Age and sex differences in the cortisol stress reactivity and recovery among infants exposed to prenatal psychological distress

Susanna Kortesluoma [a,b,*], Laura S. Korhonen [a,c], Juho Pelto [a], Jetro J. Tuulari [a,d,e], Linnea Karlsson [a,c,e,f], Hasse Karlsson [a,e,f]

[a] FinnBrain Birth Cohort Study, Turku Brain and Mind Center, Department of Clinical Medicine, University of Turku, Turku, Finland
[b] Institute of Biomedicine, University of Turku, Turku, Finland
[c] Department of Paediatrics and Adolescent Medicine, University of Turku and Turku University Hospital, Turku, Finland
[d] Turku Collegium for Science, Medicine and Technology, University of Turku, Turku, Finland
[e] Department of Psychiatry, University of Turku and Turku University Hospital, Turku, Finland
[f] Centre for Population Health Research, University of Turku and Turku University Hospital, Turku, Finland

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ABSTRACT

Background: Altered hypothalamic-pituitary-adrenal axis (HPA) functioning is one of the potential mechanisms bridging exposure to maternal prenatal psychological distress (PPD) and later risk for offspring psychiatric illness. Research on infant cortisol stress reactivity, on scarcely studied recovery and their associations with maternal PPD is needed to clarify these mechanisms. Knowledge on sex differences in prospective settings is largely lacking. We aimed at filling these gaps by building upon our previous report showing that exposure to maternal prenatal depressive and anxiety symptoms associates with slower cortisol recovery among 10-week-old female infants.
Methods: In all, 363, 205 and 263 infants at 10 weeks, six and 14 months of age from the FinnBrain Birth Cohort Study participated in a stress test comprising of venipuncture and nasopharynx sampling. Five saliva cortisol samples were collected during each visit to measure cortisol reactivity and recovery. PPD was assessed from maternal self-reports for depressive, anxiety and pregnancy-related anxiety symptoms at gestational weeks 14, 24 and 34.
Results: An 11% enhanced recovery among 14-month-old females was associated with higher depressive and anxiety symptoms (95% CI = 1–23%) and pregnancy-related anxiety symptoms (2–21%). No alterations in the female cortisol reactivity or male cortisol stress responses were observed.
Conclusions: The opposite directions in the associations between the PPD exposure and infant cortisol recovery among 10-week-old and 14-month-old females suggest sex- and age-dependent associations between HPA axis functioning and PPD exposure among healthy infants. Follow-up is needed to characterize the impact of this altered negative feedback mechanism on later health.

1. Introduction

In line with the Developmental Origin of Health and Disease (DOHaD) hypothesis (Barker, 2007), evidence from birth cohorts shows that prenatal maternal depressive, anxiety and stress symptoms associate with emotional and behavioral problems and depression in offspring (Betts et al., 2015; O'Donnell et al., 2014; Pearson et al., 2013). According to the DOHaD, or fetal programming theory, the development of a fetus can be shaped by environmental factors, such as increased exposure to maternal glucocorticoids, that may mediate long-lasting

effects of the prenatal environment on offspring health (Moisiadis and Matthews, 2014). Meta-analyses show dysregulated hypothalamic-pituitary-adrenal (HPA) axis functioning in patients with major depression or anxiety disorder across the lifespan (Zorn et al., 2017). Emerging prospective studies suggest that altered diurnal HPA functioning might be one of the mechanisms behind the increased risk for the mental disorders after prenatal psychological distress (PPD) exposure (Van Den Bergh et al., 2008), but there is still a lack of longitudinal studies on cortisol stress responsiveness from infancy onwards to better support this theory in humans.

It is crucial to understand the normal variation in infant cortisol stress responsiveness during the typical course of development to be able to detect possibly aberrant trajectories. It is known that cortisol response to acute physical stressors, such as vaccination, decreases with age during the first year of life in healthy infants (Davis and Granger, 2009; Jansen et al., 2010). Infant age does not seem to moderate the cortisol stress reactivity to psychological stressors, e.g., separation, from 3 to 18 months of age, although the application of different psychological stressors within limited age ranges among the studies hamper comparability and limit the ability to draw comprehensive conclusions (Puhakka and Peltola, 2020). Exposure to maternal PPD most commonly increases cortisol reactivity to a variety of stressors in children from neonates to 6-year-olds (Hunter et al., 2011). Still, a lower cortisol response to mother-infant separation in exposed infants has also been reported (Galbally et al., 2019). Especially, in a longitudinal study using four different age-appropriate stressors, exposure to maternal PPD associated with increased cortisol stress reactivity in 5-week-olds but lower reactivity at the ages of 8 weeks and 1 year (Tollenaar et al., 2011). Another longitudinal study found that the cortisol reactivity of infants of antenatally depressed mothers did not differ from controls at 2 months but had increased reactivity at 1 year of age (Osborne et al., 2018). It is challenging to interpret the mixed results, as it is difficult to differentiate between the possible true effects of PPD exposure, variation in child age and stressor type on the observed variation in the child cortisol stress response. To clarify the issue, we have followed up on the development of the cortisol stress responsiveness among the PPD-exposed infants by using the same stressor at different ages.

Sexually dimorphic presentation in the prevalence of some psychiatric disorders have been suggested to be partly a result of sex-dependent HPA functioning, which, in turn, could be programmed differently by PPD depending on child's sex (Hicks et al., 2019). In line with that, sexually dimorphic alterations in cortisol stress reactivity (Giesbrecht et al., 2017; Stroud et al., 2016; Yong Ping et al., 2015) and recovery (Kortesluoma et al., 2021a, 2021b) among PPD-exposed children have been reported. In these studies, females have typically been more vulnerable to the influence of PPD than males. In addition, alterations in the cortisol stress response seem to be in opposite directions between men and women with current major depression or anxiety disorder (Zorn et al., 2017). Sexually dimorphic glucocorticoid metabolism in placenta (Jahnke et al., 2021) and gonadal hormones that interact with the HPA axis (Handa and Weiser, 2014) might in part explain these observations. A transient surge of gonadal hormones occurring in under one-year-old infants, including at mini-puberty at the age of 1–3 months (Kuiri-Hänninen et al., 2014), overlaps with the time of rapid infant brain and adrenal gland development. However, sex differences in the developmental trajectory of the HPA axis have not been characterized, and it is not known how age relates to the sex differences in the association between prenatal distress and cortisol stress response among infants.

Although the importance of an adequate recovery from stress to well-being is well known (McEwen, 1998; Nederhof et al., 2015), to our knowledge, our recent report about PPD-exposed females expressing slower cortisol recovery after the acute stressor at the age of 10 weeks (Kortesluoma et al., 2021a, 2021b) is the only study on the cortisol recovery among PPD-exposed infants. To the best of our knowledge, there are no studies on the possible developmental changes in cortisol recovery across infancy. Further, the two existing and contradicting findings on age-dependent, hypo- and hyperreactive cortisol stress responses in PPD-exposed infants should be confirmed by using the same stressor longitudinally. Therefore, we built on our earlier study on 10-week-old infants and continued to explore the role of maternal prenatal depressive, general anxiety and pregnancy-related anxiety symptoms at gestational weeks (gwks) 14, 24 and 34 on the infant cortisol stress reactivity and recovery at the infant ages of six and 14 months. To detect age-related changes, we used the similar acute stressor in each age group as we used in our baseline measurement at 10 weeks

(Kortesluoma et al., 2021a). We hypothesized that the higher PPD exposure associates with increased cortisol stress reactivity to acute stressor independent of age. We also hypothesized that our previously observed sex differences in cortisol recovery would be a stable phenotype across the follow-up.

2. Material and methods

The study is a part of the FinnBrain Birth Cohort Study of 3808 families (www.finnbrain.fi), which aims to study prospectively the effects of PPD on child development and health. Research nurses recruited the families to the Cohort by contacting personally women attending a free-of-charge ultrasound at gestational week 12 at maternal welfare clinics. The recruitment was done between December 2011 and April 2015 in the Southwest Hospital District and the Åland Islands in Finland. Sufficient knowledge of either Finnish or Swedish and a normal ultrasound screening result were required for participation (Karlsson et al., 2018).

The study was approved by the Ethics Committee of the Hospital District of Southwest Finland. Written informed consents were required from the parents during the recruitment for the FinnBrain Birth Cohort and again before each infant study visit on behalf of the infant.

2.1. Sample selection

462 infants participated in this longitudinal study for a saliva cortisol stress response during an acute stressor at the age of 10 weeks (363 infants), 6 months (205 infant) and 14 months (263 infants). They were drawn from the nested case-control sample of the FinnBrain Focus Cohort study (N = 1219). The Focus Cohort was established to compare mothers exposed to PPD with their non-exposed controls. The criteria for the Focus Cohort were determined by using the first 500 FinnBrain Birth Cohort participant mothers' questionnaire data in exploratory analyses and establishing the cut-off points for the approximately highest and lowest 25th percentiles of maternal PPD during pregnancy (Karlsson et al., 2018).

Maternal PPD was measured at gwks 14, 24 and 34 using validated self-report questionnaires for depressive (Edinburgh Postnatal Depression Scale, EPDS) (Cox et al., 1987; Wisner et al., 2002), overall anxiety (Symptom Checklist-90, SCL-90, anxiety subscale) (Derogatis et al., 1973; Holi et al., 1998) and pregnancy-related anxiety symptoms (Pregnancy-Related Anxiety Questionnaire-Revised, PRAQ-R2) (Huizink et al., 2016, 2004; Van den Bergh, 1990). The Cronbach's alphas were 0.87–0.89 (EPDS), 0.88–0.90 (SCL-90 anxiety subscale) and 0.86–0.88 (PRAQ-R2) in the questionnaires at gwks 14, 24 and 34 in our sample.

The total sum score cut-off points for PPD cases and controls were as follows: ≥ 12 and ≤ 6 for the EPDS, ≥ 10 and ≤ 4 for the SCL-90 anxiety subscale and ≥ 34 and ≤ 25 points for the PRAQ-R2. Scoring above the selected threshold twice on any nine measurement (3 questionnaires x 3 time points) during pregnancy was required to be included in the case group. The controls had to score below the selected threshold at all measurements. In addition, all mothers reporting the use of reuptake inhibitors (SSRIs) during their pregnancy were also included as cases. After the collection of the pregnancy data of the whole cohort, the potential PPD case target group comprised 20% and the control group 27% of the pregnant women in the Cohort (Karlsson et al., 2018).

2.2. Sample characteristics

Mothers' age (at the expected date of delivery), education, parity, smoking, alcohol, illicit drug use and medication use during pregnancy were collected from the self-report questionnaires at gwks 14 and 34. Data regarding infant age (from expected date of delivery), birth weight for gestational age (Sankilampi et al., 2013), gestational age at birth, Apgar scores at 1 min and 5 min and the mother's antenatal
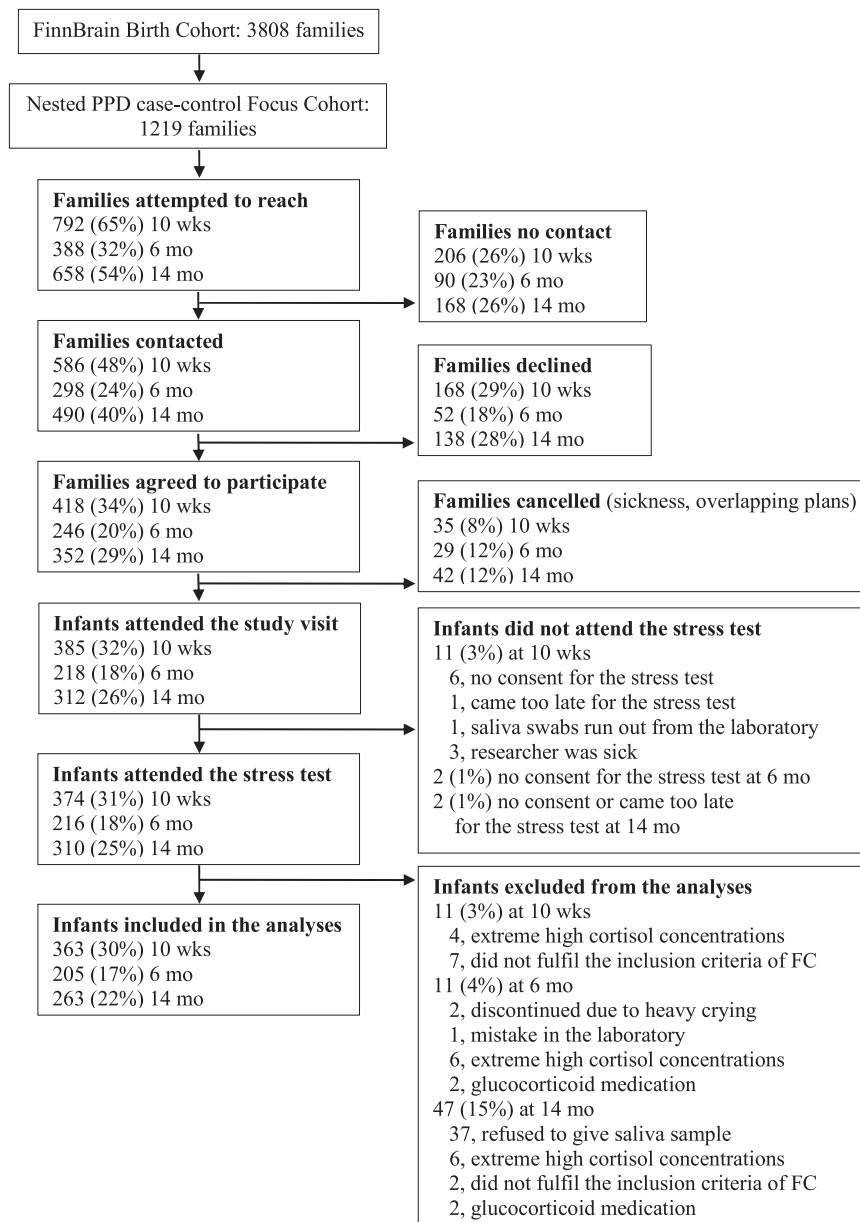
Fig. 1. Flowchart of the study participants. PPD = prenatal psychological distress, FC = Focus Cohort.

corticosteroid treatment and smoking during pregnancy were drawn from the Medical Birth Register of National Institute for Health and Welfare. The mother was categorized as a prenatal smoker, if she reported any smoking during pregnancy based on the self-report questionnaires and/or the Medical Birth Register.

Mother and infant characteristics that may influence cortisol levels during the study day were inquired from the mothers at the beginning of the study visit including information about the time of last sleeping and feeding and any medications used by the infant. Moreover, information about the usage of caffeine, alcohol and smoking during the previous 12 h and about any medications taken by the mothers was requested to control for the possible effects of these substances on the breastfed infants. Postpartum smoking was also asked by the self-report questionnaire sent to the mother's address, when the infant was three months old. Breastfeeding status was asked by the questionnaires at six, 12 and 24 months of age and categorized based on the type of breastfeeding at the time of the study visit.

2.3. Infant stress test for cortisol saliva sampling

As the current study builds upon our previous report on 10-week-old infants (Kortesluoma et al., 2021a) with the aim to compare the developmental changes during the follow-up, relevant methodical details about the baseline at 10 weeks have been included in the following sections to indicate the continuity in the study protocol and statistical analyses throughout the follow-up.

To recruit the infants for the study visit (details in the Fig. 1), the research personnel contacted the families from the Focus Cohort in the order of the infant age within each age group. Due to project logistics, all the families could not be attempted to be contacted. Several families could not be contacted despite several attempts by phone, email and text messages. Eventually 374 (31%), 216 (18%) and 310 (25%) infants at 10 weeks, six months and 14 months of age from the Focus Cohort (N = 1219), respectively, attended the stress test. Of those infants, some were excluded from the cortisol analyses for having extremely high cortisol concentrations (> 400 nmol/l) (Bae et al., 2019), for not fulfilling the Focus Cohort criteria for high or low maternal PPD exposure, for

Table 1

Number of infants participating in the stress tests at different ages.

10 weeks	6 months	14 months	Number of infants (%)
x	x	x	101 (22)
x	x		71 (15)
x		x	79 (17)
x			112 (24)
	x	x	17 (4)
	x		16 (3)
		x	66 (14)
			462 total

refusing to keep the saliva sampling device in their mouth or due to glucocorticoid medication. Finally, the analyses included 363 (30%), 205 (17%) and 263 (22%) infants at 10 weeks and six and 14 months of age, respectively. 101 (22%) infants out of 462 in total participated in all three stress tests. The rest of the infants participated in one or two stress tests with different combinations of ages (Table 1).

Infant stress test study visits for the collection of the cortisol samples were conducted at the research facilities during 2012–2017. During the visits, research personnel completed a protocol record form to keep track of timing and events to ensure consistency between the visits over the years.

In the beginning, the research personnel interviewed the mother for their background health information and asked for written informed

Table 2

Subject characteristics. Values are mean (standard deviation) for continuous variables and number of cases (%) for discrete variables.

		10 wks N^1	Mean (SD) orN (%)	Range	6 months N^2	Mean (SD) or N (%)	Range	14 months N^3	Mean (SD) or N (%)	Range
Mother										
PRAQ-R2	gwk 24	354	22.7 (7.7)	10–45	202	22.6 (7.2)	10–42	258	22.0 (7.4)	10–46
	gwk 34	347	22.3 (7.2)	10–47	200	22.3 (7.2)	10–47	256	21.8 (7.1)	10–47
SCL-90 (anxiety)	gwk 14	358	3.7 (4.9)	0–30	199	3.6 (5.0)	0–30	258	3.3 (4.6)	0–24
	gwk 24	354	4.3 (5.3)	0–26	202	4.3 (5.4)	0–28	258	4.1 (5.4)	0–28
	gwk 34	347	3.4 (4.9)	0–33	200	3.4 (5.0)	0–33	257	3.2 (4.9)	0–33
	3 mo	325	2.9 (4.0)	0–24	182	3.0 (4.0)	0–17	244	2.8 (4.2)	0–24
	6 mo				174	3.8 (5.1)	0–28	227	3.2 (4.8)	0–28
EPDS	gwk 14	358	5.2 (4.8)	0–26	200	4.9 (4.6)	0–26	258	4.8 (4.5)	0–22
	gwk 24	354	5.0 (4.8)	0–25	202	4.8 (4.8)	0–25	258	4.7 (4.8)	0–25
	gwk 34	347	4.8 (4.7)	0–20	200	5.0 (4.9)	0–20	257	4.7 (4.6)	0–20
	3 mo	325	4.4 (4.0)	0–19	183	4.5 (3.8)	0–16	244	4.0 (3.8)	0–19
	6 mo				173	5.4 (5.0)	0–24	227	4.6 (4.6)	0–23
	12 mo							205	5.3 (5.0)	0–23
Age	year		30.7 (4.5)	18–45		30.3 (4.6)	18–44		30.8 (4.3)	19–44
Education	low, < 12 y	359	116 (32)		201	65 (32)		258	71 (28)	
	mid, 15 y		102 (28)			54 (27)			84 (33)	
	high, > 15 y		141 (39)			82 (40)			103 (40)	
Parity	Primiparous	358	194 (53)		204	111 (54)		261	129 (49)	
Smoking	gwk 14–34	355	48 (13)			22 (11)			23 (9)	
Smoking	3 mo		36 (10)		202	23 (11)		256	11 (4)	
Smoking	< 12 h before		18 (5)		204	13 (6)		251	7 (3)	
Breastfeeding	None	335	5 (2)		197	1 (1)		252	2 (1)	
	Ceased		25 (8)			26 (13)			126 (50)	
	Partial		55 (16)			115 (58)			124 (50)	
	Exclusive		250 (75)			55 (28)			0 (0)	
Infant										
Age	Week		10.6 (2.0)	4–19		26.9 (2.2)	21–33		60.5 (2.6)	54–76
Sex	Boys		189 (52)			108 (53)			139 (53)	
Time since last feeding before baseline	min	362	53.8 (36.0)	1–220		65.8 (41.3)	-5–212		75.8 (42.0)	2–230
Infants fed during the study visit			161 (44)			75 (37)			9 (3)	
Time since last sleeping before baseline	min		47.8 (45.8)	-15–255	204	66.7 (58.4)	2–342		108.2 (65.6)	-15–320
Time of day at baseline	hh:mm		12:24 (1:54)	8:40–16:57		11:17 (1:38)	8:20–15:15		11:52 (2:23)	8:38–16:52
Exposure group	Cases		157 (43)			88 (43)			100 (38)	
Gwk at birth			39.9 (1.5)	34–42		40.0 (1.3)	36–42		39.9 (1.4)	33–42
Gwks < 37 at birth			14 (4)			5 (2)			10 (4)	
Birth weight for gestational age	SGA	359	4 (1)		204	3 (2)		261	2 (1)	
	AGA		348 (96)			196 (96)			253 (97)	
	LGA		7 (2)			5 (3)			6 (2)	
Apgar 1 min < 7		358	28 (8)			22 (12)		261	17 (7)	
Apgar 5 min < 7		360	7 (2)			7 (3)		262	8 (3)	

Gwk = gestational week; mo = month; EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Check List-90 (anxiety subscale); PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised; SGA/AGA/LGA = small/appropriate/large birth weight for gestational age.
If not otherwise stated: N^1 = 363 (10 weeks), N^2 = 205 (6 months), N^3 = 263 (14 months).

Table 3

Mixed model results for the association between maternal prenatal depressive and anxiety (EPSD+SCL) or pregnancy-related anxiety (PRAQ-R2) symptoms and infant cortisol stress reactivity and recovery during the acute stressor at the ages of 6 months and 14 months. Estimates are presented as a relative change in the cortisol concentration (nmol/l) min^{-1} ratio. Reactivity describes the 15-min post-stress/baseline ratio and the recovery the 15-min post-stress/35-min post-stress ratio. Estimates for females and males are extracted from the sex interaction models.

	Reactivity			Recovery		
6 Months						
EPDS+SCL	est	p	95% CI	est	p	95% CI
Females	0.88	0.256	0.70–1.10	1.04	0.555	0.91–1.19
Males	1.03	0.768	0.86–1.23	0.95	0.149	0.88–1.02
Interaction	1.17	0.286	0.87–1.57	0.91	0.229	0.79–1.06
PRAQ-R2						
Females	0.99	0.923	0.81–1.21	1.08	0.165	0.97–1.22
Males	1.03	0.775	0.83–1.28	1.02	0.641	0.93–1.12
Interaction	1.04	0.787	0.77–1.40	0.94	0.422	0.81–1.09
14 Months						
EPDS+SCL	est	p	95% CI	est	p	95% CI
Females	1.10	0.321	0.91–1.34	1.11	**0.034**	1.01–1.23
Males	0.93	0.313	0.82–1.07	0.96	0.414	0.88–1.05
Interaction	0.85	0.159	0.67–1.07	0.86	**0.034**	0.76–0.99
PRAQ-R2						
Females	1.03	0.776	0.83–1.28	1.11	**0.014**	1.02–1.21
Males	0.89	0.113	0.78–1.03	1.03	0.622	0.92–1.16
Interaction	0.87	0.282	0.67–1.12	0.93	0.289	0.80–1.07

EPDS+SCL combines both questionnaires at gwks 14–34 (z-score), N = 205 (6 months) and N = 263 (14 months). PRAQ-R2 combines PRAQ-R2 at gwks 24–34 (z-score), N = 204 (6 months) and N = 262 (14 months). Models were adjusted for mother's age, education, smoking, breastfeeding, infant's sex, age, time of the day during the baseline sampling, time since previous naps before baseline sampling, feeding during the study visit and the cortisol EIA kit version used. See Table 2 for the categories of the confounders.

EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Check List-90 (anxiety subscale); PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised; gwks = gestational weeks. p < 0.05 has been indicated in bold.

consent. This calm period of 15 min was used to normalize the cortisol baseline among all the infants. The cortisol baseline sample was taken after the 15 min of rest. Next, at the age of 10 weeks and 14 months, the pediatrician met with the parents and recorded the infant's health information. Next, the infant was stripped naked for a standardized pediatric examination. At six months, the family met the nurse instead and received information about the treatments of common infant illnesses. At the end of each visit, venipuncture and nasopharynx sampling was collected and, for the purposes of the current study, used as an acute stressor for the infants. Consequently, these measures can be considered as a mild physical discomfort. The saliva samples at 0, 15, 25 and 35 min after the stressor were collected to quantify cortisol reactivity and recovery (Ramsay and Lewis, 2003; Tollenaar et al., 2011).

Salimetrics infant swabs (Stratech, Suffolk, UK) were used for the saliva sample collection. The polymer swab was kept in the infant's mouth for 2 min with occasional pauses for a few seconds during the collection if the infant was restless. The swabs were kept in a refrigerator in swab storage tubes during the study visit. Saliva was collected by centrifuging the tubes (15 min, 1800g, 4 °C) and freezing at − 70 °C immediately.

2.4. Analysis of cortisol

Cortisol saliva samples were assayed using the Cortisol Saliva Luminescence Immunoassay (kits RE62011/2012 and RE62111/2015, IBL International, Hamburg, Germany) in the Work Environment Laboratories of the Finnish Institute of Occupational Health, Helsinki, Finland. As the available kit version changed during the data collection, samples from 54%, 92% and 94% of children at the age groups of 10 weeks, 6 and 14 months, respectively, were measured using the newer kit RE62111. Comparability across the two immunoassay kit versions was validated using LC-MS/MS as previously reported (Kortesluoma et al., 2021a). All five samples from each child per study visit were analyzed in the same batch. Intra-assay and inter-assay variations were 5% and 7% at the level of 10 nmol/l.

2.5. Statistical analyses

Group comparisons between sexes concerning subject characteristics employed a *t*-test, Mann-Whitney *U* test, chi-square or Fisher's test depending on the variables.

The PPD case group comprised of heterogenous group of mothers with different levels in total scores among the three types of measured PPD. We used continuous scores to assess what amount of exposure was needed for a certain amount of change in the cortisol stress response. Log-transformation for cortisol was used to normalize the distributions (See Fig. A1 for original and transformed values in the Appendix).

Cortisol *reactivity* was defined as the ratio between the 15-min, post-stress cortisol level (highest level on average) and the baseline cortisol level (−30 min). Cortisol *recovery* was defined as the ratio between the 15-min- and 35-min post-stress cortisol levels. The reason to use ratios instead of differences followed from our choice to use log transformed cortisols in the analyses, e.g. log (15-min cort) – log (baseline cort) = log (15-min cort/baseline) which becomes the ratio 15-min cort/baseline after the exponent transformation. As the result, estimates *above* one in the results section and Tables 3, A4 and A6 indicate higher PPD being associated with *faster* reactivity and recovery, while estimates below one indicate the opposite direction of the associations.

Associations between PPD exposure and infant cortisol reactivity and recovery were analyzed by first fitting a mixed model, separately for each age group, using all the available cortisol data. Next, these models were used to estimate the associations between PPD and cortisol reactivity and recovery. Finally, 95% confidence intervals and p values related to these estimates were calculated using bootstrap.

The mixed models had log-transformed cortisol as the outcome and the following structure for fixed- and random effects:

Fixed effects: *Sex + PPD + TimeTerms + Sex×PPD + Sex×TimeTerms + PPD×TimeTerms + Sex×PPD×TimeTerms + Covariates*

Random effects: *(Intercept +) TimeTerms per each infant*

PPD variable for symptoms of depression and general anxiety was computed by first calculating the means of EPDS and SCL-90 over the gwks 14, 24 and 34, then by standardizing and summing them and
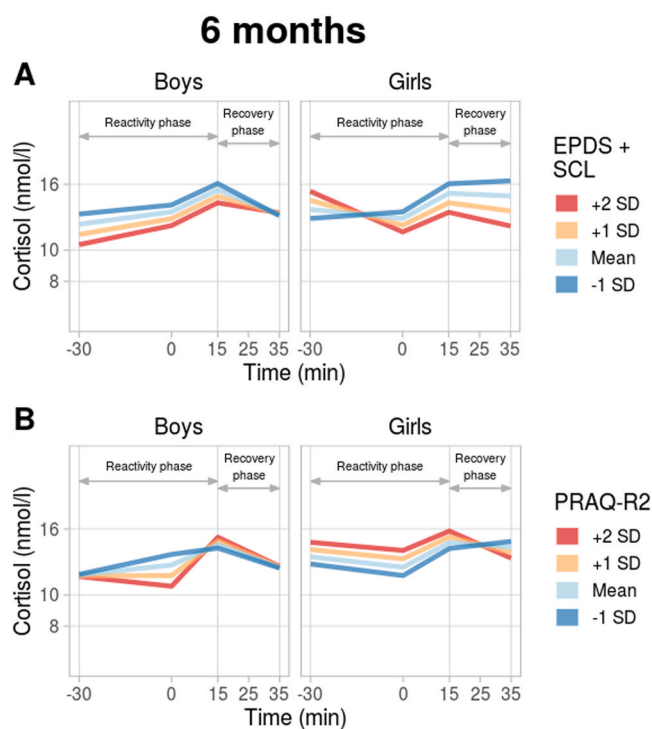
Fig. 2. Estimated average infant cortisol responses based on the mixed models drawn for different levels of maternal stress. Symptom total scores were modeled as continuous variables, but for illustrative purposes, the estimates are presented for four selected values (mean – 1 SD, mean, mean + 1 SD and mean + 2 SD). The curves illustrate the associations between prenatal maternal (A) depressive and general anxiety (EPDS+SCL) and (B) pregnancy-related anxiety (PRAQ-R2) symptom total scores with infant saliva cortisol reactivity and recovery phases of the stress response during the acute stressor at the age of six months. EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Checklist-90, anxiety subscale; PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised 2.

Fig. 3. Estimated average infant cortisol responses based on the mixed models drawn for different levels of maternal stress. Symptom total scores were modeled as continuous variables, but for illustrative purposes, the estimates are presented for four selected values (mean – 1 SD, mean, mean + 1 SD and mean + 2 SD). The curves illustrate the associations between prenatal maternal (A) depressive and general anxiety (EPDS+SCL) and (B) pregnancy-related anxiety (PRAQ-R2) symptom total scores with infant saliva cortisol reactivity and recovery phases of the stress response during the acute stressor at the age of 14 months. EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Checklist-90, anxiety subscale; PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised 2.

finally standardizing the sum. The PPD variable for pregnancy-related anxiety symptoms was calculated as standardized mean of PRAQ-R2 sum scores at gwks 24 and 34. In the case of missing values at some gwks (see Table 1), the means in the EPDS/SCL and PRAQ measures were based on those values that were observed resulting in N = 636 and N = 630 (10 weeks), N = 205 and N = 204 (6 months) and N = 263 and N = 262 (14 months), respectively. As the PRAQ-R2 at gwks 14 was included in the study protocol later than the other questionnaire measurement time points, the sample comprised of only less than 90 mothers and was omitted from the analyses.

Correlations between EPDS and SCL anxiety subscale total scores at gwks 14–34 (r = 0.551–0.709) were higher compared to corresponding correlations between EDPS and PRAQ-R2 (r = 0.452–0.539) or between SCL and PRAQ-R2 (r = 0.454–0.492) (Table A1) among mothers of 6-month-old infants. Similarly, among mothers of 14-month-old infants, corresponding correlations between EPDS and SCL anxiety subscale total scores at gwks 14–34 (r = 0.511–0.697) were higher compared to correlations between EDPS and PRAQ-R2 (r = 0.426–0.491) or between SCL and PRAQ-R2 (r = 0.381–0.500) (Table A2). Thus, EPDS and SCL were combined and analyzed separately from PRAQ-R2. In addition, pregnancy-related anxiety have been suggested to be a distinct pregnancy-specific aspect of anxiety (Bayrampour et al., 2016) with separate health outcomes from general anxiety and depression (Acosta et al., 2019; Kataja et al., 2017).

TimeTerms are the terms needed for the piece-wise linear function used to model the cortisol responses with respect to the time of the stressor. The breakpoints of the piece-wise function were at − 30 min (baseline), 0 min and 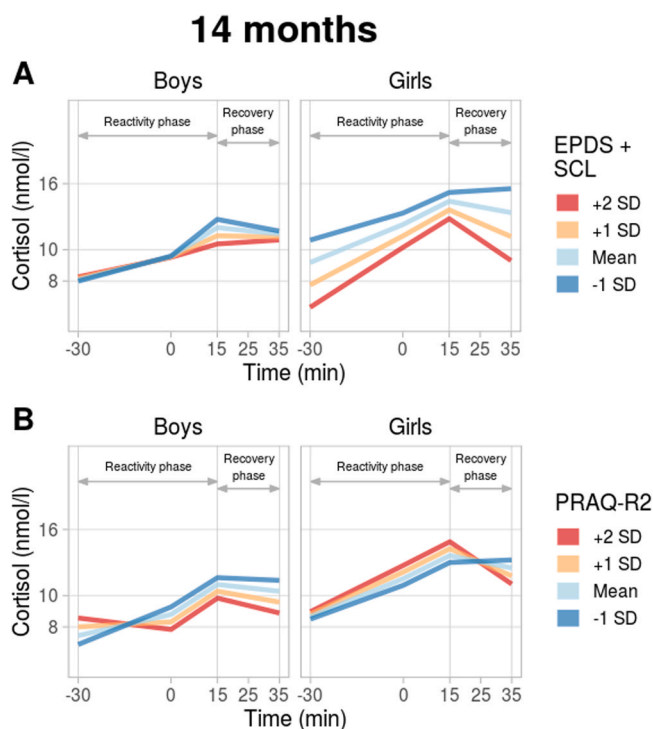15 min post-stressor. The choice of the breakpoints was based on the exploratory analysis of the data. *TimeTerms* were also included in the random effects to let the form of the cortisol responses vary among the infants. However, to avoid an overly complex random effects structure, the breakpoint at − 30 min was omitted from the random effects.

Covariates were chosen based on the earlier literature (Kudielka et al., 2009) and on the characteristics of our sample. The models were adjusted for feeding during the study visit; time since last sleeping before baseline sampling; the EIA kit version used; the mother's age, education, smoking, breastfeeding; the infant's age and the time of the day during the baseline sampling. Allowing also covariates to vary by sex did not change the results thus the interaction terms (*Sex×Covariate*) were omitted from the analyses to maintain the mixed models more straightforward.

Sensitivity analyses without SSRI-medicated mothers (i.e., any usage from gwk 14–14 months postpartum) were performed as SSRI exposure might alter infant HPA axis functioning (Oberlander et al., 2008).

1000 bootstrap samples were generated by sampling the infants, after which, the estimates were calculated for each of the association of interest, in the log scale, on each bootstrap sample. P-values and CI were calculated by assuming normality of the bootstrap distributions in the log scale, after which, the CI were transformed to the original units. The mixed models were fitted using R package lme4 (Bates et al., 2015). Statistical analyses were performed using IBM SPSS Statistics version 26, 27 and R 4.0.5 (R Core Team, 2021). A two-sided p-value < .05 was considered statistically significant, and raw p values are reported. Results are given in the original cortisol units (nmol/L).
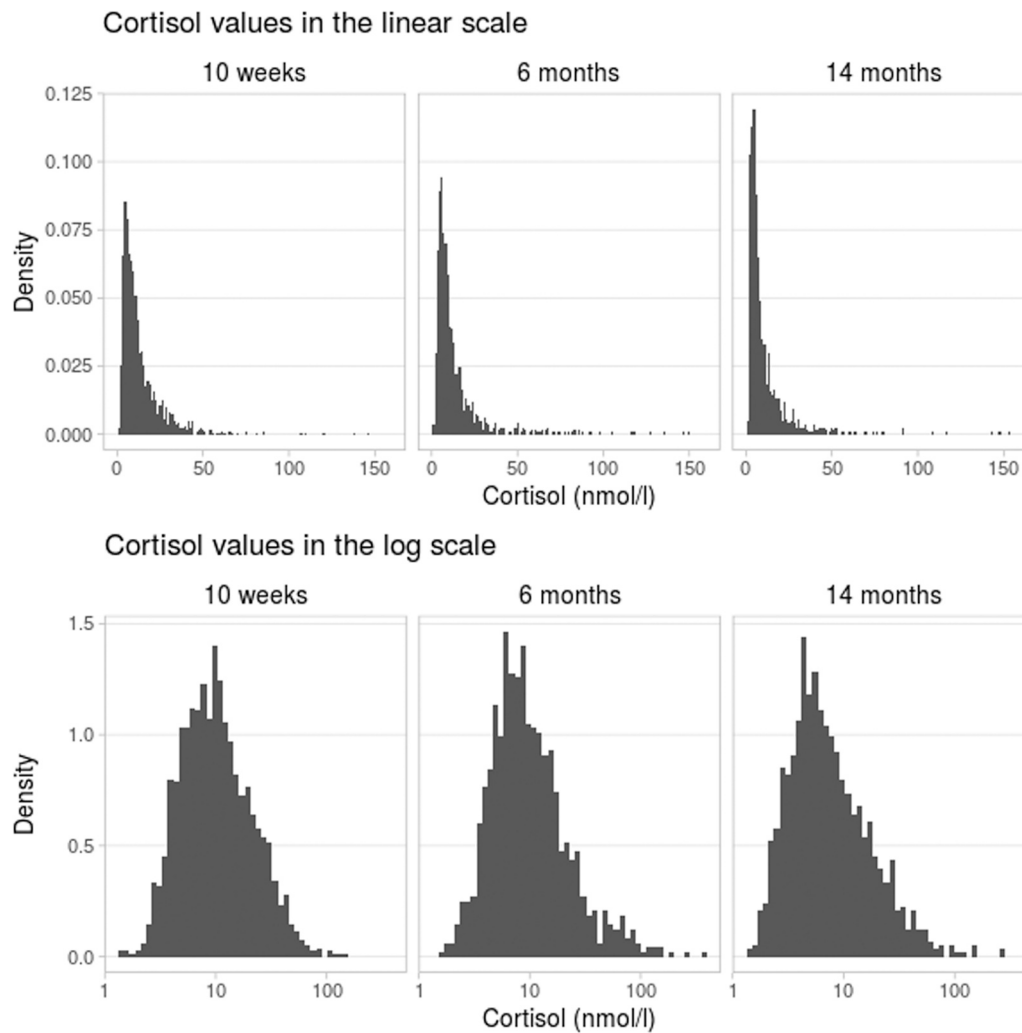
Fig. A1. Distributions of the cortisol values presented in the linear scale (above) and in the log scale (below). The values have been corrected for the two immunoassay kit versions that were used. Furthermore, to illustrate the distributions more clearly, 3 and 2 extreme values (183–357 nmol/l) have been removed from the 6-month and 14-month linear scale figures, respectively. The figures illustrate how log transformation normalizes the distributions.

Table A1

Spearman correlations between the EPDS, SCL anxiety subscale and PRAQ-R2 total scores at gestational weeks 14, 24 and 34 among mothers of 6-month-old infants.

6 months	EPDS gwks 14	EPDS gwks 24	EPDS gwks 34	SCL gwks 14	SCL gwks 24	SCL gwks 34	PRAQ gwks 24	PRAQ gwks 34
EPDS gwks 14	1.000	.681	.639	.683	.575	.551	.452	.482
EPDS gwks 24		1.000	.663	.668	.709	.563	.539	.518
EPDS gwks 34			1.000	.580	.607	.646	.472	.492
SCL gwks 14				1.000	.752	.672	.454	.461
SCL gwks 24					1.000	.715	.492	.487
SCL gwks 34						1.000	.463	.491
PRAQ gwks 24							1.000	.815
PRAQ gwks 34								1.000

Table A2

Spearman correlations between the EPDS, SCL anxiety subscale and PRAQ-R2 total scores at gestational weeks 14, 24 and 34 among mothers of 14-month-old infants.

14 months	EPDS gwks 14	EPDS gwks 24	EPDS gwks 34	SCL gwks 14	SCL gwks 24	SCL gwks 34	PRAQ gwks 24	PRAQ gwks 34
EPDS gwks 14	1.000	.702	.648	.675	.585	.552	.458	.441
EPDS gwks 24		1.000	.698	.564	.697	.607	.491	.426
EPDS gwks 34			1.000	.511	.584	.650	.439	.435
SCL gwks 14				1.000	.667	.620	.404	.381
SCL gwks 24					1.000	.744	.481	.401
SCL gwks 34						1.000	.500	.452
PRAQ gwks 24							1.000	.760
PRAQ gwks 34								1.000

Table A3

Maternal report of illicit drug, alcohol and medication use with possible relevance to the infant saliva cortisol levels prenatally (gestational weeks 14 and 34) and at the time of stress tests (postnatal) in each age group. Data on antenatal corticosteroid treatment were from the medical birth register.

		10 weeks N (%)	6 months N (%)	14 months N (%)
Illicit drugs	Pre/postnatal	0	0	0
Alcohol (median 1 dose/mo)	gwk 14	83 (23)	43 (22)	60 (23)
	gwk 34	34 (10)	17 (9)	26 (10)
	3 months	204 (63)	111 (60)	149 (61)
SSRI	Prenatal	40 (11)	29 (14)	24 (10)
	Postnatal	26 (7)	20 (10)	26 (10)
Glucocorticoids	Prenatal	20 (6)	12 (6)	17 (7)
	Postnatal	13 (4)	6 (3)	11 (4)
Thyroxine	Prenatal	28 (8)	14 (7)	26 (11)
	Postnatal	25 (7)	9 (4)	23 (9)
Hormonal contraceptives	Postnatal	68 (19)	58 (28)	61 (27)
Antenatal corticosteroid treatment		11 (3)	3 (2)	9 (3)

Table A4

Mixed model results for the association between maternal prenatal depressive and anxiety (EPSD+SCL) or pregnancy-related anxiety (PRAQ-R2) symptoms and infant cortisol stress reactivity and recovery during the acute stressor at the ages of 10 weeks. Estimates are presented as a relative change in the cortisol concentration (nmol/l) min^{-1} ratio. Reactivity describes the 15-min post-stress/baseline ratio, and the recovery is the 15-min post-stress/35-min post-stress ratio. Estimates for females and males are extracted from the sex interaction models.

	Reactivity			Recovery		
10 Weeks	est	p	95% CI	est	p	95% CI
EPDS+SCL						
Females	0.87	0.101	0.73–1.03	0.90	0.051	0.82–1.00
Males	1.03	0.654	0.90–1.18	1.06	0.097	0.99–1.13
Interaction	1.19	0.423	0.95–1.48	1.17	**0.010**	1.04–1.32
PRAQ-R2						
Females	0.91	0.243	0.77–1.07	0.92	0.142	0.82–1.03
Males	0.99	0.934	0.86–1.15	1.02	0.705	0.94–1.10
Interaction	1.10	0.423	0.87–1.38	1.10	0.168	0.96–1.27

EPDS+SCL combines both questionnaires at gwks 14–34 (z-score), N = 363. PRAQ-R2 combines PRAQ-R2 at gwks 24–34 (z-score), N = 360. Models were adjusted for mother's age, education, smoking, breastfeeding, infant's sex, age, time of the day during the baseline sampling, time since previous naps before baseline sampling, feeding during the study visit and the cortisol EIA kit version used. See Table 2 for the categories of the confounders.

EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Check List-90 (anxiety subscale); PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised; gwks = gestational weeks. p < 0.05 has been indicated in bold.

3. Results

Characteristics of the study population per age group are described in Table 2. Recent infant medication use in the assessments for the ages of 6- and 14-months included mainly vitamin D (92% / 88%), probiotics (42% / 33%), antibiotics (2% / 4%) and paracetamol or nonsteroidal anti-inflammatory drugs (2% / 5%). Maternal prenatal and postnatal medications and alcohol consumption are reported in Appendix Table A3. As was the case with the age group of 10 weeks (Kortesluoma et al., 2021a, 2021b), mothers of male infants from the age groups six and 14 months reported more commonly postpartum alcohol consumption (57% vs. 43%, p = .048% and 59% vs. 41%, p = .029), when the infants were three months old. There were no sex differences in the maternal and child medication use or type of breastfeeding in any age group.

Breastfeeding changed considerably during the follow-up.
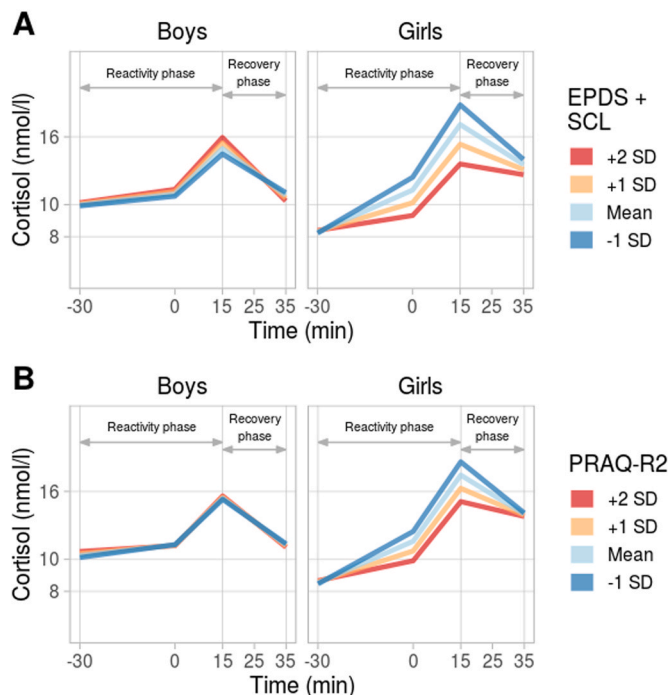

Fig. A2. Estimated average infant cortisol responses based on the mixed models drawn for different levels of maternal stress. Symptom total scores were modeled as continuous variables, but for illustrative purposes, the estimates are presented for four selected values (mean – 1 SD, mean, mean + 1 SD and mean + 2 SD). The curves illustrate the associations between prenatal maternal (A) depressive and general anxiety (EPDS+SCL) and (B) pregnancy-related anxiety (PRAQ-R2) symptom total scores with infant saliva cortisol reactivity and recovery phases of the stress response during the acute stressor at the age of 10 weeks. EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Checklist-90, anxiety subscale; PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised 2.

Breastfeeding was omitted from the models in our previous study (Kortesluoma et al., 2021a, 2021b), because 90% of the infants were still breastfed at 10 weeks. As breastfeeding was included in the models for six and 14 months, we remodeled the data of 10-week-olds with breastfeeding status (see Appendix Table A4, Fig. A2) to gain comparability between the models. The results remained the same.

Variation in the cortisol levels within the age groups was considerable as expected (Appendix, Table A5). The stress test elicited a cortisol stress response in 71%, 63% and 66% of infants in the age groups of 10 weeks, 6 months and 14 months, respectively, when the classification criteria of > 15.5% baseline-to-peak increase was used (Miller et al., 2013).

3.1. PPD associations with cortisol reactivity and recovery

Results for the PPD exposures predicting cortisol reactivity and recovery slopes at the age of 6 months and 14 months with sex interactions are presented in Table 3 and Figs. 2 and 3.

3.1.1. Cortisol reactivity and recovery at 6 months of age

We did not find any evidence for the association between the PPD exposures and infant cortisol stress reactivity or recovery among either sex (Table 3, Fig. 2).

3.1.2. Cortisol reactivity and recovery at 14 months of age

None of the PPDs predicted infant cortisol stress reactivity, but we observed sex differences in the association between exposures and

Table A5

Infant saliva cortisol concentrations (nmol/l) at 10 weeks, 6 months and 14 months of age during the stress test. The samples were collected at baseline (−30 min) and 0, 15, 25 and 35 min after the stressor.

	10 weeks Median (Q25–Q75)	Range	N	6 months Median (Q25–Q75)	Range	N	14 months Median (Q25–Q75)	Range	N
All									
Baseline	7.3 (4.6–11.1)	1.4–146.4	353	8.0 (5.4–15.4)	1.6–182.7	204	5.7 (3.6–8.5)	1.5–116.3	263
0 min	9.4 (5.8–15.3)	1.3–80.9	356	8.0 (5.1–13.6)	1.8–149.7	201	5.9 (3.8–11.6)	1.5–280.0	239
15 min	13.7 (7.9–24.7)	1.6–119.8	339	10.5 (6.5–17.7)	1.8–272.8	197	7.5 (4.4–15.5)	1.7–142.3	231
25 min	10.7 (6.6–18.8)	2.6–109.7	307	9.3 (5.6–14.9)	2.2–357.3	191	6.7 (4.3–13.5)	1.8–258.3	220
35 min	8.4 (5.4–13.3)	2.4–85.9	288	8.6 (5.6–14.2)	1.8–146.4	187	7.0 (4.3–13.3)	1.5–75.8	209
Females									
Baseline	6.9 (4.3–11.4)	1.6–132.4	167	8.1 (4.9–15.6)	2.2–182.7	97	6.1 (3.8–10.3)	1.5–116.3	124
0 min	9.5 (5.4–16.3)	1.3–77.7	169	7.2 (4.7–13.6)	2.5–118.2	95	7.7 (4.3–13.0)	1.5–154.0	109
15 min	16.2 (8.8–26.5)	2.2–103.1	158	9.8 (6.3–17.0)	2.7–272.8	96	8.7 (4.4–18.1)	1.7–73.5	105
25 min	12.0 (8.0–21.1)	2.5–105.3	143	9.5 (5.2–14.1)	2.4–357.3	95	7.7 (4.5–15.1)	1.8–63.7	96
35 min	9.7 (6.5–15.6)	2.4–82.4	133	8.9 (5.9–15.4)	2.3–146.4	93	8.1 (4.4–14.8)	1.5–51.3	91
Males									
Baseline	7.6 (5.1–11.1)	1.4–146.4	186	8.0 (5.7–15.2)	1.6–91.9	107	5.4 (3.5–7.7)	1.6–45.4	139
0 min	9.3 (5.9–13.8)	1.9–59.3	187	8.6 (5.8–14.2)	1.8–149.7	106	5.0 (3.8–9.4)	1.8–280.0	130
15 min	12.3 (7.3–21.6)	1.6–119.8	181	10.9 (7.1–17.9)	1.8–116.5	101	6.6 (4.3–12.9)	1.8–142.3	126
25 min	9.7 (5.9–16.8)	2.6–75.1	164	9.1 (6.1–15.1)	2.2–92.7	96	6.4 (4.2–12.8)	1.8–258.3	124
35 min	8.0 (4.9–11.5)	2.7–66.1	155	8.4 (5.6–13.0)	1.8–79.8	94	6.4 (4.3–12.3)	1.7–75.8	118

Table A6

Mixed models without the SSRI-medicated mothers. Results for the association between maternal prenatal depressive and anxiety (EPSD+SCL) or pregnancy-related anxiety (PRAQ-R2) symptoms and infant cortisol stress reactivity and recovery during the acute stressor at the ages of 10 weeks, 6 months and 14 months. Estimates are presented as a relative change in the cortisol concentration (nmol/l) min⁻¹ ratio. Reactivity describes the 15-min post-stress/baseline ratio, and the recovery is the 15-min post-stress/35-min post-stress ratio. Estimates for females and males are extracted from the sex interaction models.

	Reactivity			Recovery		
10 Weeks						
EPDS+SCL	est	p	95% CI	est	p	95% CI
Females	0.87	0.174	0.72–1.06	0.88	0.051	0.77–1.00
Males	1.01	0.889	0.85–1.20	1.07	0.199	0.97–1.18
Interaction	1.16	0.286	0.88–1.52	1.22	**0.020**	1.03–1.44
PRAQ-R2						
Females	0.92	0.342	0.78–1.09	0.94	0.259	0.84–1.05
Males	0.99	0.860	0.84–1.16	1.02	0.583	0.94–1.12
Interaction	1.07	0.581	0.85–1.34	1.09	0.223	0.95–1.26
6 Months						
EPDS+SCL	est	p	95% CI	est	p	95% CI
Females	0.95	0.754	0.68–1.33	1.04	0.674	0.86–1.27
Males	1.06	0.595	0.86–1.31	0.93	0.169	0.83–1.03
Interaction	1.12	0.578	0.76–1.65	0.89	0.292	0.71–1.11
PRAQ-R2						
Females	1.00	0.983	0.81–1.22	1.05	0.462	0.93–1.18
Males	1.04	0.755	0.82–1.30	1.01	0.919	0.91–1.11
Interaction	1.04	0.807	0.76–1.42	0.96	0.618	0.82–1.12
14 Months						
EPDS+SCL	est	p	95% CI	est	p	95% CI
Females	1.23	0.084	0.97–1.56	1.15	**0.022**	1.02–1.30
Males	0.87	0.149	0.73–1.05	0.99	0.852	0.86–1.13
Interaction	0.71	**0.028**	0.52–0.96	0.86	0.100	0.72–1.03
PRAQ-R2						
Females	1.07	0.590	0.85–1.34	1.11	**0.027**	1.01–1.21
Males	0.88	0.088	0.77–1.02	1.03	0.659	0.91–1.16
Interaction	0.83	0.181	0.63–1.09	0.93	0.341	0.80–1.08

EPDS+SCL combines both questionnaires at gwks 14–34 (z-score), N = 320 (10 weeks), N = 174 (six months) and N = 233 (14 months). PRAQ-R2 combines PRAQ-R2 at gwks 24–34 (z-score), N = 320 (10 weeks), N = 174 (six months) and N = 233 (14 months). Models were adjusted for mother's age, education, smoking during pregnancy, breastfeeding, infant's sex, age, time of the day during the baseline sampling, time since previous naps before baseline sampling, feeding during the study visit and the cortisol EIA kit version used. See Table 2 for the categories of the confounders.

EPDS = Edinburgh Postnatal Depression Scale, SCL = Symptom Check List-90 (anxiety subscale), PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised, gwks = gestational weeks. p < 0.05 has been indicated in bold.

cortisol recovery. In females, higher EPDS+SCL scores predicted enhanced recovery (est = 1.11, 95% CI 1.01–1.23) as did higher PRAQ scores (est = 1.11, 95% CI = 1.02–1.21). We did not observe any association between PPD exposure and cortisol recovery in males (Table 3, Fig. 3).

3.1.3. Sensitivity analyses

Both exposures still predicted enhanced female cortisol recovery at 14 months, although the sex interaction in the association between EPDS+SCL scores and cortisol recovery did not remain statistically significant after excluding the SSRI-medicated mothers (details in Appendix Table A6).

4. Discussion

We hypothesized that PPD exposure would be associated with an altered developmental trajectory of the infant HPA axis functioning towards a more reactive phenotype with slower stress recovery compared to a typical development with reduced cortisol reactivity to a physical stressor along with the age. Surprisingly, we did not observe any association between PPD exposure and infant cortisol stress reactivity at either six or 14 months of age. Instead, higher PPD exposure in females associated with enhanced recovery at 14 months. The direction of the association was opposite compared to the age of 10 weeks, where the exposure associated with a slower recovery (Kortesluoma et al., 2021a, 2021b).

Our results suggest that a negative feedback mechanism that regulates the cortisol recovery could be more sensitive to the effects of PPD compared to reactivity, because recovery associated more strongly with PPD exposure despite the relatively low level of PPD in our sample. Sex differences emphasizing vulnerability to the PPD exposure in females have been linked to the sexually dimorphic placental functioning such as its glucocorticoid metabolism (Jahnke et al., 2021). Consistently high prenatal depressive symptoms have been shown to associate with elevated hair cortisol levels reflecting long-term cortisol concentrations in pregnant mothers (Mustonen et al., 2019). Observed age- and sex-dependent associations between PPD and cortisol recovery might be related to the rapid development of the brain, the adrenal cortex and gonadal hormone secretion during early life. As the fetal zone of the infant adrenal cortex disappears by apoptosis during the first six months, the secretion of dehydroepiandrosterone drops to the low levels seen in infants from six months onwards (Kamin and Kertes, 2017), while gonadal hormone levels transiently peak due to mini-puberty (Kuiri-Hänninen et al., 2014). These hormones modulate HPA axis

functioning, and together with cortisol, regulate the development and functioning of the brain circuits, which, in turn, regulates the secretion of these hormones via the HPA and hypothalamus-pituitary-gonadal (HPG) axes (Kamin and Kertes, 2017; Oyola and Handa, 2017). In addition, infants express varying and increasing levels of stranger fear around six months of age and forward (Brooker et al., 2013), which might moderate the experienced stressfulness of the study visit and recovery from the similar stressor used at each age group. It could be speculated that a prenatally programmed, slower cortisol stress recovery among 10-week-old females led to the heightened cortisol exposure during the following months and altered the interactions between developing the brain, HPA and HPG axes. At the age of 14 months, the originally hyposensitive negative feedback mechanism would have become hypersensitive to compensate for the effect of PPD.

The clinical relevance of our results can be based on the earlier study where a flatter cortisol recovery predicted the first onset psychiatric disorders among adolescents (Nederhof et al., 2015). In their study, the origin of the altered cortisol stress recovery could not be stated. Our sex-specific observations on potentially altered development of infant cortisol stress recovery among PPD-exposed females might also relate to the growing evidence that has showed that exposure to increased maternal PPD and/or prenatal cortisol associates with the various child characteristics only in females, such as more fearful temperament, that may explain the observed higher risk for later affective problems among females compared to males (for example, Sandman et al., 2013). In line with that, maternal prenatal cortisol levels predicted affective problems only among the female children, and these associations were mediated by the connectivity and volume of the child's amygdala, which, in turn, had sexually dimorphic associations with maternal prenatal cortisol levels (Buss et al., 2012; Graham et al., 2019). Variations in amygdala volume have also been associated with variations in cortisol stress responses in early childhood (Fowler et al., 2021).

Postnatal maternal characteristics, such as maternal depressive and anxiety symptoms, the quality and predictability of parental care and the mother-child attachment, have been associated with the infant cortisol stress response in addition to maternal PPD (Hackman et al., 2018). For these factors to have a marked effect on the infant cortisol stress response, the quality of these characteristics should be pronouncedly poor or at least long lasting. This is most likely not the case in our study population, which consisted of relatively highly educated and healthy mother-child dyads with low postnatal levels of any symptoms. Mothers have been selected for this study based on the level of prenatal, not postnatal, distress. As the maternal PPD strongly associates with the postnatal psychological distress, it would be difficult to differentiate the effects of these two factors in the same model considering the type of our study sample and design. In order to do that, one would preferably need mothers with high PPD and low postnatal distress and vice versa and mothers with both distress types being low or high. The high correlation between the prenatal and postnatal EDPS and SCL total scores in our data suggests that this is not the case here. The PPD has quite well established possible direct biological routes affecting the fetus via the placenta based on animal studies and has been increasingly supported by human-based studies (Van den Bergh et al., 2020). However, mechanisms linking maternal postnatal distress with offspring outcomes are different, although they include also biological routes, e.g., via care-taking behavior (Oyetunji and Chandra, 2020). It could be argued that it is not beneficial to add two predictors, being pre- and postnatal symptoms, with a distinct theoretical mechanism that links them to the outcome in the same model. That said, Braithwaite et al. (2020) have reported that association between postnatal maternal depressive symptoms and child emotional problems were moderated by maternal prenatal depressive symptoms in a sex-dependent manner. The mismatched combination of the low maternal prenatal and high postnatal depressive symptoms and vice versa associated with higher emotional problems among the females compared to males, but the matched combination of high maternal prenatal and postnatal depressive

symptoms associated with higher emotional problems in males compared to females. The authors suggested a glucocorticoid mechanism behind the observed effect. Future studies with a sufficient sample size should consider the combined role of the prenatal and postnatal symptom exposure in the sex-specific development of the child HPA axis functioning by predicting cortisol stress responses of the children with the similar three-way interaction models.

The same venipuncture and nasopharynx sampling as a stressor for every age group increases the confidence that the observed changes in cortisol stress responses during the follow-up was due to age and not the stressor type. The comprehensive register record and questionnaire background information of the participants from early pregnancy to 14 months postpartum; the protocol for keeping the consistency of the study visits during the years; the LC-MS/MS validated EIA cortisol assay; the recommended mixed model analyses for repeated measurements and a large sample size, which allowed for the statistics of the sex interaction, are all strengths of our study.

It is possible that not having the pediatric physical examination of the infant as part of the study visit at the age of six months decreased the stressfulness of the visit for the infant to some degree. Only 101 (22%) of infants participating in all three stress tests is another limitation.

In conclusion, exposure to maternal PPD potentially associates with the development of the recovery phase of the infant cortisol stress response in an age- and sex-specific manner. To fully capture the role of HPA axis functioning in the mechanisms linking the PPD exposure with later mental health, we recommend including both phases of the cortisol stress response in the analyses instead of concentrating on the reactivity alone, which is common in the existing literature. This approach could also be beneficial to the development of age-appropriate, preventive and treatment policies. Follow-up is needed to better elucidate the role of the altered cortisol stress recovery phase for a child's psychosocial developmental and other health outcomes.

Role of the funding source

CRediT authorship contribution statement

Susanna Kortesluoma: Formal analysis, Investigation, Writing – original draft. **Laura Korhonen**: Investigation, Writing – review & editing. **Juho Pelto**: Formal analysis, Visualization, Writing – original draft. **Jetro**: Investigation, Writing – review & editing. **Linnea Karlsson**: Conceptualization, Project administration, Writing – review & editing. **Hasse Karlsson**: Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

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Declarations of interest

None.

Appendix

Statistics

Distributions of the cortisol values. see Fig. A1.

Correlations between the EPDS, SCL anxiety subscale and PRAQ-R2 total scores at gestational weeks 14, 24 and 34, see Table A1 and Table A2

Results

Sample characteristics. See Table A3

Cortisol reactivity and recovery at 10 weeks of age. No associations between any PPD exposures and infant cortisol stress reactivity were observed. Instead, cortisol recovery associated differently with combined EDPS+SCL total scores depending on sex. In females, the higher EPDS+SCL scores associated with slower recovery (est = 0.90, 95% CI 0.82–1.00), while among males, we did not observe any associations (Table A4, Fig. A2).

Infant saliva concentrations, see Table A5

Sensitivity analyses for SSRI-medicated mothers. See Table A6

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