	0.95	0.003 (0.046)	0.95	0.008 (0.047)	0.86
Cortisol	0.180 (0.380)	0.63	0.264 (0.322)	0.41	0.274 (0.353)	0.44	0.001 (0.001)	0.35
Linear	0.047 (0.008)	< .001	0.029 (0.003)	< .001	0.038 (0.004)	< .001	0.048 (0.011)	< .001
EPDS	−0.002 (0.003)	0.50	−0.001 (0.003)	0.79	−0.002 (0.003)	0.56	−0.002 (0.003)	0.56
Cortisol	**−0.055 (0.025)**	**< .05**	−0.003 (0.022)	0.88	**−0.056 (0.024)**	**< .05**	−0.000 (0.000)	0.08
Quadratic	−0.000 (0.000)	< .001	−0.000 (0.000)	< .001	−0.001 (0.000)	< .001	−0.001 (0.000)	< .001
EPDS	0.000 (0.000)	0.83	−0.000 (0.000)	0.92	0.000 (0.000)	0.90	0.000 (0.000)	0.86
Cortisol	0.001 (0.000)	0.07	0.000 (0.000)	0.92	**0.001 (0.000)**	**< .05**	0.000 (0.000)	0.07
Random effects								
Level 2 (individual)								
Intercept variance	0.058(0.017)	< .001	0.056(0.017)	< .001	0.058(0.017)	< .001	0.058(0.017)	< .001
Linear slope variance	0.000(0.000)	< .001	0.000(0.000)	< .001	0.000(0.000)	< .001	0.000(0.000)	< .001
Intercept/Linear slope covariance	−0.001(0.000)	< .01	−0.001(0.000)	< .01	−0.001(0.000)	< .01	−0.001(0.000)	< .01
Level 1 (occasions)								
Intercept variance	0.055(0.008)	< .001	0.057(0.008)	< .001	0.055(0.008)	< .001	0.056(0.008)	< .001

Note: Cortisol diurnal indices are examined separately in Model 1-4. CAR, cortisol awakening response, AUCg, area under the curve with respect to the ground.

schizophrenia, in the resulting offspring (e.g. Estes and McAllister, 2016), the extent to which there is a programming effect of maternal cytokines is unknown. Head circumference is considered a marker for intrauterine brain development and is associated with later cognitive function (Broekman et al., 2009). Thus, our findings are consistent with results from animal studies (e.g. Meyer et al., 2006) and preliminary brain imaging studies in humans (Graham et al., 2017; Rasmussen et al., 2018) suggesting that maternal inflammation can affect fetal growth and neural development. Whether directly transferred through the placenta or indirectly affecting the fetal development through placental inflammation, elevated cytokines are hypothesized to act at multiple levels of the fetal brain, affecting neurogenesis, gliogenesis and the neurotransmitter systems (reviewed in Rakers et al., 2017). An alternative mechanism is that epigenetic mechanisms could mediate the impact of prenatal maternal inflammation on fetal brain development with potentially long-lasting effects as suggested by results of animal studies (Kundakovic and Jaric, 2017). Future efforts are needed to elucidate the role of maternal IL-6 levels in influencing rates of fetal growth and brain development in humans.

Consistent with previous findings (Osborne and Monk, 2013), we reported a positive association between IL-6 levels and self-reported depressive symptoms in a low-risk sample of women free from any medication or medical conditions. This result was specific for maternal depression, rather than anxiety, and is in line with accumulating evidence for a role of inflammation in the vulnerability for depression (Miller and Raison, 2016), thus encouraging further research into a possible inflammatory pathway from prenatal depressive symptoms to altered offspring outcomes. It is important to note that self-reported depressive symptoms were not associated with birth outcomes. This is consistent with a number of studies that show null or weak associations between prenatal continuous depression measures and adverse birth outcomes in low-risk samples (Goedhart et al., 2010; Grote et al., 2010).

Higher maternal prenatal sAA levels at awakening and throughout the day were associated with higher infant birth weight. Our findings are partially in line with the only published study on the link between maternal sAA and birth outcomes (Giesbrecht et al., 2015), which showed an association between greater birth weight and higher maternal sAA waking levels and decrease after waking. Replication in larger cohorts is needed before the significance of these preliminary findings can be discussed. We might speculate that mild elevations in maternal sAA indicate an increased SNS involvement to ensure maternal and fetal energetic resources for growth during late normative pregnancy, in line with evidence (e.g. DiPietro et al., 2011) suggesting that adjustments in maternal neuroendocrine functioning over gestation are mainly generated and supported by the feto-placental unit.

Lastly, the lack of significant associations between birth outcomes and cortisol levels is in line with results of larger studies (e.g. Goedhart et al., 2010).

4.2. Prenatal maternal influences and newborns' stress reactivity

Infants displayed the expected increase in cortisol levels and behavioural distress in response to the heel-stick in the neonatal period. In line with our hypotheses, findings support a role of variations in maternal diurnal cortisol rhythm, and in particular in maternal CAR and diurnal slope, during late pregnancy in influencing offspring's stress reactivity.

Specifically, maternal CAR was related to newborns' cortisol reactivity, with infants prenatally exposed to a higher maternal CAR showing a flatter cortisol response to stress. Only two studies have examined the role of maternal prenatal CAR on infant stress reactivity. In line with our findings, Giesbrecht et al. (2017) reported a significant association between a higher maternal CAR and blunted cortisol reactivity in 3-month old boys, whereas De Weerth et al. (2013) related a higher maternal CAR to impaired infants' cortisol habituation, as shown by a stable cortisol response over repeated maternal separations. Taken together, these findings might suggest that an increased maternal CAR during pregnancy might be associated with an impairment of the infants' capacity to flexibly react to stress.

Maternal cortisol diurnal slope during pregnancy was significantly associated with newborns' behavioral response to the heel-prick, with a smaller decline in maternal cortisol levels throughout the day being associated with greater infant behavioral reactivity. These findings are novel; while previous studies have shown greater behavioral stress reactivity in infants prenatally exposed to higher maternal cortisol (e.g. Davis et al., 2011; Werner et al., 2013), none of these studies included diurnal cortisol measures.

Both the CAR and diurnal slope are key components of the diurnal

cortisol rhythm that are relatively maintained and sensitive to psychological distress during pregnancy (Kivlighan et al., 2008). However, their role in fetal programming is still largely unknown. Thus it is unclear why different measures of maternal prenatal diurnal cortisol relate to different aspects of infants stress reactivity (i.e. physiological or behavioral). Interestingly, in line with previous reports (e.g. De Weerth et al., 2013) maternal CAR and cortisol decline were moderately correlated, with higher CARs being related to flatter cortisol declines. Therefore, it would seem that an alteration of maternal diurnal cortisol pattern is, to a certain extent, associated with both a dampened cortisol reactivity and an amplified behavioral response to stress in the infants soon after birth. As a lack of coordination between adrenocortical and behavioral responses has been associated with later psychopathology (e.g. Quas et al., 2000), it would be important to evaluate whether neonatal profiles of reactivity persist and are predictive of later patterns of stress reactivity and later emotional or behavioral dysregulation.

Questions remain about the mechanisms underlying the associations between diurnal variation in maternal cortisol diurnal measures and patterns of infants stress reactivity. On the one hand, we might speculate that both a higher than typical CAR or a flatter cortisol diurnal decline might result in a greater amount of cortisol reaching the fetus and potentially alter the set-point of fetal stress response systems. Consistently with this, animal studies suggest that the rise in glucocorticoids at the circadian peak saturates the 11β-HSD2 enzyme, leading to greater placental transfer of maternal glucocorticoids to the fetus (Venihaki et al., 2000). Alternatively, while the stress reactivity patterns observed a few hours after birth can be considered largely independent of postnatal influences, hereditary transmission might still explain the observed associations, with mother-infant shared genes accounting for variation in both maternal diurnal cortisol patterns and newborns' stress response (e.g. Van Hulle et al., 2012).

Some non-significant results are worth mentioning. First, in contrast with previous reports (e.g. Davis et al., 2011), we did not find any effect of maternal depressive or anxiety symptoms on infant stress regulation. There are a number of reasons for these null findings, including the limited sample size which might have reduced the chance to detect a true effect, and the possibility that mild variations in maternal distress have little to no effect on the developing foetus, as compared to stronger exposures (e.g. Vedhara et al., 2012). It is also possible that the effects of prenatal maternal distress on offspring stress reactivity might emerge later in development (O'Donnell et al., 2013). It is noteworthy that few studies examined both antenatal anxiety and depression in the same sample and generally comparable associations between anxiety or depression and children stress-related physiology have been reported (e.g. Davis et al., 2011; Werner et al., 2013; O'Donnell et al., 2013). However, few reports showed a stronger effect for maternal antenatal anxiety symptoms as compared to depressive in predicting children cortisol diurnal patterns (O'Connor et al., 2005) and reactivity (Grant et al., 2009). More studies are needed to determine the prenatal risk phenotype that might be more implicated in child development, as well as elucidate the underlying mechanisms.

Second, consistent with prior work (e.g. Davis et al., 2011; Baibazarova et al., 2013) maternal prenatal cortisol and self-reported symptoms were not significantly associated with infant outcomes, thus calling into question the role of cortisol as a mediator of fetal programming. 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, relatively low levels of distress might not significantly affect the HPA axis regulation. Nonetheless, it is noteworthy that we do report a significant association between maternal depressive symptoms and IL-6 levels, thus possibly suggesting that inflammatory markers might be a more promising avenue for future research.

Our preliminary results do not support a role of the SNS and the IRS in influencing newborn stress reactivity. To our knowledge, only Osborne et al., (2018) investigated the role of maternal prenatal inflammation on offspring's stress reactivity in humans and reported unadjusted positive correlations between prenatal maternal inflammatory markers and 12-month-olds' cortisol levels following the immunization in a sample of clinically depressed women and healthy controls. While sample size was comparable to the current one, methodological heterogeneity in the composition of the samples and in infants' ages might account for different results. Further replication of the current findings in different cohorts is needed. Similarly, prenatal maternal sAA levels were not associated with newborns' stress reactivity. Only two published studies have thus far examined this association, reporting significant links between maternal sAA levels in early-mid gestation and, respectively, 2-month-olds' negative emotionality (Braithwaite et al., 2017) and 6-month-olds stress-reactivity profiles (Rash et al., 2016) but no significant links with sAA in late pregnancy (Rash et al., 2016).

4.3. Limitations

Despite the strengths of the present study, such as a prospective design, the inclusion of multiple stress markers, several confounders and the neonatal assessment soon after birth, the interpretation of findings should be cautious due to several limitations. First, data are based on a relatively small middle-high SES community sample evaluated in late pregnancy and levels of self-reported distress were relatively low, thus limiting generalizability of results to high-risk populations and different gestational windows. We focused on late pregnancy because it is a period of particularly rapid fetal growth and brain development including neuronal differentiation, synaptogenesis and myelination (Grossman et al., 2003), which make the fetus particularly susceptible to environmental influences. We cannot rule out that effects earlier in pregnancy may be different. Secondly, we cannot determine whether the observed associations are specific to the third trimester or might be the result of a continuous prenatal exposure. Our results are not inconsistent with studies suggesting a stronger and specific effect of prenatal stress exposure on the outcomes examined in late pregnancy as compared to earlier exposure (e.g. Davis et al., 2011; Vedhara et al., 2012), but without measuring multiple time points across pregnancy we cannot directly address this hypothesis. Notably, Giesbrecht et al. (2017) recently reported stronger effects of early exposure to maternal diurnal cortisol on infant cortisol reactivity, as compared to later exposures. As recently highlighted by Zyjlmans and colleagues (2015), future studies should include multiple time points across gestation, in order to better characterize prenatal stress exposure and test for timing effects. Additionally, as a rise in cortisol levels has been reported as gestation progresses (Davis et al., 2011) and as cortisol levels were unrelated to psychological measures of distress, we cannot exclude the possibility that cortisol data might be primarily determined by pregnancy-related alterations.

Third, maternal salivary samples were collected at home and compliance with the protocol was not objectively measured. Fourth, as previously reported (Egliston et al., 2007), obtaining sufficient saliva volumes from newborns was a challenge, thus leading to limited sample size for cortisol analyses. HLMs were employed in order to maximize the number of infants included in the final analyses and obtain reliable estimates of effects despite missing values for one or more time-points. However, conclusions that can be drawn from the current study are to be regarded as preliminary and require replication in different and larger cohorts. Additionally, it cannot be excluded that newborns' cortisol reactivity might be masked by a still-unresolved cortisol response to delivery, although duration of labor and mode of delivery were unrelated to infants cortisol levels.

Lastly, as we did not use a relevant genetically informative design (Rice et al., 2018) and the study is observational, we cannot rule out possible pleiotropic effects of shared genes which both influence maternal stress levels and infants' outcomes, as well as unmeasured confounding, and causal inferences cannot be drawn.

4.4. Conclusions

Taken together, the current results suggest that variations in maternal stress hormones and inflammation in late pregnancy might influence fetal growth and development with possible implications for later outcomes. However, the weak or null association between psychological and biological measures of stress underlines the incomplete picture of the mechanisms through which prenatal distress may be transmitted to the foetus and affect later development, and highlights the needs for further research. We believe that a better understanding of the complex pathways underlying fetal programming will not only improve our knowledge of typical and atypical fetal development but will also have important implications for the development of effective targeted intervention.

Declarations of interest

None.

Acknowledgments

This work was supported by a PhD studentship provided by the Stanley Thomas Johnson Foundation awarded to Sarah Nazzari [grant number: 4461]. Funding were also received by Fondazione Banca del Monte di Lombardia and by Soroptimist Club di Lecco, Italy. The authors wish to thank all the families and children who participated in this study. We are extremely grateful to Dr. Stefano Norchi and Dr. Daniele Merazzi from Ospedale Valduce di Como, Dr. Rinaldo Zanini from Ospedale Mandic di Merate and Dr. Alberto Zanini from Ospedale Fatebenefratelli di Erba, as well as to all the midwives and nurses from the hospitals involved for their help in recruitment and data collection. The assistance of Enrita Pozzi, Marco Redaelli and Marta Calcinati in data collection is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.018.

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