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# Sex differences in the associations between maternal prenatal distress and infant cortisol reactivity and recovery

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ABSTRACT

Previous research suggests that maternal prenatal psychological distress (PPD) is related to altered cortisol reactivity in the exposed child. There are indications for the sex differences in vulnerability for prenatal adversities that depend on the exposure and child outcome. Still, it is not known whether the association between maternal PPD and infant cortisol stress response is moderated by sex. In addition, the recovery phase of the cortisol stress response has not been given as much attention as reactivity. Our aim was to study the sex differences in the associations between self-reported maternal prenatal depressive-, anxiety- and pregnancy-related anxiety symptoms through gestational weeks 14, 24 and 34 and the saliva cortisol reactivity to and recovery from the acute stress among 10-week-old infants. The study population comprised of 363 mother-infant pairs from the FinnBrain Birth Cohort Study. We found evidence for sex-dependent associations between PPD exposure and infant cortisol response. A less steep recovery slope (-10 % per one SD increase in PPD [95 % CI = -18 to -2 %] and -8 % [-16 to 0 %] depending on the exposure) and a possibly less steep reactivity slope (-14 % [95 % CI = -25 to 0 %] and -10 % [-21 to 3 %]) were associated with higher PPD exposure in females. Of the PPD measures, the strongly intercorrelated, and thus combined, depressive and anxiety symptom score provided the most robust prediction of infant cortisol recovery. Our results demonstrate sexually dimorphic alterations in the functioning of the infant hypothalamus-pituitary-adrenal axis and especially in the functioning of the negative feedback loop of the axis after prenatal PPD exposure among healthy babies.

## 1. Introduction

Exposure to maternal prenatal psychological distress (PPD) is reportedly linked to alterations in brain development and later in child developmental outcomes, such as behavioral, motor and emotional problems and also in impaired cognitive functions (Van den Bergh et al., 2017). Additionally, there is evidence for associations between maternal PPD exposure and an increased risk of childhood asthma, allergic diseases and recurrent respiratory infections (Flanigan et al., 2018; Korhonen et al., 2019; Zijlmans et al., 2017). Knowledge of the mechanisms behind the observed effects in humans is scarce. One of the main hypotheses for the mechanism bridging PPD exposure with later health outcomes of the child is the altered development of the hypothalamus-pituitary-adrenal (HPA) axis due to elevated cortisol levels of distressed mothers causing altered fetal programming during pregnancy (Sandman et al., 2011). The HPA axis regulates and interacts with essential homeostatic systems, and a dysfunction of the HPA axis itself is linked with psychiatric diseases (Zorn et al., 2017). Therefore, the HPA axis may play an important role in fetal programming (Glover, 2014; Howland et al., 2017; Pearson et al., 2015; Sandman et al., 2011; Van Den Bergh et al., 2008). Fetal programming refers to the process where environmental signals during pregnancy influence the development of the fetus with long-lasting effects on physiological functions and health (Barker, 1990).A widely used measure for the HPA axis

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functioning is the cortisol response to perceived stress, which is commonly depicted by reactivity and recovery phases. Cortisol levels usually increase during reactivity, as a response to a stressor, and eventually decrease back to the basal levels during recovery due to a negative feedback loop of the HPA axis. It is important to note that both heightened and decreased cortisol reactivity to a stressor as well as delayed recovery from a stressor have all been characterized as potentially maladaptive, which, over time, might lead to an allostatic load that increases the risks for numerous diseases (Epel et al., 2018; McEwen, 1998; Nederhof et al., 2015).Young infants are preferred in studying prenatal programming of the HPA axis in order to diminish the postnatal confounding effects (Gunnar and Donzella, 2002). Altered cortisol stress responses have been observed among infants exposed to prenatal depressive, general anxiety and pregnancy-related anxiety symptoms as well as in anxiety and major depressive disorders (MDD) (Fernandes et al., 2015; Giesbrecht et al., 2017; Grant et al., 2009; Osborne et al., 2018; Stroud et al., 2016; Tollenaar et al., 2011), although not always (Braithwaite et al., 2016; Davis et al., 2011; Osborne et al., 2018). The most common finding has been increased cortisol reactivity or total output after a stressor. Previous research has mostly focused on reactivity by analyzing the differences between two cortisol levels, being before and after a stressor (Davis et al., 2011; Fernandes et al., 2015; Giesbrecht et al., 2017; Osborne et al., 2018; Tollenaar et al., 2011). This limits the understanding of the effects of PPD exposure on cortisol response and might lead to an underestimation of the association when part of the response is not covered in the analyses. Stroud et al. (2016) covered the entire response as measured by the area under the curve (AUC), where the challenge might be that very similar sized AUCs can be obtained from different combinations of various reactivity and recovery patterns. From the two infant studies that have analyzed the overall pattern of the cortisol response curve (Braithwaite et al., 2016; Grant et al., 2009), only the latter study showed preliminary support for a link between prenatal anxiety and an infant cortisol stress response pattern.

Sex differences have been repeatedly observed in numerous offspring outcomes after a variety of prenatal exposures, and male fetuses have often been reported to be more vulnerable (DiPietro and Voegtline, 2017). Specifically, among prenatal stress exposures, animal studies support the sex differences in the offspring outcomes. Typically, female offspring show more often increased stress responses and anxiety- and depression-like behavior, while males express more likely altered learning and memory performances (Glover and Hill, 2012). In humans, altered neural structure and functioning and elevated negative emotionality and reactivity are more often observed in female children in a manner that confers risk for affective pathology, while maturational delays, decreased negative emotionality and reactivity are seen in males (Sandman et al., 2013; Sutherland and Brunwasser, 2018). Developmental profiles from prenatal maternal stress to offspring psychopathology with different risk factors may operate in concert to predict adolescent depression, and these risk factors may differ depending on sex (Maxwell et al., 2018). The combination of PPD exposure and offspring cortisol stress response is scarcely studied in this regard. A systematic review on sex differences in prenatal programming of the HPA axis in humans suggests increased vulnerability to maternal stressors in female HPA axis functioning in 14 out of 23 studies, but the exposures or the markers of exposure for prenatal stress were mostly others beside PPD (Carpenter et al., 2017). Out of four studies concerning particularly PPD exposure, only the study of Yong Ping et al. (2015) found a “sex x exposure” interaction. They found that maternal post-traumatic symptoms after a flood during pregnancy associated with elevated cortisol stress reactivity in female toddlers only. Carpenter et al. (2017) suggested that PPD exposure in other studies might have been too broad, not severe enough or that maternal hormonal response to prenatal stress is needed for sex-specific prenatal programming. In addition, higher cortisol stress response was found only in female infants of mothers with prenatal MDD episodes compared to controls (Stroud et al., 2016). Adding complexity to the issue, maternal prenatal diurnal cortisol and distress levels might have both independent and interactive effects on infant cortisol reactivity with different outcomes between sexes (Giesbrecht et al., 2017). Taken together, it seems that sex specificity in vulnerability for prenatal adversities depends on the type and/or combination of exposure and child outcome measured.

Our aim was to study sex differences in the associations between maternal PPD and infant saliva cortisol stress reactivity and recovery phases at the age of 10 weeks. In this study, PPD was determined by self-reported depressive-, general anxiety- and pregnancy-related anxiety symptoms assessed at gestational weeks (gwk) 14, 24 and 34. We hypothesized that PPD exposure is associated with: 1) increased reactivity, i.e., a steeper reactivity slope and/or 2) a slower recovery, i.e., a less steep recovery slope and that 3) these associations are moderated by sex, so that the effect is observed only in females.

## 2. 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. Recruitment for the FinnBrain Birth Cohort took place at maternal welfare clinics by research nurses, who personally contacted women attending a free-of-charge ultrasound at gestational week 12. Recruitment was done between December 2011 and April 2015 in the South-Western 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. Separate written informed consents were required from the parents during the recruitment for the FinnBrain Birth Cohort and again before the infant study visit on behalf of the infant.

### 2.1. Study population

The study population comprised of 363 10-week-old infants with maternal PPD exposure (N = 157) and their non-exposed controls (N = 206) drawn from the nested case-control sample of the FinnBrain Focus Cohort study. 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).

The participants chose the Finnish or Swedish version and either postal or online questionnaires. The questionnaires for depressive (Edinburgh Postnatal Depression Scale, EPDS), overall anxiety (Symptom Checklist-90, SCL-90, anxiety subscale) and pregnancy-specific anxiety symptoms (Pregnancy-Related Anxiety Questionnaire-Revised, PRAQ-R2) at gwks 14, 24 and 34 were used for defining maternal PPD (cases). The total sum score cut-off points for cases and controls were as follows: $\geq 12$ and $\leq 6$ for the EPDS, $\geq 10$ and $\leq 4$ for the SCL-90 anxiety subscale and $\geq 34$ and $\leq 25$ points for the PRAQ-R2. Scoring above the selected threshold on two different questionnaires or twice on one instrument during pregnancy was required in order to be included in the case group. The controls had to score below the selected threshold at all measurements. In addition, according to the design of the main cohort and its Focus Cohort, all mothers reporting the use of reuptake inhibitors (SSRIs) during their pregnancy were also included as cases, as SSRI usage is one of the specific exposures of interest in the cohort. In this particular study, the SSRI status was a parameter in the sensitivity analyses. 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).

Mothers’ age (at expected date of delivery), education, parity,

**Table 1**
Subject characteristics. Values are mean (standard deviation) for continuous variables and number of cases (%) for discrete variables.

| MOTHER | | N* | ALL mean (SD) or N (%) | Range | N** | CASES mean (SD) or N (%) | Range | N*** | CONTROLS mean (SD) or N (%) | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| PRAQ-R2 | gwk 24, total | 354 | 22.7 (7.7) | 10–45 | 148 | 28.41 (8.1) | 10–45 | | 18.6 (3.9) | 10–29 |
| | factor 1 | | 6.6 (2.8) | 3–15 | | 8.0 (3.1) | 3–15 | | 5.5 (1.9) | 3–15 |
| | factor 2 | | 8.5 (3.8) | 4–20 | | 10.9 (4.2) | 4–20 | | 6.8 (2.3) | 4–15 |
| | factor 3 | | 7.7 (3.2) | 3–15 | | 9.5 (3.5) | 3–15 | | 6.3 (2.0) | 3–13 |
| | gwk 34, total | 347 | 22.3 (7.2) | 10–47 | 141 | 27.6 (7.6) | 10–47 | | 18.7 (4.1) | 10–28 |
| | factor 1 | | 6.7 (2.6) | 3–15 | | 8.1 (2.9) | 3–15 | | 5.8 (1.8) | 3–13 |
| | factor 2 | | 8.5 (3.7) | 4–20 | | 10.6 (4.1) | 4–20 | | 7.0 (2.5) | 4–16 |
| | factor 3 | | 7.2 (3.0) | 3–15 | | 9.0 (3.3) | 3–15 | | 6.0 (2.1) | 3–12 |
| SCL-90 (anxiety) | gwk 14 | 358 | 3.7 (4.9) | 0–30 | 152 | 7.4 (5.6) | 0–30 | | 1.0 (1.2) | 0–4 |
| | gwk 24 | 354 | 4.3 (5.3) | 0–26 | 148 | 8.7 (5.5) | 0–26 | | 1.1 (1.3) | 0–4 |
| | gwk 34 | 347 | 3.4 (4.9) | 0–33 | 141 | 7.0 (5.8) | 0–33 | | 0.9 (1.2) | 0–4 |
| | 3 mo | 325 | 2.9 (4.0) | 0–24 | 132 | 5.3 (4.9) | 0–24 | 193 | 1.2 (1.9) | 0–12 |
| EPDS | gwk 14 | 358 | 5.2 (4.8) | 0–26 | 152 | 9.0 (5.0) | 0–26 | | 2.4 (1.7) | 0–6 |
| | gwk 24 | 354 | 5.0 (4.8) | 0–25 | 148 | 9.0 (4.8) | 0–25 | | 2.1 (1.7) | 0–6 |
| | gwk 34 | 347 | 4.8 (4.7) | 0–20 | 141 | 8.8 (4.8) | 0–20 | | 2.1 (1.8) | 0–6 |
| | 3 mo | 325 | 4.4 (4.0) | 0–19 | 132 | 7.0 (4.2) | 0–19 | 193 | 2.7 (2.7) | 0–13 |
| Age | year | | 30.7 (4.5) | 18–45 | | 30.2 (4.6) | 19–42 | | 31.2 (4.3) | 19–45 |
| Education | low, < 12 y | 359 | 116 (32) | | 153 | 63 (41) | | | 53 (26) | |
| | mid, 15 y | | 102 (28) | | | 42 (28) | | | 60 (29) | |
| | high, > 15 y | | 141 (39) | | | 48 (31) | | | 93 (45) | |
| Parity | primiparous | 358 | 194 (53) | | 153 | 94 (61) | | 205 | 100 (49) | |
| Smoking | gwk 14–34 | 355 | 48 (13) | | | 35 (22) | | | 13 (6) | |
| Smoking | 3 mo | | 36 (10) | | | 25 (16) | | 205 | 11 (5) | |
| Breastfeeding | none | 335 | 5 (2) | | 139 | 2 (1) | | 196 | 3 (2) | |
| | ceased | | 25 (8) | | | 15 (11) | | | 10 (5) | |
| | partial | | 55 (16) | | | 22 (16) | | | 33 (17) | |
| | exclusive | | 250 (75) | | | 100 (72) | | | 150 (77) | |
| INFANT | | | | | | | | | | |
| Age | week | | 10.6 (2.0) | 4–19 | | 10.8 (2.0) | 4–17 | | 10.5 (2.0) | 5–19 |
| Sex | boys | | 189 (52) | | | 85 (54) | | | 104 (51) | |
| Time since last feeding before baseline | min | 362 | 53.8 (36.0) | 1–220 | 156 | 51.2 (33.5) | 1–163 | | 55.73 (37.6) | 1–220 |
| Infants fed during the study visit | | | 161 (44) | | | 73 (47) | | | 88 (43) | |
| Time since last sleeping before baseline | min | | 47.8 (45.8) | −15–255 | | 49.2 (46.8) | −5–227 | | 46.6 (45.1) | −15–255 |
| Time of day at baseline | hh:mm | | 12:24 (1:54) | 8:40–16:57 | | 12:39 (1:50) | 8:44–16:57 | | 12:13 (1:56) | 8:40–16:49 |
| Exposure group | cases | | 157 (43) | | | | | | | |
| Gwk at birth | | | 39.9 (1.5) | 34–42 | | 39.9 (1.4) | 34–42 | | 39.9 (1.5) | 35–42 |
| Gwks < 37 at birth | | | 14 (4) | | | 4 (3) | | | 10 (5) | |
| Birth weight for gestational age | SGA | 359 | 4 (1) | | 154 | 2 (1) | | 205 | 2 (1) | |
| | AGA | | 348 (96) | | | 149 (97) | | | 199 (97) | |
| | LGA | | 7 (2) | | | 3 (2) | | | 4 (2) | |
| Apgar 1 min < 7 | | 358 | 28 (8) | | 155 | 17 (11) | | 203 | 11 (5) | |
| Apgar 5 min < 7 | | 360 | 7 (2) | | 155 | 6 (4) | | | 1 (1) | |

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 = 363 (all), **N = 157 (cases), ***N = 206 (controls).

smoking, alcohol, illicit drug use and medication use during pregnancy were collected from the self-reported 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 smoking during pregnancy were drawn from the Medical Birth Register of National Institute for Health and Welfare (Table 1). The mother was considered to be a prenatal smoker, if she reported smoking at all during pregnancy based on the self-reported questionnaires at gwks 14 and 34 and the information from the Medical Birth Register.

Mother and infant characteristics that may influence cortisol levels at 10 weeks 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 any medications taken by the mothers was requested in order to control for the possible effects of these substances on the breastfed infants. Postpartum smoking and breastfeeding were also asked by the self-report questionnaire sent to the mother's address, when the infant was three and six months of age, respectively. The mother was considered to be a postpartum smoker, if she reported any smoking during the three months after birth. Breastfeeding was categorized based on the type of breastfeeding at the time of the study visit.

Due to project logistics not systematically related to any family characteristics, we could not attempt to contact all the families from the Focus Cohort (N = 1219). Overall, an attempt was made by the research personnel to reach 792 (65 %) families from the Focus Cohort by phone in order to recruit the infants for the study visit that included a stress test to measure saliva cortisol stress response profiles for an acute stressor. Of these, 206 (26 %) could not be contacted despite several attempts. 586 (48 %) families were contacted, after which, 418 (34 %) agreed to participate. Eventually 374 infants (31 %), including two twins, attended a stress test. Eleven (3 %) infants were excluded from the cortisol analyses for having extremely high cortisol concentrations (> 1000 nmol/l) or not fulfilling the Focus Cohort criteria for high or low maternal PPD exposure. Finally, the analyses included 363 infants (30 %) from the Focus Cohort (Fig. 1). The study sample (N = 363) resembles the Focus Cohort (N = 1219) with maternal education (study

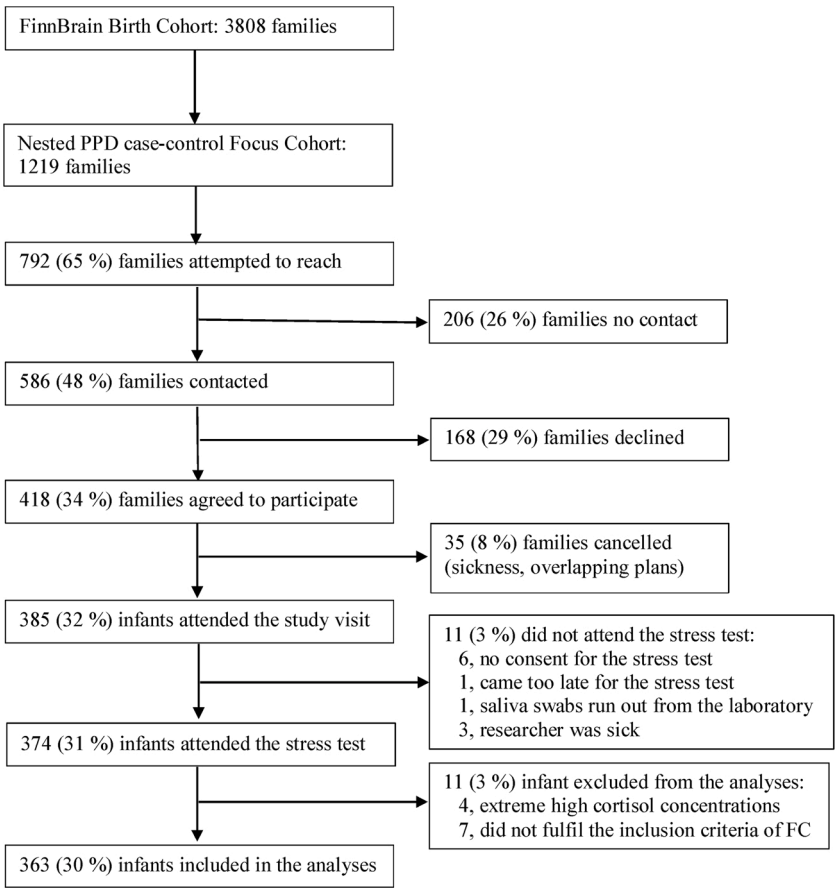


**Fig. 1.** Flowchart of the study participants. PPD = prenatal psychological distress, FC = Focus Cohort.

sample: low = 32 %, middle = 28 %, high = 39 % vs. the Focus Cohort: low = 37 %, middle = 30 %, high = 33 %, $\chi^2$ (2) = 5.3, p = .070) and the total scores of EPDS, SCL and PRAQ-R2 at gwks 14, 24 and 34 (p-values from .121 to .995).

### 2.2. Edinburgh postnatal depression scale (EPDS)

EPDS is a self-reported questionnaire, which consists of 10 questions scored on a 4-point Likert scale (0–3 points/item) (Cox et al., 1987). The total scores ranged between 0 and 30. The EPDS has proven to be a valid, reliable and effective screening tool for identifying patients at risk for perinatal depression (Cox et al., 1987; Wisner et al., 2002). The Cronbach's alphas in our sample were 0.88–0.89 in the questionnaires at gwks 14, 24 and 34.

### 2.3. Symptom Checklist-90 (SCL-90) anxiety subscale

Symptoms of overall anxiety were assessed with the anxiety subscale of the self-rated SCL-90 (Derogatis et al., 1973; Holi et al., 1998). The anxiety subscale consists of 10 items scored on a 5-point Likert scale (0–4 points/item), and the range of the total sum score is 0–40. The Cronbach's alphas in our sample were 0.89–0.91 in the questionnaires at gwks 14, 24 and 34.

### 2.4. Pregnancy-Related anxiety questionnaire, revised 2 (PRAQ-R2)

The PRAQ-R2, a version of the PRAQ-R suitable for both nulliparous and multiparous women (Huizink et al., 2016), was used in the study. It is a 10-item self-report being a shortened version of the 34-item PRAQ (Huizink et al., 2004; Van den Bergh, 1990). Scores on each item ranged from 1 (definitely not true) to 5 (definitely true). The items of PRAQ-R2 can be divided into three subscales: Fear of Giving Birth (Factor 1, F1), Worries about Bearing a Physically or Mentally Handicapped Child (Factor 2, F2) and Concern about Own Appearance (Factor 3, F3). For the PRAQ-R2, the total sum score was calculated. The Cronbach's alphas in our sample were 0.86–0.87 in the questionnaires at gwks 24 and 34. The PRAQ-R2 at gwks 14 was included in the study protocol later than the other questionnaire measurement time points. Consequently, the study sample comprised only of 81 mothers in the PRAQ-R2 gwk 14 measurement and, for this reason, this measurement was omitted from the analyses.

### 2.5. Saliva cortisol sampling during the stress test

Cortisol samples of 10-week-old infants were collected during the Focus Cohort infant stress test study visit. Study visits were carried out between October 2012–February 2016 from 8:30 a.m. to 6:00 p.m. at the research facilities. During the visits, a research nurse completed a protocol record form in order to keep track of timing and events to ensure consistency between the visits over the years.

The study visit started with a peaceful period of 15 min for the infants in order to normalize the cortisol baseline among all the infants. During this time, the research personnel interviewed the mother for their background health information and asked for written informed consent, while the infant was resting on mother's lap, in a child seat or on the blanket on the floor next to the mother. After cortisol baseline sampling, the pediatrician discussed with the parents and recorded the infant's health information. After that, a standardized pediatric examination was performed, where the infant was stripped naked. At the end, venipuncture and nasopharynx sampling were collected and used as an acute stressor for the infants. The infant's skin had a patch for local anesthesia, and she/he was given glucose to pacify the pain.

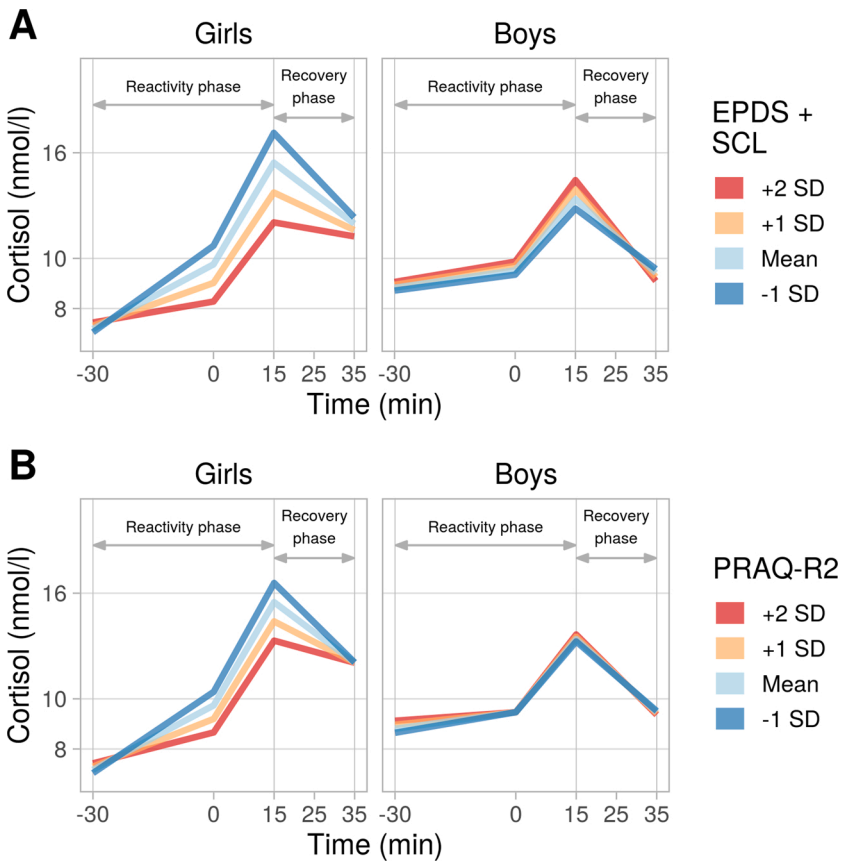


**Fig. 2.** Estimated average infant cortisol responses based on the mixed models drawn for different levels of maternal prenatal distress. Symptom total scores were modelled as continuous variables, but for illustrative purposes, the estimates are presented for four selected values (mean – 1 SD, mean, 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.

Consequently, these measures can be considered as a mild physical discomfort. The procedure was done in the same order for all infants. The saliva samples at 0, 15, 25 and 35 min after the stressor were collected for cortisol reactivity and recovery. The time from cortisol baseline to 0-min post stressor saliva sampling took 30 min (SD 7.4 min) on average. The saliva samples were collected using Salimetrics infant swabs (Stratech, Suffolk, UK) by research personnel with the help of the mother if needed. The polymer swab was kept in the infant's mouth for two minutes with occasional few second pauses during the collection, if the infant was restless. The swabs were placed in swab storage tubes and kept in a refrigerator (max. 1.5 h) during the study visit. Saliva was collected by centrifuging the tubes (15 min, 1800 g, 4°C) and freezing at −70 °C immediately after.

### 2.6. Immunological analysis of cortisol

Cortisol in saliva samples was determined 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. Samples from 168 children were measured using the kit RE62011, and samples from 195 children were measured using the newer kit RE62111, as the manufacturing of the previous assay version ended before the data collection was finished. All samples from each child were analyzed in the same batch. Intra-assay and inter-assay variations were 5 % and 7 % at the level of 10 nmol/l.

### 2.7. Immunoassay validation

Comparability across the two immunoassay versions was validated by analyzing a set of same samples with both assays and LC–MS/MS. In 2016, cortisol saliva samples (N = 32) of seven 10-week-old children were analyzed with both immunoassays. In 2017, the same validation was made with a new set of cortisol saliva samples (N = 33) of four 10-week-old children and three six-month-old children and also additionally analyzed with LC–MS/MS. Samples for the validation were selected based on having a sufficient volume of saliva to perform all the analyses.

### 2.8. LC–MS/MS analysis of cortisol

A sample of 50 µL of saliva was taken for the LC–MS/MS analysis. The sample was vortexed after 150 µL of water; 50 µL of 0.2 mM deuterated cortisol (IsoSciences, King of Prussia, PA, USA), as an internal standard and 700 µL of dichloromethane were added to the sample. After centrifuging at 3500 g for 5 min and cooling in a refrigerator, 400 µL of the organic phase was collected and evaporated to dryness. Dry samples were stored in a freezer and dissolved in 100 µL mixture of water and methanol (1:1) prior to analysis. Calibration samples made of cortisol (Sigma-Aldrich, Steinheim, Germany) and deuterated cortisol in the same solvent mixture were used to create a calibration curve for quantitative analysis.

A Thermo Surveyor liquid chromatograph system connected to a Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer (San Jose, CA, USA) was used for the quantitative analysis of cortisol. A Waters XTerra MS C18 3.5 µm, 2.1 × 150 mm column (Milford, MA, USA) was used for the chromatographic separation. Water, methanol and formic acid (95/5/0.1 v/v/v) and water, methanol and formic acid (5/95/0.1 v/v/v) were the eluents in a gradient run, and the flow rate was 250 µL/min. Analytes were ionized by positive electrospray ionization (ESI), argon was the collision gas and selected reaction monitoring (SRM) was used for analyzing the target compounds. The transitions monitored were $m/z$ 363.2 → 121.0 for cortisol and $m/z$ 367.2 → 121.0 for deuterated cortisol.

**Table 2**
Results from the mixed models with and without sex interactions for the associations of two types of overall PPD exposure with cortisol reactivity and recovery in 10-week-old infants during the acute stress test. 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 being the 15-min, post-stress / 35-min, post-stress ratio. Estimates for females and males are extracted from the interaction models.

A. Models without sex interactions

| | ALL INFANTS | | |
|---|---|---|---|
| REACTIVITY | Est | p | 95 % CI |
| EPDS + SCL | 0.95 | .212 | 0.87–1.03 |
| PRAQ-R2 | 0.94 | .149 | 0.86–1.02 |
| RECOVERY | | | |
| EPDS + SCL | 0.99 | .785 | 0.94–1.05 |
| PRAQ-R2 | 0.97 | .299 | 0.92–1.03 |

B. Models with sex interactions

| | ALL INFANTS | | | FEMALES | | | MALES | | |
|---|---|---|---|---|---|---|---|---|---|
| REACTIVITY | Est | p | 95 % CI | Est | p | 95 % CI | Est | p | 95 % CI |
| EPDS + SCL | 1.19 | .061 | 0.99–1.43 | 0.86 | .046 | 0.75–1.00 | 1.03 | .603 | 0.92–1.15 |
| PRAQ-R2 | 1.10 | .304 | 0.92–1.31 | 0.90 | .134 | 0.79–1.03 | 0.99 | .906 | 0.88–1.12 |
| RECOVERY | | | | | | | | | |
| EPDS + SCL | 1.18 | .003 | 1.06–1.31 | 0.90 | .021 | 0.82–0.98 | 1.06 | .067 | 1.00–1.13 |
| PRAQ-R2 | 1.11 | .079 | 0.99–1.24 | 0.92 | .058 | 0.84–1.00 | 1.02 | .652 | 0.95–1.09 |

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 during pregnancy, 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 1 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.

### 2.9. 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 mothers with a variety of combinations of different levels in total scores among the three types of PPD that were measured. Using continuous scores instead of binary exposure categories made it possible to assess in more detail what amount of exposure (in scores) was needed for a certain amount of change in the cortisol stress response. Pregnancy-related anxiety symptoms were studied separately from the other symptoms, as it has been suggested to be a distinct aspect of anxiety that is specific for pregnancy (Bayrampour et al., 2016). These symptoms have associated with health outcomes of children and pregnant women separate from general anxiety and depression (Acosta et al., 2019; Kataja et al., 2017).

Mixed models with the following structure were used to analyze the associations between different types of PPD exposures and infant cortisol reactivity and recovery:

Fixed effects: $Sex + PPD + TimeTerms + Sex \times PPD + Sex \times TimeTerms + PPD \times TimeTerms + Sex \times PPD \times TimeTerms + Feeding + Kit + SleepTimeTerms + Other\ Covariates$

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

- Log-transformed cortisol was used as the response variable. Originally strongly skewed distribution approximated normality rather well after the log transformation.
- *PPD* was a standardized variable consisting of either EPDS and SCL-90 anxiety subscale sum scores or PRAQ-R2 sum scores. In the former case, the variable was computed by first calculating the means of EPDS and SCL-90 over the gwks 14, 24 and 34, then standardizing and summing them and finally standardizing the sum. In the latter case, the variable was the standardized mean of PRAQ-R2 sum scores at gwks 24 and 34. In case of missing values at some gwks (see Table 1), the means were based on those values that were observed leading N = 636 and N = 630 in the EPDS/SCL and PRAQ measures, respectively. In addition to the reasoning discussed above for the separate analyses with PRAQ-R2 from EDPS and SCL, EPDS and SCL scores were also combined, as they are known to correlate strongly. Indeed, correlations in our sample between PRAQ-R2 and EPDS (r = .437–.521) or between the PRAQ-R2 and SCL anxiety subscale (r = .421–.561) total scores were somewhat smaller compared to associations between the EPDS and SCL anxiety subscales (r = .551–.718) by observation.
- *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 (see Fig. 2). The choice of the breakpoints was based on the exploratory analysis of the data (see Fig. A1). *TimeTerms* were also included in the random effects to let the form of the cortisol responses vary between infants. However, to avoid an overly complex random effects structure, the breakpoint at -30 min was omitted from the random effects.
- *Feeding* was a binary variable indicating whether the infant was fed before each cortisol measurement.
- *Kit* was a binary variable indicating which cortisol EIA kit version, RE62011 (older) or RE62111 (newer), was used to analyze each saliva sample. The variable was included to take into account the clear systematic difference between the cortisol values measured with the two kits.
- *SleepTimeTerms* are the terms of a piece-wise linear function (with breakpoints at 50 min and 100 min before the cortisol baseline sampling at awakening) needed to model the impact of time, since last sleeping, on baseline cortisol.
- *Other Covariates* were mother's age, education and smoking during her pregnancy, the infant's age and the time of the day during the baseline sampling.
- When analyzing the associations in all infants independently of sex, all the terms in the model that included interaction with sex were omitted (Table 2A). The separate estimates for males and females were extracted from the interaction model (Table 2B).

Although the response variable in the mixed models was the log-transformed cortisol, all the results are given in the original units (nmol/L). The quantities of interest were defined as follows:

- Cortisol *reactivity* was defined as the ratio between the 15-minute, post-stress cortisol level (highest level on average) and the -30-minute, baseline cortisol level.

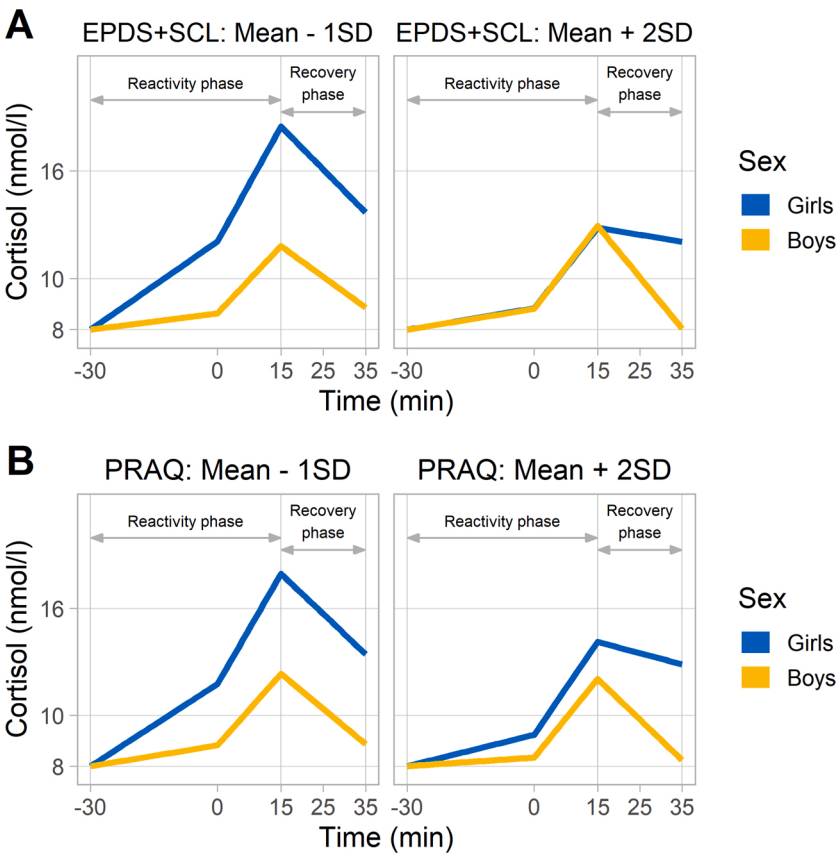


**Fig. 3.** Estimated average infant cortisol responses based on the mixed models drawn for different levels of maternal prenatal distress by sex. Symptom total scores were modelled as continuous variables, but for illustrative purposes, the estimates are presented for two selected values (mean – 1 SD 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. Note that, for illustrative purposes, each curve is vertically shifted so that the baseline level (at -30 min) equals to 8.0 nmol/L.

- Cortisol *recovery* was defined as the ratio between the 15-minute and 35-minute (the end of the experiment) post-stress cortisol levels.

The estimates for reactivity and recovery were extracted from the model, and the corresponding confidence intervals (CI) and p-values were computed using bootstrap. That is, first, 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.

A sensitivity analysis was also performed by re-running each analysis without SSRI-medicated mothers (i.e., any usage from gwk 14 to 3 months postpartum, N = 46), as SSRI exposure might alter infant HPA axis functioning (Oberlander et al., 2008; Pawluski et al., 2012).

Statistical analyses were performed using IBM SPSS Statistics version 26 and R 3.6.2 (R Core Team, 2019). A two-sided p-value $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Immunoassay validation

Cortisol concentrations measured twice from the same saliva samples using the two immunoassays RE62011 (older) and RE62111 (newer) in year 2016 were highly correlated (r = .801). Accordingly, similar measurements in year 2017 resulted in an even stronger correlation (r = .955). The correlation between cortisol concentrations measured using the RE62011 immunoassay and LC–MS/MS was r = .799, and between RE62111 and LC–MS/MS, the correlation was r = .896 in year 2017. Combining the data between cortisol concentrations measured by the two immunoassays from year 2016 and 2017 also yielded a consistent result (r = .754).

### 3.2. Subject characteristics

The characteristics of the sample of mothers and infants are presented in Table 1. The PPD measures were mainly evenly distributed with the exception of PRAQ-R2 scores being slightly higher among mothers of male infants in contrast with mothers of female infants at gwks 24 (total score: 23.7 vs. 21.7, p = .025; Factor 2: 9.0 vs. 8.0, p = .009) and at gwk 34 (total score: 23.1 vs. 21.5, p = .041). Concerning the other sample characteristics, the Apgar scores at 1 min were more often < 7 among males than in females (10.7 % vs. 4.7 %, $\chi^2$ (1) = 4.5, p = .034).

There were no illicit drug users in this sample, and 82 (23 %) and 34 (9 %) mothers reported some alcohol consumption (median = one dose, 1–2 times/month) at gwks 14 and 34, respectively. 204 (56 %) mothers reported some alcohol consumption (median = one dose, <1 times/month) 3 months postpartum. Maternal reports of any prenatal and postnatal (3 months) use of medication with possible relevance to the infant saliva cortisol levels included, respectively, SSRIs (9 % / 7 %), other medication with central nervous system effects (3 % / 1 %), glucocorticoids (4 % / 4 %), thyroxine (6 % / 7 %) and hormonal contraceptives (19 %). The use of maternal medications, alcohol and breastfeeding was equally distributed between male and female infants with the exception of slightly more mothers of male infants reporting postpartum alcohol consumption (68 % vs 57 %, p = 0.035). The most common medication and other products in use for the infants were vitamin D (82 %), probiotics (42 %), simethicone/dimethicone (11 %) and antibiotics (1 %).

### 3.3. Associations between PPD and cortisol reactivity and recovery

Results for the PPD exposures predicting cortisol reactivity and recovery slopes of the stress response without and with sex interactions are presented in Table 2 and Fig. 2 and 3. When analyzing these associations

**Table 3**
Sensitivity analyses without SSRI-exposed infants. Results from the mixed models with and without sex interactions for the associations of two types of overall PPD exposure with cortisol reactivity and recovery in 10-week-old infants during the acute stress test. 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 15-min, post-stress / 35-min, post-stress ratio. Estimates for females and males are extracted from the interaction models.

A. Models without sex interactions

| | ALL INFANTS | | |
|---|---|---|---|
| REACTIVITY | Est | p | 95 % CI |
| EPDS + SCL | 0.94 | .228 | 0.84–1.04 |
| PRAQ-R2 | 0.94 | .212 | 0.85–1.04 |
| RECOVERY | | | |
| EPDS + SCL | 0.98 | .683 | 0.91–1.06 |
| PRAQ-R2 | 0.98 | .591 | 0.93–1.05 |

B. Models with sex interactions

| | ALL INFANTS | | | FemaLES | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|
| REACTIVITY | Est | p | 95 % CI | Est | p | 95 % CI | Est | p | 95 % CI |
| EPDS + SCL | 1.18 | .147 | 0.94–1.47 | 0.88 | .140 | 0.75–1.04 | 1.04 | .598 | 0.90–1.20 |
| PRAQ-R2 | 1.05 | .626 | 0.86–1.28 | 0.95 | .488 | 0.83–1.09 | 1.00 | .990 | 0.87–1.14 |
| RECOVERY | | | | | | | | | |
| EPDS + SCL | 1.20 | .025 | 1.02–1.41 | 0.89 | .063 | 0.78–1.01 | 1.06 | .213 | 0.96–1.17 |
| PRAQ-R2 | 1.09 | .154 | 0.97–1.23 | 0.95 | .241 | 0.86–1.04 | 1.03 | .433 | 0.96–1.11 |

EPDS + SCL combines both questionnaires at gwks 14–34 (z-score), N = 317. PRAQ-R2 combines PRAQ-R2 at gwks 24–34 (z-score), N = 317. Models were adjusted for mother's age, education, smoking during pregnancy, 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 1 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.

in all infants independently of sex, all the estimates were small, and none of the results were statistically significant (Table 2A).

### 3.3.1. *Sex differences in the association between PPD exposure and cortisol reactivity*

We found some evidence for sex differences in the association between combined EPDS + SCL scores and cortisol reactivity (19 %, 95 % CI = -1–43 %, p = .061). In females, 1 SD higher EPDS + SCL score was estimated to associate with 14 % less steep reactivity slope (95 % CI = -25–0 %, p = .046), while in males the estimate was close to zero (3%, 95 % CI = -8–15 %, p = .603) (Table 2B, Figs. 2A, 3A). No convincing evidence was found for the sex interaction between PRAQ scores and cortisol reactivity (10 %, 95 % CI = -8–31 %, p = .304) (Table 2B, Figs. 2B, 3B).

### 3.3.2. *Sex differences in the association between PPD exposure and cortisol recovery*

Instead, for the cortisol recovery, clear evidence for sex differences in the EPDS + SCL associations was found (18 %, 95 % CI = 6–31 %, p = .003). Our results suggested that in females, a higher EPDS + SCL score associated with a less steep recovery slope (-10 %, 95 % CI = -2 to -18 %, p = .021), while interestingly in males, the association may be in the opposite direction (6 %, 95 % CI = 0–13 %, p = .067) (Table 2B, Figs. 2A, 3A). The evidence for sex interaction in the case of PRAQ was much weaker (11 %, 95 % CI = -1–24 %, p = .079) and the estimate was clearly smaller but the direction was similar than in case of EPDS + SCL (Table 2B, Figs. 2B, 3B).

### 3.3.3. *Sensitivity analyses*

Next, we conducted a sensitivity analysis using the same analyses as presented above without 46 SSRI-medicated mothers (i.e., any medication taken during gwk 14–3 months postpartum, 29 % of PPD mothers). The association between the EPDS + SCL sum score and cortisol reactivity in females did not remain significant, but otherwise the results remained essentially unaltered (Table 3). The changes in the results were likely partly due to a decreased sample size.

Altogether, the associations were weak, and the minimum effects (in 95 % CIs) were close to zero. The variation in cortisol stress response profiles between infants was substantial, of which, the PPD explained only a small proportion (Fig. A1, Table A1).

## 4. Discussion

Our study contributes to the existing literature about the link between PPD exposure and infant HPA axis functioning by investigating sex differences in the associations between maternal prenatal depressive, anxiety and pregnancy-related anxiety symptoms through gwks 14, 24 and 34 and the saliva cortisol reactivity to and recovery from the acute stress among 10-week-old infants. Taken together, we did not observe sex-independent associations between PPD exposures measured and the infant cortisol response. In line with our hypotheses, we found evidence for the moderating effect of the child's sex on the association between PPD exposure and infant cortisol recovery. We also found some evidence for the moderating effect of sex on the association between PPD exposure and reactivity, but this result was less robust. In addition, according to our hypothesis, a higher PPD exposure was associated with a slower recovery. Contrary to our hypothesis, we did not observe increased reactivity after PPD exposure. In fact, when any association between PPD exposure and infant cortisol reactivity was observed, the tendency was towards decreased reactivity. Associations were weak, but one would not expect to see substantial changes in the rate of cortisol kinetics in a relatively healthy sample of infants as a function of overall low levels of PPD exposure.

The effect of PPD exposure on infant cortisol response was opposite depending on the sex resulting in the observed less steep reactivity and recovery slopes in females and a potentially steeper reactivity and recovery slopes in males. These potentially opposing effects might explain partly why associations were not observed, when the moderating effect of sex was not taken into account. Nevertheless, based on the confidence intervals, it was difficult to state the direction for the associations among males. Alternatively, if the PPD exposure indeed associates with altered cortisol stress response only in female infants, the sex-independent association was probably diminished due to the lack of effect in males.

Our results support the earlier sex-specific finding of an altered cortisol stress response to a mother-toddler separation test in 30-month-old females exposed to maternal prenatal objective- and subjective stress due to a flood (Yong Ping et al., 2015). On the other hand, Yong Ping et al. observed increased reactivity, while we found a tendency towards decreased reactivity. This difference could be due to differences in exposure, stressor and/or age of the child in these studies. We further showed that also the recovery is altered in females after the prenatal

depressive and anxiety exposure. Yong Ping et al. measured cortisol levels up to 45 min post-stressor but could not analyze recovery, as the cortisol levels kept increasing among females making a comparison impossible.

Another study very similar to ours by design and sample found decreased cortisol reactivity to vaccination among eight-week-old infants exposed to PPD, while we found a tendency in the same direction in females (Tollenaar et al., 2011). Although they measured prenatal general anxiety, pregnancy-related anxiety, pregnancy-specific daily hassles and the circadian cortisol levels of the mother, only the PRAQ factor: "Fear of Bearing a Handicapped Child" was linked to decreased infant cortisol reactivity. We observed a similar tendency toward decreased reactivity in the PRAQ and even more so with combined depression and anxiety. Based on the PRAQ scores, there seems to be no essential differences in the severity of exposure among these two studies. However, comparison between these two studies is hampered as the measure of general anxiety differed between the studies and the depressive symptom levels in their sample are unknown. By combining the measures of anxiety and depressive symptoms, we might have captured more of those mothers, whose quality and level of PPD was sufficient to affect the infant's cortisol reactivity compared to the study of Tollenaar et al., who used anxiety symptoms alone.

Earlier studies have also reported increased infant cortisol reactivity after PPD exposure or no associations. The discrepancy between the reactivity types between those studies and ours might be explained by the different ethnicity and socioeconomic status of the mothers, or a more severe or different type of exposure, which could have had a different effect on the development of the HPA axis. Also, infant stress reactivity type, or lack of it, is dependent on the combination of age and the stressor used (Tollenaar et al., 2011). A lack of association might also be due to a limited power present in smaller studies or too mild exposures in higher socioeconomic, low risk samples.

Mechanisms behind the possible sex differences in the programming effect of PPD on infant HPA axis functioning are not known. There is increased evidence for the sexually dimorphic regulation and expression of placental genes, proteins, steroids and structure of the placenta indicating that the placenta might mediate the maternal stress signals to the developing fetus differently depending on the sex of the fetus. Observed sex differences in human placental cortisol metabolism suggest that the female fetus is more sensitive to the effects of glucocorticoids (Carpenter et al., 2017; Clifton, 2010; Howland et al., 2017). In addition, alterations in the methylation level of the placental *HSD11B2* gene moderated the influence of maternal prenatal MDD on infant baseline cortisol, while such an association was not seen in controls. This effect was only seen in the whole group and a stratified sample of females (Stroud et al., 2016). The 11β-hydroxysteroid dehydrogenase type 2 enzyme inactivates cortisol to cortisone and regulates the amount of maternal cortisol that reaches the fetus.

A strength of our study is that we used longitudinal repeated measures of three types of PPD from gwk 14 to gwk 34 with one of the largest sample sizes among other comparable studies. This enabled a more accurate detection of the associations between maternal PPD and infant cortisol reactivity and recovery. In contrast to previous studies, we explored the less studied recovery phase of the cortisol response in addition to reactivity. The wide intra-individual variability in cortisol among very young infants is a challenge, but our sample size increased the reliability of the findings. The sample size also enabled a better examination of possible sex differences. Versatile register records and prospective self-reported questionnaires on several factors related to pregnancy, postpartum life and the stress test among mothers and infants allowed us to check and control for many confounders that have been undetected in many of the previous studies. Immunoassay measurements were validated against the LC–MS/MS data. Although the time span of data collection was long, a detailed protocol record form was followed and completed during each visit to ensure the consistency of each visit. The families had a 15-minute resting period before the baseline cortisol sampling of the infant to minimize the effects of earlier hassles during the day on the baseline levels. This increased the correspondence of the baseline sample taken in the laboratory setting with the "true" baseline measured at home, as the infant had time to adjust to the new environment. The study sample comprised of subjects drawn from the general population instead of a clinical sample and equally from both sexes thus further improving the generalizability of the results.

One limitation of our study was the unavailability of PRAQ-R2 data at gwk 14 that prevented the study of the first trimester exposure similarly as we did with EPDS and SCL-90 anxiety subscale. Only one baseline sample per infant was collected as part of the study visit, whereas repeated sampling at the laboratory or additional sampling in the home environment would have provided more accurate baseline level estimations. Despite the instructions to feed the infant before the study visit to avoid the possible effects of feeding, during the visit, on saliva cortisol samples, not all mothers were able to time the feeding accordingly. It could be speculated that feeding, especially breastfeeding, could pacify the infant and decrease the cortisol stress reactivity or enhance the recovery depending on the timing of the feeding in relation to the stressor. Observations concerning the effect of feeding on the cortisol stress response in infants have been inconsistent (Egliston et al., 2007). As it is not ethical to prohibit the mothers from feeding their infants, efforts were made to record and statistically control for the feedings in the analyses. Our results may be partly explained by SSRI medication, as the associations between PPD exposures and infant cortisol response diminished after excluding the SSRI-medicated mothers. On the other hand, the SSRI medicated mothers reported higher levels of PPD compared to the rest of the population, so in the sensitivity analyses, these associations were reduced both by decreased level of PPD exposure and sample size, not solely due the removal of the potential effects of SSRI. Our study was concentrated on the most common types of PPD, thus the results cannot be generalized to cover the effects of all potential stress.

Adequate cortisol reactivity to a stressor is essential for well-being, and dysfunctional reactivity is linked with various diseases. Adequate recovery is important for health, as chronic stress with chronically elevated cortisol levels leads to adverse effects on health outcomes. A tendency towards a slower cortisol recovery leads to prolonged exposure to cortisol and, if accumulated over time, possible wear and tear effects of the hormone may occur heightening the risk for adverse health outcomes later in life. For instance, a flatter recovery slope after a social stress task predicted the first onset of general psychopathology in adolescents (Nederhof et al., 2015).

To conclude, our results demonstrate sexually dimorphic alterations in the functioning of the infant hypothalamus-pituitary-adrenal axis and especially in the functioning of the negative feedback loop of the axis after PPD exposure among healthy infants. Whether these observations are related to a later risk for psychiatric disorders needs to be investigated.

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## 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. **Sirpa Hyttinen:** Formal analysis, Writing - original draft. **Olli Laine:** Formal analysis, Writing - original draft. **Linnea Karlsson:** Conceptualization, Project administration, Writing - review & editing. **Hasse Karlsson:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing - review & editing.

## Declaration of Competing Interest

None.

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## Appendix A

Table A1, Fig. A1

**Table A1**
Infant saliva cortisol concentrations (nmol/l) at the age of 10 weeks during the stress test. The samples were collected at baseline and 0, 15, 25 and 35 min after the stressor.

| | Cortisol sampleS | Mean | SD | Min | Max | N |
|---|---|---|---|---|---|---|
| All | Baseline (-30 min) | 10.1 | 12.7 | 1.4 | 146.4 | 353 |
| | 0 min | 12.5 | 10.7 | 1.3 | 77.7 | 356 |
| | 15 min | 17.8 | 14.5 | 1.6 | 119.8 | 339 |
| | 25 min | 14.4 | 12.1 | 2.5 | 105.3 | 307 |
| | 35 min | 11.5 | 10.4 | 2.4 | 82.4 | 288 |
| Females | Baseline (-30 min) | 9.7 | 12.4 | 1.6 | 132.4 | 167 |
| | 0 min | 13.0 | 11.3 | 1.3 | 77.7 | 169 |
| | 15 min | 19.5 | 15.2 | 2.2 | 103.1 | 158 |
| | 25 min | 16.4 | 14.0 | 2.5 | 105.3 | 143 |
| | 35 min | 12.8 | 11.4 | 2.4 | 82.4 | 133 |
| Males | Baseline (-30 min) | 10.5 | 13.0 | 1.4 | 146.4 | 186 |
| | 0 min | 12.0 | 10.1 | 1.9 | 59.3 | 187 |
| | 15 min | 16.3 | 13.8 | 1.6 | 119.8 | 181 |
| | 25 min | 12.6 | 10.0 | 2.6 | 75.1 | 164 |
| | 35 min | 10.4 | 9.4 | 2.7 | 66.1 | 155 |

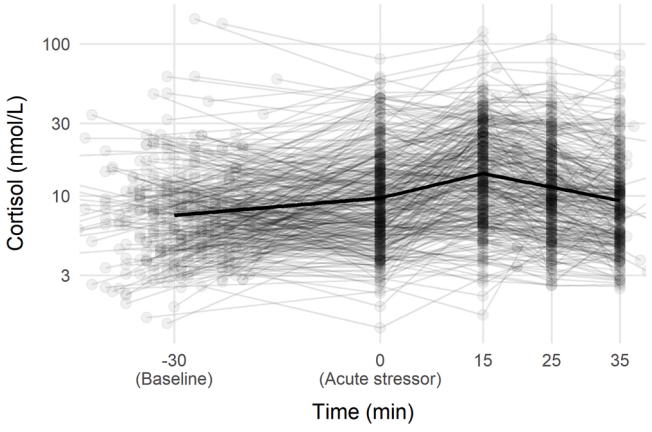


**Fig. A1.** Raw data of the saliva cortisol stress response profiles during the acute stressor from 363 infants at the age of 10 weeks. A bold line indicates a group mean and the thin lines represent individual infants with a maximum of five saliva samples (small circles). Baseline sampling occurred on average 30 min before the 0 min sampling, which was the first post-stressor sample. The effect of two EIA kits has been taken into account to make the cortisol response profiles comparable to each other.

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