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"And surely, we are all out of the computation of our age,
and every man is some months elder than he bethinks him;
for we live, move, have a being,
and are subject to the actions of the elements,
and the malices of diseases,
in that other World, the truest Microcosm,
the Womb of our Mother"

Sir Thomas Browne, Religio Medici, 1642

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ABSTRACT

The adrenal cortex secretes cortisol during stressful situations to regulate stress reactions of several other systems, such as the immune system, to enhance coping and survival. An altered cortisol stress responsiveness might be a potential mechanism linking maternal prenatal psychological distress (PPD) exposure to the increased risk for childhood negative psychosocial and developmental outcomes and later stress-related diseases. Research on the associations between maternal PPD and infant cortisol stress reactivity and recovery in a longitudinal setting with the focus on sex differences is largely lacking. The role of the infant immune system in the cortisol stress response of the PPD-exposed infant is not well understood.

We studied infant cortisol stress reactivity and recovery longitudinally at 10 weeks, 6 months and 14 months of age to detect sex- and age-related changes in infant cortisol stress response to an acute, mild physical stressor. Based on the levels of maternal self-reports on depressive, general anxiety and pregnancy-related anxiety symptoms at gestational weeks 14, 24 and 34, we selected 462 infants to the study. To see whether the virus-activated immune system alters the association between PPD exposure and the cortisol stress response, we studied 10-week-old infants with and without the subclinical rhinovirus infection.

As a result, ten-week-old female infants exposed to higher maternal prenatal depressive and anxiety symptoms had slower cortisol recovery from an acute stressor compared to males and non-exposed females. Later, the cortisol stress recovery had become faster among 14-month-old females exposed to higher maternal prenatal depressive and general anxiety symptoms compared to males and non-exposed females. In addition, female cortisol recovery was faster also after higher exposure to pregnancy-related anxiety symptoms compared to non-exposed females at the age of 14 months. Finally, PPD-exposed infants with subclinical rhinovirus infection had a blunted cortisol stress response, which was more evident in males.

Our results suggest sexually dimorphic- and age-dependent alterations in cortisol stress recovery among PPD-exposed infants. Prior infection might impair cortisol stress responsiveness in PPD-exposed infants. Follow-up is needed to assess whether the altered cortisol stress responses during infancy predict later health outcomes.

KEYWORDS: cortisol, HPA axis, depression, anxiety, prenatal stress, prenatal programming, sex differences, rhinovirus

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TIIVISTELMÄ

Lisämunuaiskuori edistää jaksamista erittämällä stressaavissa tilanteissa kortisolia, joka säätelee muiden järjestelmien, kuten immuunipuolustusjärjestelmän, stressivasteita. Muuttunut kortisolin stressivaste on yksi mahdollinen mekanismi, joka voisi selittää lapsen psykososiaalisten ja kehityksellisten häiriöiden ja stressiin liittyvien sairauksien lisääntyneen riskin, kun lapsi on altistunut äidin raskausajan stressille. Pitkittäistutkimuksia äidin raskausajan stressin yhteyksistä vauvan kortisolin stressireaktiivisuuteen ja stressistä palautumiseen huomioiden sukupuolierot ei juurikaan ole. Immuunijärjestelmän merkitys kortisolin stressivasteeseen raskausajan stressille altistuneilla vauvoilla tunnetaan huonosti.

Seurasimme 10 viikon sekä 6 ja 14 kuukauden ikäisten vauvojen kortisolin stressireaktiivisuutta ja palautumista stressistä selvittääksemme sukupuolen ja iän vaikutusta vauvojen kortisolin stressivasteeseen lyhytkestoisen stressin jälkeen. Äidin raskausviikoilla 14, 24 ja 34 raportoimien masennus- ja ahdistusoireiden sekä raskauteen liittyvien ahdistusoireiden perusteella valitsimme tutkimuksiin 462 vauvaa. Tutkimme myös, muuttaako rinoviruksen aktivoima immuunijärjestelmä raskausajan stressin ja vauvan kortisolistressivasteen välistä yhteyttä 10 viikon iässä.

Tyttövauvojen, joiden äideillä oli enemmän masennus- ja ahdistusoireilua raskauden aikana, kortisolitasot palautuivat hitaammin stressistä 10 viikon iässä, mutta palautuminen oli kehittynyt nopeammaksi 14 kuukauden iässä verrattuna poikiin ja altistumattomiin tyttöihin. Lisäksi enemmän raskauteen liittyvää ahdistusoireilua raportoineiden äitien tyttövauvoilla kortisolitasot palautuivat nopeammin stressistä verrattuna muihin tyttöihin 14 kuukauden iässä. 10 viikon ikäisillä raskausajan stressille altistuneilla vauvoilla oli heikentynyt kortisolivaste rinovirusinfektion aikana. Tämä näkyi selvemmin pojilla.

Raskausajan stressille altistuneilla vauvoilla on sukupuolesta ja iästä riippuvaisia muutoksia kortisolin palautumisessa stressistä. Rinovirusinfektio heikentää mahdollisesti raskausajan stressille altistuneiden vauvojen stressivastetta. Seurantatutkimuksia tarvitaan selvittämään, liittyykö muuttunut kortisolin stressivaste myöhemmän iän hyvinvointiin.

AVAINSANAT: kortisoli, HPA-akseli, masennus, ahdistuneisuus, raskausajan stressi, sikiöaikainen ohjelmoituminen, sukupuolierot, rinovirus

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Abbreviations

11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
ACTH	adrenocorticotropic hormone
AUC	area under the curve
AUCg	area under the curve with respect to ground
AUCi	area under the curve with respect to increase
AVP	arginine vasopressin
CAR	cortisol awaking response
CBG	corticosteroid-binding globulin
CRH	corticotropin-releasing hormone
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DOHaD	Developmental Origin of Health and Disease
DZ	definitive zone
EPDS	Edinburgh Postnatal Depression Scale
FZ	fetal zone
GWK	gestational week
GR	glucocorticoid receptor
HCC	hair cortisol concentration
HPA	hypotalamic-pituitary-adrenal
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MDD	major depressive disorder
MR	mineralocorticoid receptor
pCRH	placental corticotropin-releasing hormone
PPD	prenatal psychological distress
PRAQ-R2	Pregnancy-Related Anxiety Questionnaire Revised 2
PTSD	post-traumatic stress disorder
PVN	paraventricular nucleus
SAD	social anxiety disorder
SAM	sympatho-adrenal medullary system
SCL	Symptom Checklist
SSRI	selective serotonin reuptake inhibitor

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Laura S. Korhonen, Susanna Kortesluoma, Minna Lukkarinen, Ville Peltola, Henri Pesonen, Juho Pelto, Jetro J. Tuulari, Heikki Lukkarinen, Tytti Vuorinen, Hasse Karlsson and Linnea Karlsson. Prenatal maternal distress associates with blunted cortisol response in rhinovirus-positive infants. *Psychoneuroendocrinology*, 2019; 107: 187–190.

II Susanna Kortesluoma, Laura S. Korhonen, Juho Pelto, Sirpa Hyttinen, Olli Laine, Linnea Karlsson, Hasse Karlsson. Sex differences in the associations between maternal prenatal distress and infant cortisol reactivity and recovery. *Psychoneuroendocrinology*, 2021; 124: 105064.

Susanna Kortesluoma, Laura S. Korhonen, Juho Pelto, Sirpa Hyttinen, Olli Laine, Linnea Karlsson, Hasse Karlsson. Corrigendum to "Sex differences in the associations between maternal prenatal distress and infant cortisol reactivity and recovery" [Psychoneuroendocrinology 124 (2021) 105064]. *Psychoneuroendocrinology*, 2021; 128: 1–2.

III Susanna Kortesluoma, Laura S. Korhonen, Juho Pelto, Jetro J. Tuulari, Linnea Karlsson, Hasse Karlsson. Age and sex differences in the cortisol stress reactivity and recovery among infants exposed to prenatal psychological distress. *Psychoneuroendocrinology*, 2022; 135; 105580.

1 Introduction

One of the earliest descriptions of general physiological responses to injury came from Hans Selye (1907–1982), who called the beginning of the response as a "general alarm reaction" that leads to the "general adaptation syndrome" (Neylan, 2014; Selye, 1936). This description described how an animal adapted to a new condition or survived from the damage due to various alterations in tissues and glands or eventually died because of the irreversible changes in the organs in the case of chronic or too severe damage. The substances of this response were unknown for the early researchers but, since then, knowledge on the broad stress regulatory capacities of the hypothalamic-pituitary-adrenal (HPA) axis during stress and adaptation has increased enormously.

Glucocorticoid hormones, cortisol in humans, that are secreted by adrenal cortex of the HPA axis, have been described to have permissive, suppressive, stimulatory and a preparative role in the stress response. The influence of these hormones on metabolism and the cardiovascular, reproductive, immune and nervous systems prepares the body in advance to encounter the future stressors, activates and mediates and eventually limits the stress responses and their consequences during the stressful situation and finally supports the recovery from the stress. Glucocorticoids transmit their effects via glucocorticoid and mineralocorticoid receptors that act as gene transcription factors, which activate and inhibit different genes depending on the tissue and developmental stage of the individual (Sapolsky et al., 2000).

The type and number of stressors in modern life are different from those that our ancestors encountered thousands of years ago but still our physiology and especially our brain, over time, have not changed that much. Especially many brain areas, including the hippocampus, amygdala and prefrontal cortex that are involved in cognitive performances, such as learning, memory and emotion regulation, are vulnerable to the effects of stress, as those brain areas are rich in glucocorticoid and mineralocorticoid receptors. These areas also regulate the HPA axis functioning. Humans have a limited capacity to cope with stressful situations for a longer period of time and the importance of rest and recovery from the demands of daily life is noticed often too late, when the physical and mental health are already endangered due to the "wear and tear" effects of stress. For example, depressive disorders are

the fourth and sixth leading cause of the global disability-adjusted life year among 10–24- and 25–49-year-olds, respectively (Abbafati et al., 2020), and depression affects 280 million people worldwide (WHO, 2021). For example, in Finland, psychiatric disorders are the most common reason to grant sickness benefits for adults, which included74 300 individuals in 2018 (Finnish Institute for Health and Welfare (THL), 2021).

The development of a fetus can be altered by the environmental signals that the fetus perceives from the mother through the placenta. Increased cortisol levels or other biological pathways of the stressed mother are suggested to alter the development of the HPA axis and the brain of the fetus. Research on infant HPA axis development and functioning and on the role of maternal prenatal psychological distress in this process has increased notably during the last few decades. At the same time, researchers have encountered challenges in explaining perhaps seemingly contradictory new observations in a coherent way. Most commonly, the studies have observed increased cortisol stress reactivity in infants of mothers who have experienced psychological distress during their pregnancy, but also decreased infant cortisol reactivities have been reported. In addition, infant cortisol recovery has not been given as much attention as in cortisol reactivity. It also seems that not all infants are affected, and sex might be one of the characteristics among many other factors that could explain the differences in vulnerability to prenatal exposures such as maternal psychological distress between individuals. As the study designs have varied during the years, it is not clear how much differences in the results are due to the differences in the used methods. The quality of the cortisol stress response depends on the quality of the encountered stressor and the developmental stage of the individual. A challenge in infant stress research is to find a stressor that is not too strong and not ethically problematic but still strong enough to achieve a measurable increase in cortisol levels among the majority of the infants at different ages. During the first year, the infant brain and other biological systems change rapidly as infants age. As a result, it is not surprising that the perception of stress and the functioning of the HPA axis changes as well, and a stressor in a 3-month-old infant does not evoke a similar cortisol stress response pattern than in a 1-year-old.

To fill the gaps, we have studied infant cortisol stress reactivity and the less studied cortisol recovery longitudinally at 10 weeks, six months and 14 months of age and used the similar acute stressor in each age group to detect age-related changes in infant cortisol stress response. Infants have been drawn from the nested case-control Focus Cohort to investigate the role of maternal prenatal depressive, general anxiety and pregnancy-related anxiety symptoms at gestational weeks 14, 24 and 34 in the functioning and development of the infant cortisol stress response. Furthermore, we have studied the role of the forementioned prenatal psychological distress (PPD) exposure in cortisol stress response to acute stressor in infants with

and without the subclinical rhinovirus infection to determine whether the virus-activated immune system alters the association between PPD and the cortisol stress response. The studies are part of the FinnBrain Birth Cohort Study.

2 Review of the Literature

2.1 Hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrinological stress regulating system that helps to maintain and coordinate physiological, metabolic, cognitive, emotional and behavioral activities in response to stressful and threatening situations regardless of they are current, anticipated or imaginary. These situations can include a variety of stressors, such as the physical damage of the tissues, infections, inflammation, physical strain, pain or experienced psychological distress (Herman et al., 2016).

The HPA axis consists of a cascade of secreted hormones where each previous step activates the secretion of the next hormone (Figure 1). The cascade is activated when the hypothalamus receives a stress signal from the other part of the central nervous system. An immediate physiological disturbance, such as pain, inflammation or hypoglycemia, sends ascending stress signals directly to the hypothalamus mainly via the nucleus of the solitary tract in the brainstem. Psychological stress signals are received to the hypothalamus mainly from the limbic brain areas, such as the amygdala, hippocampus and prefrontal cortex, via intermediary neurons.

Parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) neuropeptides from their axons to the hypophyseal portal vessels in the brain. AVP enhances the effects of CRH. CRH and AVP receptors in the corticotropic cells of the anterior pituitary activate the production and secretion of adrenocorticotropic hormone (ACTH) to the systemic blood circulation from where it binds to the ACTH receptors in the adrenal glands, which are located on the top of kidneys. Activated ACTH receptors in the adrenal cortex induce the production and secretion of glucocorticoid hormones to the circulation as cortisol in humans, non-human primates and fish, whereas these hormones are corticosterone in non-primate mammals and birds (Figure 1) (Herman et al., 2016; Sopinka et al., 2015).

Glucocorticoids are a group of steroid hormones, which help animals to adapt to environmental changes and different kinds of stressors by shaping physiological

and behavioral responses. The effects of glucocorticoids in their target tissues are transmitted by glucocorticoid (GR) and mineralocorticoid receptors (MR). GR and MR are intracellular cytoplasmic receptors that form heterocomplexes with other proteins in an inactive state. There is also a membrane-associated glucocorticoid receptor that regulates the feedback functions of the glucocorticoids. When the glucocorticoid binds to the cytoplasmic receptor, the activated hormone-receptor monomer dissociates from the heterocomplex and translocates to the cell nucleus. The active receptor binds to the glucocorticoid response elements (GRE) in the DNA or transcription factors of the target genes to change the gene expression (Sapolsky et al., 2000). GRs are expressed in almost every cell and tissue type throughout the body. In the brain, they are especially found in the hippocampus, amygdala and medial prefrontal cortex (mPFC) in addition to hypothalamus and pituitary. MRs are less abundant and mostly found in adipose tissue and kidney, where they are activated mainly by aldosterone, because glucocorticoids are inactivated by 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2). In the brain, the highest levels of MRs have been reported in the hippocampus, but MRs are also found in prefrontal cortex, amygdala, lateral septum, thalamus and hypothalamus (Gjerstad et al., 2018; Gomez-Sanchez and Gomez-Sanchez, 2021; Herman et al., 2016; Lightman et al., 2020).

Secretion of the glucocorticoids follows a circadian pattern consisting of a cortisol awakening response (CAR), in the morning in diurnal animals and in the evening in nocturnal animals, causing glucocorticoid levels to briefly increase prior to awakening with a peak level typically during the first 30 minutes after awakening, after which, the levels gradually decrease during the day and reach their nadirs at night. The circadian pattern is a result of a pulsatile ultradian glucocorticoid secretion, where the amplitude and frequency of the smaller glucocorticoid pulses increase briefly after awakening and decrease towards the night. These spontaneous glucocorticoid pulses, which occur a little less than every hour in human adults, can be approximately the same size as the ones evoked by a stressor, especially in humans, which makes the recognition of the glucocorticoid stress responses challenging (Young et al., 2004). The ultradian glucocorticoid pulsatility seems to be important for GR sensitivity and maintaining stress responsiveness, as glucocorticoids are released from GRs during each nadir of the pulse in a baseline condition. MRs, which have a ten times higher affinity for glucocorticoids, are activated already at low basal levels of glucocorticoids. During the higher levels of CAR in the morning and during stress-induced higher levels of glucocorticoids, when MRs get saturated, GRs with lower affinity get occupied (Lightman et al., 2020).

Neonates do not have an adultlike cortisol circadian rhythm with only one peak in the morning, but hormone levels vary in accordance with daytime napping and

feeding rhythms. Depending on the definition and methods of modelling of the cortisol circadian pattern from the collected cortisol samples, adultlike circadian rhythms can be observed already at two months of age in some cases, but there are notable individual differences in the stability of this pattern, and, at which age, the pattern is achieved. Usually, the cortisol circadian rhythm stabilizes during early childhood when daytime napping stops (De Weerth et al., 2003; Gunnar and Vazquez, 2006).

Approximately 70% of the circulating cortisol is bound to the plasma protein corticosteroid-binding globulin (CBG) and 20% to albumin. Only less than 10% of the plasma cortisol level is bioavailable and free to enter cells and activate MRs and

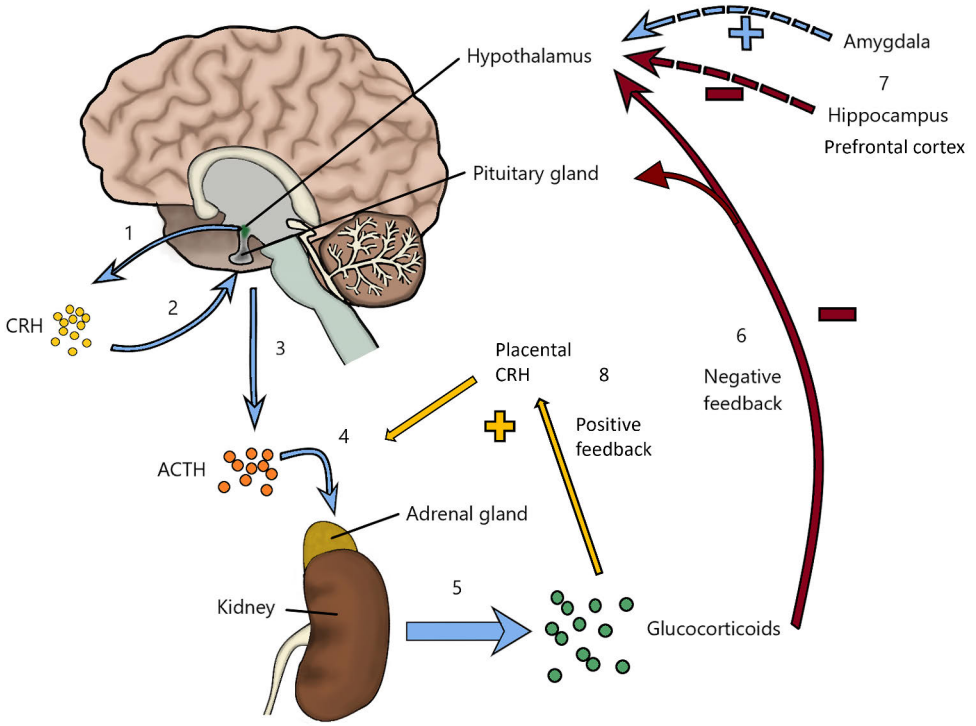

Figure 1. Hypothalamic-pituitary-adrenal (HPA) axis. 1. The hypothalamus secretes corticotropin-releasing hormone (CRH), which 2. activates the pituitary to 3. secrete adrenocorticotropic hormone (ACTH) to the systemic blood circulation. 4. ACTH activates the adrenal glands to 5. secrete glucocorticoids (cortisol in humans). 6. Eventually an increased level of blood glucocorticoids directly inhibits the secretion of CRH from the hypothalamus and ACTH from the pituitary by a negative feedback mechanism. 7. In the brain, the amygdala activates and the hippocampus and prefrontal cortex inhibit the functioning of the hypothalamus indirectly. 8. During pregnancy, an increased level of placental CRH activates the secretion of maternal and fetal glucocorticoids, which, in turn, activate the secretion of the placental CRH by a positive feedback mechanism. Modified from the original figure by Satu Lehtola, published with the permission from the author (Lehtola Satu J, 2020).

GRs. This makes the levels of binding proteins an important regulating factor for the effects of cortisol (Brien, 1981). As the free cortisol fraction is readily and reliably measured non-invasively from saliva, saliva cortisol has become a popular biomarker for HPA axis functioning (Clements, 2013).

Many aspects of the HPA axis functioning change considerably during a pregnancy and are outside of the scope of this brief overview. These aspects are further discussed in Sections 2.3.1 and 2.4.1.

2.2 The cortisol stress response

A widely used measure for HPA axis functioning, in addition to basal circadian measurements during rest, such as CAR and diurnal cortisol slope, is the cortisol response to perceived stress. It is commonly depicted by successive cortisol stress reactivity and cortisol stress recovery phases. Cortisol levels increase during a reactivity phase as a response to a stress-induced increase in ACTH levels. The magnitude of the cortisol increase depends, in addition to various other factors, on the phase of the cortisol ultradian pulse at the onset of a stressor. A stressor that occurs during the rising phase of the pulse tends to elicit a higher cortisol stress response, and during the falling phase, the cortisol stress response is inhibited (Lightman et al., 2020; Young et al., 2004).

2.2.1 Cortisol stress reactivity and regulation of stress

Neuroendocrine responses to stress involve an immediate increase in the secretion of several hormones, such as catecholamines from the sympatho-adrenal medullary (SAM) system, CRH and ACTH from the HPA axis and glucagon from the pancreas. At the same time, the secretion of gonadotropin-releasing hormone (GnRH) and gonadotropins from the hypothalamus and pituitary decrease. After some minutes, a slower response of steroid hormones begins, where the secretion of gonadal hormones, such as testosterone, estrogens and progesterone, decreases, and the levels of glucocorticoids increase. As a result, physiological and psychological changes during stress include the stimulation of the immune function, increased cardiovascular tone, increased energy supply to the muscles and brain, enhanced cognition, inhibition of reproductive behavior and a decreased appetite.

As glucocorticoid receptors exist in almost all organs and tissues, the effects of glucocorticoids are widespread. These effects are divided into permissive, suppressive, stimulating and preparative actions depending on their influence and timing with respect to the other stress response elements (Sapolsky et al., 2000). Permissive actions in tissues occur already before stress and help the immediate stress responses of the endocrine systems to elicit their actions. After the onset of

stress, when glucocorticoid levels have increased, their suppressive actions prevent the stress responses from being overactive and protects the body from the adverse effects of the stress response. Opposingly, the stimulating actions of glucocorticoids enhance the effect of the stress response further. Preparative actions regulate the subsequent stress responses in advance.

Glucocorticoids permissively enhance the effects of catecholamines on the cardiovascular system so that blood pressure and cardiac output increase as a response to a stress, and blood circulation and delivery of glucose (i.e., energy) and oxygen for muscles are enhanced. Infections, but also psychological stressors, rapidly activate immune stress responses, such as cytokine secretion from immune cells. Cytokines, in turn, activate the HPA axis and increase glucocorticoid secretion. For example, cytokine IL-1 induces the secretion of CRH and ACTH. Cytokines can enhance the release of glucocorticoids directly from the adrenal cortex as well (Herman et al., 2016). Many cytokines are cytotoxic, and, over time, their levels are controlled by anti-inflammatory and immunosuppressive actions of glucocorticoids, which inhibit the synthesis, release and efficacy of cytokines and functioning of the leucocytes. Stress levels of glucocorticoids suppress the levels of TNF-α and IL-6, but if glucocorticoid levels have been increased days before the exposure to a toxin that activates the immune system, glucocorticoids enhance the levels of TNF-α and IL-6, permissively. In this way, glucocorticoids can improve resistance to infection.

Appetite is suppressed during stress, probably centrally by CRH, and the suppressive effect of glucocorticoids on this stimulates appetite. This glucocorticoid effect starts slowly and lasts for days, therefore an increased appetite after the energy consuming stress aids to recover the energy storages of the liver and muscles and can be considered also as a preparative effect for the next stressor. Only high glucocorticoid levels during a stress induce the secretion of insulin that in turn inhibits appetite. High glucocorticoid levels also suppress glucose transport and utilization in tissues in the periphery and in the brain.

In addition, high levels impair learning and memory functions of the hippocampus via GRs by disrupting synaptic plasticity. Effects on memory are also partly due to decreased glucose levels as glucose is needed in memory formation. Chronically high levels of glucocorticoids induce neuronal loss in the hippocampus. Opposingly, basal levels of glucocorticoids via MRs enhance hippocampal synaptic plasticity and thus learning and memory. At the beginning of the stress stimuli, cognitive performance is enhanced, which is suggested to help to recall the earlier behavior that was successful in coping with previous stress as well to consolidate the memory to help avoid the similar situation in the future (Sapolsky et al., 2000).

The terms being “hyperreactive,” “hyporeactive” or “blunted” cortisol stress reactivity are often used. There are no commonly agreed definitions to those terms, as it is not known what amount of cortisol increase in general should be considered

as typical or “healthy” to say that an observed cortisol reactivity would be “hyper” or “hypo” by comparison. Usually, the terms are used in the context of the reference group of less exposed or healthy controls present in the study in question. This means that the same magnitude of the cortisol stress reactivity that is called “hyporeactive” in one study, could be called “hyperreactive” in another study depending on the magnitude of the cortisol stress reactivities of the reference groups in these studies. It is more likely that there is no all-encompassing definition for the typical magnitude of the cortisol stress reactivity, but, rather, it is context specific and depends on the sex and age of the individual as well as the type, duration and severity of the stressor and habituation at least. A healthy cortisol stress reactivity would be the one that is sufficient in size and duration for coping with the stress in a particular context. Both a heightened and decreased cortisol reactivity to a stressor have 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). Whether cortisol stress reactivity becomes hyperreactive or hyporeactive is suggested to depend on the type of the stressor, genetics of the individual, developmental time in which the stress has occurred and the intensity and duration of the stress (Engel and Gunnar, 2020).

2.2.2 Cortisol stress recovery and the negative feedback mechanism

A cortisol stress response that sustains health and well-being has been commonly described as one where a stress-induced increase in cortisol levels eventually decreases back to the basal levels during the recovery phase of the cortisol stress response. A lack of recovery from the stress results in continued elevation of cortisol after the stress is over. A delayed recovery eventually leads back to a basal cortisol level, but still, the individual has been exposed to a higher cortisol level for a prolonged period. Similar to exaggerated cortisol reactivity, an impaired cortisol recovery might lead to adverse health outcomes as well due to overexposure to cortisol that is energetically costly.

The cortisol stress response is tightly regulated by a set of negative feedback mechanisms of the HPA axis of which few are described here. The increased level of blood cortisol inhibits the secretion of CRH and ACTH via GRs in the hypothalamus and pituitary, respectively. In addition, GRs mediate inhibitory signals from the prefrontal cortex, hippocampus and amygdala. As a result, glucocorticoid levels decrease. In addition, there is evidence that cortisol could rapidly inhibit its own synthesis and/or secretion by a local feedback mechanism in adrenals. Rodent models have characterized two negative feedback mechanisms. Faster, nongenomic regulation happens within seconds to minutes. Genomic feedback mechanisms are

sometimes divided into an intermediate, i.e., transcription-independent mechanism and delayed, i.e., transcriptional feedback mechanism, and the effect of these on glucocorticoids takes hours to days (Gjerstad et al., 2018; Herman et al., 2016).

Nongenomic fast inhibition of the PVN in the hypothalamus occurs via cell membrane-bound GRs that activate endocannabinoid release, which inhibits the glutamatergic drive from other brain areas to the PVN by activating presynaptic cannabinoid receptors. This mechanism occurs also in the hippocampus, amygdala and prefrontal cortex, which, in turn, regulate the PVN. In addition, glucocorticoids inhibit CRH gene transcription, translational activity and CRH mRNA stability in CRH-secreting cells. Fast nongenomic effects of glucocorticoids on ACTH secretion occur by regulating the electropotential properties of the ACTH-secreting cells and decreasing their bursting capabilities. Glucocorticoids also inhibit ACTH synthesis (Gjerstad et al., 2018).

Most of the research on negative feedback mechanisms at the neuronal level have been made in male animals. Research using both sexes have observed differences between the male and female glucocorticoid feedback mechanisms. For example, a selective deletion of GRs in the hippocampus, medial prefrontal cortex and basolateral amygdala has induced an impairment in the GR-mediated central feedback mechanism with altered glucocorticoid levels after the restrain stress only in male mice compared to male controls. This suggest alternative, possible compensatory, mechanisms in the central glucocorticoid control system among the females (Solomon et al., 2012).

2.2.3 Cortisol stress response indicators

Most of the research, especially infant research, has focused on reactivity only by analyzing the difference between two cortisol measurements being a baseline cortisol level before a stressor and a cortisol level after a stressor. This limits the understanding of the cortisol stress response and might lead to an underestimation of the association between HPA axis functioning and other factors of interest when recovery of the stress response is not covered in the analyses. Another challenge in the practice of measuring reactivity as a difference between the two cortisol levels only is to determine a suitable time point for the single post-stress cortisol measurement. If measured too early or too late, the peak cortisol level will not be captured and the measure of the cortisol stress reactivity will be too small, get unnoticed or be actually measured during the recovery phase. Individuals also differ in their timing of peak cortisol, such that the single, post-stress measurement will capture the peak cortisol level only in a proportion of assessed individuals from a group. Measuring saliva cortisol concentration around 20 minutes after the stressor has become a common practice (Gunnar et al., 2009), as many early studies observed

a cortisol peak after 15–30 minutes of mild laboratory stress (Kirschbaum and Hellhammer, 1989; Ramsay and Lewis, 2003), but it can occur as late as 40 minutes after a stressor (Goldberg et al., 2003). These differences in the timing of peak cortisol probably depend on the intensity and nature of the stressor as well as the characteristics of the studied individuals. Depending on the study aims and research questions, the capture of the exact peak cortisol is not always necessary as long as a sufficient cortisol reactivity is observed among the majority of the individuals.

To alleviate the above-mentioned challenges in pre-stress to post-stress cortisol measurement study designs and to include the recovery phase of the cortisol stress response in the analysis, multiple cortisol samples per individual during the study visit can be collected. The entire cortisol stress response can be quantified by calculating Area Under the Curve (AUC) of the cortisol stress response. AUC is divided into two aspects of the cortisol response being the area under the curve with respect to ground (AUCg) and the area under the curve with respect to increase (AUCi). AUCg is used to summarize the total cortisol output, or intensity of the total cortisol secretion, during a stressful situation by taking into account the distance from the ground with zero as a reference. AUCi uses the first measurement as a reference value and depicts the change over time in cortisol levels in relation to the reference baseline value. AUCi is considered to be a measure of sensitivity of the cortisol secretion profile over time (Fekedulegn et al., 2007; Pruessner et al., 2003).

The challenge is that similar sized AUCs can be obtained from different combinations of reactivity and recovery patterns. As a result, both robust and dampened cortisol stress responses can be quantified with identical AUCi values (Atkinson et al., 2016). In this case, AUCi cannot be used to separate between the two individuals with the different cortisol responses, which, in turn, might have different consequences in the individual and associate differently with other characteristics and factors in question.

2.3 HPA axis development

2.3.1 Fetal adrenal cortex and cortisol production

There are important differences in the gestational physiology and the anatomy of the adrenal cortex among species that brings challenges to translate research findings from animal-based prenatal stress models to humans. Rodents have been the predominant choice in the modelling of prenatal stress and offspring HPA axis functioning. For example, the fetal zone of the adrenal cortex is a structure that has been found only in primates, including humans. In addition, placental-derived CRH (pCRH) is only found in humans and some other primates. Among non-human primates, the timing of the synthesis and release of pCRH differ from humans

(Sandman et al., 2011). For these reasons, child developmental studies starting from pregnancy, also in humans, are needed.

During a pregnancy, the maternal cortisol is an important source of cortisol for the fetus and the placenta plays a major role in the regulation of the cortisol levels of the mother and the fetus. pCRH increases the cortisol secretion from the maternal and the fetal adrenals and, in turn, instead of inhibiting the secretion of the pCRH, as it would inhibit the hypothalamic CRH, cortisol induces the secretion of the pCRH by a positive feedback mechanism. This further increases the levels of pCRH and cortisol (Figure 1). The placental 11β-HSD2 enzyme converts maternal cortisol to inactive cortisone and protects the fetus from the overexposure to maternal cortisol. (Sandman et al., 2011).

A rudimentary human fetal adrenal gland is observable as a distinct structure from its surroundings at 33 days post-conception. Around days 50–52, two zones are recognizable in the cortex being an outer, definitive zone (DZ) and inner, fetal zone (FZ) based on the different morphologies of the cells that they contain. The enzymes for the cortisol biosynthesis pathway appear mainly in the FZ. The enzyme 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) that is needed for *de novo* synthesis of cortisol can be mostly detected between the two zones. After midgestation, this area will develop into the third middle zone of fetal adrenal cortex called the transitional zone (TZ) (Ishimoto and Jaffe, 2011; Melau et al., 2019; Pihlajoki et al., 2015).

At gestational weeks (gwk) 8–9, there is a peak in the level of 3β-HSD2, which then starts to decrease until the enzyme cannot be detected anymore at gwk 14. At the same time, cortisol levels decrease by 50%. The expression of the enzyme is detectable again at gwks 23–24, but this time in the DZ and also in the TZ, which have developed during the second trimester (Ishimoto and Jaffe, 2011). The TZ between the DZ and the FZ contains a mixture of DZ and FZ cells (Melau et al., 2019). 3β-HSD2 is found mainly in the FZ during the first trimester, and the purpose of this short period of cortisol synthesis during that time is unknown. It is suggested that the cortisol secretion inhibits the ACTH secretion, and by this way, safeguards the normal female development from virilization, where increased ACTH induces adrenal androgen secretion and the development of male-like external genitalia in females (Goto et al., 2006). Opposingly, a significant increase in cortisol production from the first to second trimester has also been noted and supports the hypothesis that the negative feedback regulation of ACTH is not restricted to early pregnancy only (Melau et al., 2019).

During the gwks 8–10, the adrenal gland increases its weight almost 10-fold, and at gwk 20, it is as large as the fetal kidney. Ten weeks later, its relative size is 10–20 fold bigger than the adult adrenal gland, and its size even doubles after that. By term, the fetal adrenal weighs about 3–5 g. This rapid growth is almost entirely in the FZ,

which dominates the gland during gwks 16–20. By gwk 30, the DZ and the TZ begin to resemble the zona glomerulosa and the zona fasciculata of the adult adrenal cortex (Ishimoto and Jaffe, 2011).

After birth, the fetal adrenal involutes due to the rapid disappearance of the FZ, and the total weight of the glands decreases by 50%. During the first 2 weeks, the size of the adrenal gland decreases to its normal size for infants. During the early postnatal months, the development of zona glomerulosa and the zona fasciculata continues. The former produces the mineralocorticoid aldosterone and the latter glucocorticoids (Ishimoto and Jaffe, 2011). The human adrenal cortex matures and achieves its final zonation and functioning during puberty, when the innermost third layer zona reticularis matures and production of adrenal androgens begins in a process called adrenarche (Auchus and Rainey, 2004; Pihlajoki et al., 2015). All the adrenocortical hormones, such as aldosterone, cortisol, dehydroepiandrosterone (DHEA), its sulfate (DHEAS) and androstenedione, are synthesized from cholesterol in zone-specific pathways by cytochrome P450 and hydroxysteroid dehydrogenase enzymes depending on the type of the enzymes produced in each separate zone (Miller and Auchus, 2011).

2.3.2 Cortisol stress responsiveness during infancy

The fundamentals of typical infant cortisol stress reactivity and its development during the first years are well characterized (Engel and Gunnar, 2020). This knowledge is important in detecting the possible alterations during infant HPA axis development. Increased cortisol levels during stress are measurable already in neonates (Davis et al., 2011). Young, healthy infants until 3–6 months of age respond readily to mild physical (e.g., bathing, examination) and pain (e.g., vaccination) stressors after which the cortisol stress reactivity decreases with age during the following year (Davis and Granger, 2009; Gunnar et al., 1996; Lewis and Ramsay, 1995; Tollenaar et al., 2010). Similar age-dependent diminishing of the cortisol stress reactivity to the psychological stressors at 3–18 months of age has not been observed. On the other hand, a mother-infant separation protocol, the only one that has been associated with a consistent increase in infant cortisol, has mostly been used in infants around 13 months of age, whereas frustration, novelty and interaction disruption procedures have been a more common choice for a stress test at 5–7 months of age. Limited age ranges that have been used in different psychological stress tests limit the conclusions (Puhakka and Peltola, 2020; Tollenaar et al., 2010).

To the best of our knowledge, there are no related studies on the possible developmental changes in cortisol recovery across infancy.

2.4 Altered cortisol stress response and health

Stress in its various forms is a well-known risk factor for many diseases, such as psychiatric and metabolic disorders and cardiovascular disease, but the association between stress and disease is not straightforward. According to decades of stress research, many factors seem to be needed to be in place for stress exposure to develop into a health problem. Prolonged, too strong and/or too frequently experienced stress and resulting activation of stress systems of the body during a developmentally sensitive time, such as pregnancy, infancy, childhood and adolescence, in individuals with genetic and epigenetic vulnerability to stress have been associated with an increased risk for a myriad of diseases, especially when there are additional adverse environmental factors present. Similarly, protective environmental factors and genetic resilience against stress diminishes the association between stress exposure and negative health outcomes (Chrousos, 2009).

Psychiatric disorders, especially depression and anxiety, are one of the most actively studied diseases that have been associated with altered HPA axis functioning. Meta-analyses support the numerous separate observations that have been made during the last several decades concerning dysregulated HPA-axis stress responses in adult patients with a major depressive disorder (MDD) or anxiety disorders (Zorn et al., 2017) and in 9–16-year-old depressive children and adolescents (Lopez-Duran et al., 2009).

Zorn et al. (2017) analyzed in their meta-analysis the role of sex and current symptomatic state in the cortisol stress response during an acute psychosocial stress among patients with psychiatric disorders. Adult women with current depression had more likely a blunted cortisol stress response in the AUCi and in the AUCg to a psychosocial stressor such as the Trier Social Stress Test compared to controls. The difference was less clear among women with remitted MDD compared to controls and was detectable in the AUCg only. Interestingly, the alterations in the cortisol stress response in male patients were opposite with an increased response in the AUCg during a current disease, and there was no difference between remitted MDD versus control males. When comparing all MDD patients in general to controls, the MDD group had a lower cortisol stress response in the AUCi than in healthy individuals, who, in turn, did not differ from current or remitted MDD patients separately in either cortisol stress response measures (Zorn et al., 2017).

Further, there were no differences in cortisol stress responses in patients with an anxiety disorder, including social anxiety disorder (SAD), post-traumatic stress disorder, panic disorder and a mixed anxiety disorder, compared to healthy controls in general. Women with an anxiety disorder had a lower AUCi compared to controls, whereas males with SAD only had a higher AUCg. Women with SAD did not differ from controls (Zorn et al., 2017).

The results suggest that associations between these psychiatric disorders and HPA axis stress responsiveness in adults would be sex dependent. In addition, the disease state being current versus remitted and the used cortisol measure indicator being the AUCi or the AUCg might impact the results. A low number of other anxiety disorders, except SAD among the included studies, did not allow separate meta-analyses to further investigate possible disorder-specific associations with HPA axis functioning (Zorn et al., 2017). It is also problematic to compare all different anxiety disorders as one group to healthy controls, because the anxiety category is heterogenous. In addition, the comorbidity of depression and anxiety is a common phenomenon, therefore differentiating their own associations with the cortisol stress response is challenging. In light of these results, if they turn out to be the true associations with observed directions, it is not surprising that many earlier studies have obtained inconsistent results as the methods have varied, among many other things, in the way the status of the disease, the cortisol stress reactivity measure and sex have been taken into account.

Lopez-Duran et al. (2009) focused on pediatric depression in their meta-analytic review on the HPA-axis functioning studies that had used different methods to activate the HPA axis. Children and adolescents with MDD or dysthymia showed higher basal cortisol levels during the day, in 17 studies, and increased cortisol secretion after a dexamethasone suppression test in 17 studies compared to non-MDD control peers. Instead, ACTH and cortisol secretion did not differ between the groups after CRH administration in 4 studies. As the authors stated, this suggests similar pituitary activation by CRH and adrenal cortex activation by ACTH in both depressive and non-MDD control children but with a less sensitive negative feedback mechanism, with less suppression, for cortisol in the hypothalamus among depressed children. Two studies using a psychological stress test to activate the HPA axis, instead of a pharmacological activation, found a higher reactivity and prolonged recovery in depressed adolescents compared to controls, which could also fit to the possibility of an increased CRH drive from the hypothalamus due to a sensitivity to stress signals and/or insensitivity to negative feedback and recovery. Sex differences were not noted (Lopez-Duran et al., 2009).

Still, based on these kinds of studies, one cannot say whether the altered HPA axis stress responsiveness was a symptom of a disease or a part of the pre-existing mechanism that resulted in a disease. Being sick causes stress in itself and especially chronic illnesses, such as psychiatric disorders, could potentially lead to altered HPA axis stress responses due to the stress it places on everyday life and because of the interactions with other physiological systems that are activated because of the original illness. Potentially, both options could occur at the same time where altered HPA axis stress responses in a certain context with other risk factors would lead to

disease, which, in turn, would further induce changes in the functioning of the HPA axis.

Prospective studies are needed to elucidate the order of the events in these associations, although they cannot inform about the direct cause and effect between HPA axis stress responsiveness and disease. There is increasing evidence from the prospective studies on healthy adults that both blunted and hyperreactive responses of the HPA axis and SAM system to acute psychological stress predict psychiatric and physical health outcomes. Increased HPA axis stress reactivity has been associated with later cardiovascular disease and decreased telomere length. Blunted HPA axis stress reactivity has predicted depression and post-traumatic stress disorder (PTSD) symptoms, greater musculoskeletal pain and regulatory T-Cell percentage, poorer physical disability and lower bone mass (Turner et al., 2020).

The cortisol stress recovery has not been noted often in past studies. A prospective observation in humans regarding cortisol stress recovery from a social stress test showed that flatter cortisol stress recovery predicted the first onset of psychiatric disorders during three years of follow-up in adolescents. The association was smaller and statistically non-significant with anxiety disorders, major depression or dependence disorders separately. Altered cortisol recovery was thus suggested to be a general risk marker for psychopathology instead of being disorder-specific (Nederhof et al., 2015).

Although bringing an important research contribution to the relationship between HPA axis stress response and health, prospective study designs focusing on healthy adults are limited in their capacity to explain the origin of the observed blunted or hyperreactive HPA axis stress response. Adults have already been exposed for decades to various life events and environmental factors that could have contributed to the altered HPA stress responsiveness in addition to the genetic composition of the individual. All the major sensitive periods of development from pregnancy to adolescence have occurred, and retrospective research on all these factors has its well-known restrictions such as a recollection bias (Epel et al., 2018). Consequently, even if these studies have found supporting evidence for the altered HPA stress response being a predictor for the quality of later health, these studies are weaker in informing the potential mechanisms behind the origin of altered HPA axis. That information could be used for the development of preventive actions for the diseases. How the HPA axis stress response becomes altered in, for now, healthy individuals, remains unclear.

2.4.1 Prenatal programming of the HPA axis

To account for the aforementioned limitations, and as the role of early life stress from childhood has been increasingly noted to be an important factor in the

development of stress-related chronic diseases (Heim et al., 2019; Koss and Gunnar, 2018), many longitudinal birth cohorts have been launched to find mechanisms behind the diseases and to better characterize the vulnerable individuals. Especially, the Developmental Origin of Health and Disease (DOHaD) hypothesis by David Barker has gained much attention and shifted the studies from postnatal early life stress of childhood to now account for prenatal stress of the mother and exposed fetus as well. The Barker hypothesis, known also as the fetal programming theory, suggests that signals from the prenatal environment influence the development of an offspring by programming their long-lasting effects into the fetal physiology, which in certain circumstances, increases the risks for diseases later in life (Barker, 1990; Barker, 2007). There are many suggestions about what these prenatal environmental signals might be and how the signals would shape the development of the fetal physiological systems and which systems would be particularly important in further mediating the impact of prenatal environment to the well-being of the offspring.

Above all, based on the vast amount on animal studies and parallel promising results from human studies, increased maternal prenatal glucocorticoid levels seem to play a crucial role as an environmental signal in prenatal programming. The reasoning is that the HPA axis of a stressed, pregnant mother gets activated, and increased levels of maternal cortisol can pass through the placenta into the fetus where it influences the development of different tissues. The most vulnerable target is considered to be the developing brain where the amygdala, hippocampus, hypothalamus and cortex are rich in GRs and MRs. These same areas regulate the functioning of the HPA axis. Thus, prenatal programming of these brain areas by maternal adverse signals might alter the development and functioning of the infant HPA axis and increase the risk for several diseases. Cortisol-GR and -MR complexes act as transcription factors and influence the transcription of many genes and the developing epigenome by demethylation, for example (Moisiadis and Matthews, 2014).

Showing an association between maternal, prenatal psychological stress and cortisol levels has proven to be difficult, and the found associations have been weak. Recently, hair cortisol concentration measures have been utilized with the idea that measuring long-term cortisol accumulation from the hair would be a better indicator for maternal cortisol levels in relation to PPD, which is also a chronic situation, compared to saliva or blood cortisol levels, which measure the cortisol level at the moment. Although the associations between maternal PPD and hair cortisol levels in human studies have not been consistent, partly due to methodological reasons (Mustonen et al., 2018), a consistently high pattern of maternal prenatal depressive symptoms has been associated with increased maternal hair cortisol levels during a pregnancy (Mustonen et al., 2019). Stress responsiveness of maternal HPA axis attenuates, while cortisol levels gradually increase toward the end of a pregnancy

due to a positive feedback loop of the pCRH secretion in humans (see Figure 1 and Section 2.3.1). Resulting in increased levels of pCRH towards the end of a pregnancy regulate the timing of the spontaneous labor and may control the rate of fetal development (McLean et al., 1995; Sandman et al., 2011). For this reason, it is possible that increased maternal prenatal cortisol levels are not the direct, or only, signal per se for the programming the development of the fetus. The functioning of the placenta, e.g., blood flow and transport of nutrients and oxygen, pCRH and the placental 11β-HSD2 enzyme that converts the maternal cortisol to inactive cortisone has an important role in regulating prenatal cortisol and other environmental signals to the fetus (Moisiadis and Matthews, 2014; O'Donnell et al., 2009; Sandman et al., 2011). Preliminary evidence in humans has shown that maternal prenatal dysregulated cortisol levels associate with decreased placental 11β-HSD2 expression, which associates with higher cortisol stress reactivity in two-month-old infants (Jahnke et al., 2021). During stress, placental 11β-HSD2 activity has typically been found to be increased and protect the fetus from stress-induced elevated levels of maternal cortisol levels. It is possible that during chronic stress, the regulation of the 11β-HSD2 enzyme activity might become disturbed and the protective barrier between the mother and her fetus declines (Welberg et al., 2005).

Other possible biomarkers mediating maternal prenatal stress on offspring development and health are increased levels of pCRH; maternal catecholamines, such as adrenalin, noradrenalin of the SAM system and an altered balance of immune system markers, such as increased pro-inflammatory cytokines and low levels of anti-inflammatory cytokines, which can pass the placenta and influence the fetus. All these systems interact with each other, therefore, it is possible that maternal prenatal cortisol has some role also in these pathways. In addition, a stressed pregnant mother might smoke and use alcohol more commonly, have sleeping problems, a less healthy diet and worse physical activity compared to non-stressed mothers. Especially pCRH in maternal circulation integrates her various stress signals, not limited to PPD only, such as nutritional deprivation, immune markers and hypertension (Beijers et al., 2014; Howland et al., 2017; Pearson et al., 2015; Sandman et al., 2011).

Observations from birth cohorts during the last 10 years have supported the DOHaD hypothesis by finding associations between prenatal depressive, anxiety and stress symptoms of the mother and the emotional and behavioral problems and depression in their offspring (Betts et al., 2015; O'Donnell et al., 2014; Pearson et al., 2013; Van den Bergh et al., 2008). Associations between maternal PPD exposure and the development of immune-related disorders, such as an increased risk of childhood asthma, allergic diseases, recurrent respiratory infections and atopic disorders, have also been found (Andersson et al., 2016; Flanigan et al., 2018; Korhonen et al., 2019; Zijlmans et al., 2017).

Knowledge of the mechanisms behind the observed effects in humans is scarce. Some evidence supporting the hypothesis that altered HPA axis functioning of the offspring might mediate that association between PPD exposure and disease later in life in humans have been found. A flattened diurnal cortisol profile in adolescents has been associated with maternal prenatal anxiety exposure, and this altered HPA axis functioning associated with depressive symptoms in females (Van Den Bergh et al., 2008). There are no similar longitudinal human studies on cortisol stress responsiveness from infancy onwards to show that altered HPA axis stress responsiveness in PPD-exposed infants would mediate an increased risk for psychiatric or other diseases.

2.5 Prenatal psychological distress exposure and the infant cortisol stress response

In research that concentrates on the role of prenatal stress exposure in the HPA axis development and functioning, young infants are a preferred study population to minimize the confounding effects of the postnatal factors such as maternal postnatal psychological distress and quality of care and attachment (Gunnar and Donzella, 2002). At least two systematic reviews exist that cover older, related studies until 2010 (Hunter et al., 2011; Pearson et al., 2015), therefore focusing the review of the literature to the latest infant studies with the similar exposure than what was used in our studies is reasonable. The previous systematic reviews have supported the association between PPD exposure and altered infant cortisol stress responsiveness, which most commonly have been increased reactivity from neonates to six-year-olds, but with the typical commentary that heterogeneity among the studies limits conclusions and further studies are needed.

Twelve studies on infants or young children exposed to maternal PPD have been conducted to characterize the possible alterations in their cortisol stress responses during the last 10 years. The summary of the studies is listed in Table 1. Despite concentrating on the similar studies than ours, with exposure type and/or infant age, the studies are quite heterogenous in their study design and other characteristics including severity and quality of the PPD exposure, sample size, characteristics of the mother and the child, stressor used to elicit the infant cortisol stress response, timing of cortisol sampling, definition of the cortisol stress response indices and statistics.

The most common finding among the PPD-exposed infants has been the increased cortisol stress reactivity or total cortisol output after a stressor (Fernandes et al., 2015; Giesbrecht et al., 2017; Osborne et al., 2018; Stroud et al., 2016; Tollenaar et al., 2011; Yong Ping et al., 2015). Decreased cortisol reactivity in PPD-exposed infants has also been reported (Galbally et al., 2019; Tollenaar et al., 2011).

In some studies, the elevated cortisol stress response in PPD-exposed infants was observed only in the context of low level of prenatal partner support (Luecken et al., 2013) or only among infants with higher temperamental negativity (Luecken et al., 2015). In one study, a higher cortisol stress response was observed among exposed female infants, when higher maternal PPD was combined with a lower maternal prenatal cortisol awaking response (CAR). Instead, a blunted female infant cortisol stress response was observed when a higher PPD was combined with a higher maternal prenatal CAR (Giesbrecht et al., 2017). A few studies have not observed the association between PPD and the infant cortisol stress response (Braithwaite et al., 2016; Davis et al., 2011), some where the association has been somewhat obscure (Grant et al., 2009) or some studies did not observe the association among all the infant age groups studied (Osborne et al., 2018; Tollenaar et al., 2011). Based on the studies, the results seem to be context specific. To better understand the role of maternal PPD in the shaping of the development of the infant cortisol stress response, one needs to understand the differences between the studies, in the context, and how those differences potentially explain the differences in the observed results.

2.5.1 Type of PPD exposure

The severity and quality of the PPD in these studies varies from the different levels of prenatal depressive, general anxiety and pregnancy-related anxiety symptoms and daily hassles to the diagnosed anxiety and major depressive disorders. Although the number of different kind of symptoms measured was not that many, several different instruments for these measurements were used. Evaluations were mostly based on maternal self-reports, but four studies used a more objective assessment that was based on a structured interview. Individuals differ in their capacity and sensitivity to observe and verbalize their own state of mind, and each use their own subjective scale to determine which level of symptoms one determines as high or low. Questionnaires also differ in how much they concentrate on the physical symptoms compared to psychological ones and how long period of time they cover. For example, the Profile of Mood States self-report consists of questions concerning feelings during the previous 30 minutes, whereas the Edinburgh Postnatal Depression Scale (EPDS) covers the latest week. Some instruments, such as the Pregnancy-Related Anxiety Questionnaire (PRAQ), are not limited to a specific shorter time frame but to the overall situation, being pregnancy. Although PPD exposures included here are narrowly defined, it is likely that the quality of the experienced PPDs by the mothers and hence the quality of the infant exposures are heterogenous among the studies and partly explain the variations in the results.

2.5.2 Timing of PPD exposure

The existing research has not found support for the certain sensitive prenatal period for the fetal development concerning the HPA axis stress responsiveness. Although the third trimester has been the most common and often the only measurement time point for the maternal PPD, two measurement time points during pregnancy or even all three trimesters have been covered in few studies. The first trimester at less than 14 gwks has been studied at least in this regard. Studies that measured maternal PPD longitudinally from early to late pregnancy suggest that both early and late pregnancy are potentially a sensitive time for the influences of the PPD exposure to the development of the infant HPA axis. Giesbrecht et al. (2017) found a stronger association between early pregnancy (gwk 15), PPD exposure and infant cortisol stress response compared to later PPD exposure (gwk 33), although the role of timing was minor in general. In their study, the higher maternal PPD alone at gwk 15 was associated only with the higher infant baseline cortisol, not reactivity, in females. A three-way interaction between PPD, maternal prenatal CAR and infant sex associated with infant cortisol stress reactivity only at gwk 15. Galbally et al. (2019) reported an association with lower infant cortisol in the AUCg with the PPD exposure during the third trimester only, although they had measured PPD also at gwk of less than 20. They stated that maternal anxiety and depressive symptom levels were relatively stable across pregnancy, therefore it could have been also a matter of choice for their cross-lagged panel models. Similarly in other studies that measured only the late pregnancy maternal PPD and found the association between the infant PPD exposure and cortisol stress response, it is possible that they could have observed similar associations also from earlier pregnancy time points if the level of PPD experienced by the mother was relatively stable. An exhaustive longitudinal maternal PPD characterization with five measurement time points from gwks 15 to 37 by Davis et al. (2011) did not find any associations between PPD and cortisol stress response among neonates. The PPD exposure throughout a pregnancy associated only with a slower rate of behavioral recovery that was also measured. Instead, higher maternal prenatal plasma cortisol levels during the late second and third trimesters predicted the elevated neonate cortisol stress response. The physiology of the neonates differs in many ways from the older infants (Sharma et al., 2011), and the age (13–35 hours after birth) of study subjects might partly explain the absence of the association between PPD exposure and cortisol stress response, if there were to be one.

It is very challenging to define whether there is a certain sensitive time during a pregnancy when the possible effects of the PPD exposure to the infant cortisol stress response would be the most influential based on the existing studies. Defining an actual time of PPD exposure is somewhat problematic because the psychological distress is not usually strictly restricted to a certain time window. It is more likely

continuous, and it might not be always possible to determine when it starts and ends. The level of PPD seems to be stable across a pregnancy among the majority of the mothers although trajectories with increasing and decreasing levels of symptoms in some pregnant mothers have also been observed (Korja et al., 2018; Lahti et al., 2017). In the study of Yong Ping et al. (2015), floods in Iowa were an excellent opportunity to explore the role of PPD exposure in maternal PTSD-related symptoms and timing as the mothers were random at different trimesters of a pregnancy when the natural disaster occurred. Researchers found that the second half of apregnancy was more critical for HPA axis development, as the later PPD exposure associated with the higher infant cortisol stress response (AUCi). The timing explained 5.2% of the variance in the infant cortisol stress response that could not be explained by the objective stress being the impact of the flood on surroundings or subjective PPD exposure. The authors stated that they cannot exclude the possibility that the observed effect was partly due to the stress that most likely continued during the postpartum period. The children were 2.5 years old at the time of cortisol stress response measurements, and they had been living their early life in the environment affected by the flood.

In the study of Stroud et al. (2016), cortisol stress responses of the infants from the maternal prenatal MDD group was compared to the pre-conception-only MDD- and control groups. This way, the researchers could separate, at least to some degree, the effects of lifetime depressive episodes before a pregnancy, i.e., the possible effects of MDD on the maternal pre-pregnancy physiology and the genetic factors associated with the vulnerability of depression from the effects of prenatal major depressive episodes on the infant cortisol stress response. Although this study did not shed light on the question about the sensitive period for the prenatal programming of the infant HPA axis within a pregnancy, its results support the hypothesis that pregnancy, as such, is a sensitive period for the development of the infant cortisol stress response. This is in line with the results from the systematic review on human prospective studies concerning maternal prenatal stress and several infant outcomes where no specific vulnerable timing for the prenatal stress were noted (Van den Bergh et al., 2020). Instead, the review stated that the different associations observed between prenatal stress exposure and various infant outcomes, such as neurodevelopmental, cognitive, behavioral, affective self-regulation and mental health outcomes, might depend on the developmental stage of the different biological systems of the fetus, such as central nervous, immune and stress regulation systems, when the prenatal stress occurs. The number and type of existing studies on cortisol stress response in PPD-exposed infants does not allow the conclusion as to whether the certain type of alteration in the infant cortisol stress responsiveness would depend on the timing of the exposure.

2.5.3 The cortisol reactivity in PPD-exposed infants

The direction of the association between the infant PPD exposure and the cortisol stress reactivity has varied among the studies. Out of 12 studies reviewed here, six studies have reported increased cortisol stress reactivity (Fernandes et al., 2015; Giesbrecht et al., 2017; Luecken et al., 2013; Osborne et al., 2018; Stroud et al., 2016; Yong Ping et al., 2015), and one study has reported decreased cortisol stress reactivity (Galbally et al., 2019) in PPD-exposed infants compared to less- or non-exposed infants. Two studies have observed both reactivity types in their sample, where the type of reactivity was dependent on the other factors, such as the combination of age of the infant and the type of infant stress test (Tollenaar et al., 2011) or the combination of different maternal prenatal cortisol levels with the PPD (Giesbrecht et al., 2017). No association was found in two studies (Braithwaite et al., 2016; Davis et al., 2011), and one study could not analyze the association of the PPD with infant cortisol stress reactivity because of the lack of statistically significant cortisol reactivity to the stressor (Luecken et al., 2015). Instead, the study reported total cortisol secretion during the study visit in the AUCg. Although the increased infant cortisol stress reactivity has been found to be the most common reactivity type among the PPD-exposed infants, it seems that the association between the PPD exposure and infant cortisol stress reactivity is not that straightforward.

As the study designs are heterogenous, it is challenging to point out any shared characteristics within the studies that have observed the increased infant cortisol stress reactivities, and, on the other hand, within the studies of decreased reactivities as well as differences between the studies of opposite results to explain the observed results.

The type of the PPD exposure, age of the infant and stressor that have been used to elicit increase in infants' cortisol levels, among the many other things, have varied greatly among all the studies that have reported increased cortisol reactivity. This might mean that the association of the PPD exposure with the increased infant cortisol reactivity is not strongly prenatal exposure type, infant age or stressor specific. Instead, different types of PPD exposures, such as depressive, general anxiety, PTSD and pregnancy-related anxiety symptoms, could increase the likelihood of having increased cortisol stress reactivity for variety of stressors, both physical and psychological, in infants of 1–30 months of age.

The association between the PPD and decreased infant cortisol stress reactivity or total stress response in the AUCg has been observed in two studies that both have used a mother-infant separation test for the 12-month-old infants exposed to either maternal prenatal depressive and anxiety symptoms (Galbally et al., 2019) or pregnancy-related anxiety on fear for bearing a handicapped child (Tollenaar et al., 2011). Separation from the primary caregiver is a well-known and strong stressor for the healthy infant that typically evokes the most consistent cortisol increase among

the psychological stressors used during infancy (Puhakka and Peltola, 2020). Galbally et al. noted that a decreased infant cortisol stress response in the AUCg is consistent with the cortisol stress response profiles associated commonly with chronic, not acute, stress exposures such as ongoing stress, PTSD and other stress-related illnesses. Decreased HPA activity has been associated with conditions such as allergies, asthma, chronic fatigue syndrome, fibromyalgia, other chronic pain syndromes and rheumatoid arthritis (Chrousos, 2009; Heim et al., 2000). In light of this, one might also expect to see the decreased infant cortisol stress response during the mother-child separation test among the 30-month-old infants exposed to prenatal PTSD symptoms in the context where the families had lived in the surroundings affected by the flooding, but this study found an increased infant cortisol reactivity (Yong Ping et al., 2015). In addition, in the majority of infant cortisol stress reactivity studies that contain a PPD exposure, the exposure could be considered as chronic, but still a majority of the studies have found increased infant cortisol stress reactivity. Chronic stress exposures during one's lifetime has commonly been associated with decreased cortisol stress reactivities, but these studies have mostly been performed in adults. It could be that chronic stress exposure during the fetal development might associate differently with cortisol reactivity and lead also to increased cortisol reactivity.

In addition to the observed decreased cortisol stress reactivity at 12 months of age, Tollenaar et al. (2011) also found decreased cortisol stress reactivity to the vaccination at the age of two months. Other studies that have used vaccination as a stressor for two-month-old infants have observed an U-shaped association, where low and high depressive symptoms associated with increased cortisol stress reactivity (Fernandes et al., 2015) or have not found differences in cortisol reactivity between infants of mothers with depression (Osborne et al., 2018) or higher depressive symptoms (Braithwaite et al., 2016) and control infants. Possible reasons behind the negative findings are discussed in more detail in Section 2.5.6. A study sample of Fernandes at al. (2015) differs from other Western studies in that it is drawn from the rural, developing world setting in South India with socioeconomically disadvantageous families and with different genetic backgrounds. The authors suggested that, because in that environment the everyday life is moderately stressful and more stressful compared to that in a Western society, the best prenatal environmental match with that postnatal environment for the developing fetus is the pregnant mother with a moderate level of stress, i.e., depressive symptoms, compared to a low or high level of stress. Thus, those infants exposed to a moderate level of PPD present with lower cortisol stress reactivities. On the other hand, other infant studies have published only linear associations between the PPD exposure and infant stress reactivity, therefore it is not known whether a similar U-shaped association could be found in Western samples.

When the direction of association between the PPD exposure and infant cortisol stress reactivity has depended on the age of the infant and stressor type in one study (Tollenaar et al., 2011), it seems that the direction might also depend on the magnitude of the maternal cortisol awaking response (CAR) and steepness of diurnal cortisol slope during pregnancy. As Tollenaar et al. commented in their paper while attempting to explain their results with both decreased and increased infant cortisol stress reactivities in PPD-exposed infants, there is a challenge in interpreting the results in that it is not known what should be considered as an adequate amount of cortisol stress reactivity and in which context.

2.5.4 Infant age and cortisol stress reactivity

The best way to evaluate the role of infant age in the association between PPD exposure and infant cortisol stress reactivity is through longitudinal studies that allow within individual comparisons. There are only two earlier longitudinal studies on infant cortisol stress responses in the context of PPD exposure. Tollenaar et al. (2011) tested the same infants four times during the first year of life and used four different age-appropriate stressors. Exposure to maternal PPD associated with increased cortisol stress reactivity in five-week-olds, when bathing, but lower cortisol stress reactivity at the ages of 8 weeks, with vaccination, and at 1 year with separation. At five months, no association was found with a still-face test (Tollenaar et al., 2011). A second longitudinal study did not observe any difference in cortisol stress reactivity to vaccination at 2 months of age between infants of prenatally depressed mothers and controls, but exposed infants had increased reactivity to the same vaccination stressor at 1 year of age (Osborne et al., 2018). As both studies reported also negative findings with no associations, which might relate to the age of the infant, both topics are reviewed in more detail in Section 2.5.6.

Both studies measured infants at the age of 1 year with opposite results. Vaccination is more physical, with pain, and is a rapid stressor, whereas separation from their own mother is a psychosocial stressor with a somewhat longer procedure duration. Differences due to stressor were already discussed in a previous section. In addition to that, differences in the associations could be related to the partly different brain areas that are activated due to various stressors and different developmental rates of these brain areas. More physical stressors and sensations from the body activate the HPA axis by ascending tracts via the brainstem. The frontolimbic areas are involved in anticipation or perception of stress and are activated in psychosocial stress. These areas develop slower (Engel and Gunnar, 2020).

To differentiate the role of PPD exposure, infant age and stressor type in the variation observed in the infant cortisol stress responses, the same stressor should be used in a longitudinal study design. In order to clarify the role of PPD exposure to

the development and functioning of the infant cortisol stress responses, Studies II and III, in which the same stressor was applied to infants of different ages, were conducted, and results are presented in this thesis.

2.5.5 The cortisol recovery in the PPD-exposed infants

The majority of the infant cortisol stress response studies have focused on the reactivity phase of the response. There are few publications on the recovery phase of the infant cortisol stress response. In a few studies, there might have been the possibility to analyze or at least discuss about the possible relevance of the PPD exposure to the infant cortisol recovery as well, but these are lacking. For example, the infant cortisol stress response curves of the low and high level PPD exposure groups show that the steepness of the recovery slopes are quite different (Luecken et al., 2013, Figure 1). This study concentrated on the saliva cortisol stress reactivity only. Tollenaar et al. (2011) measured infant cortisol concentrations at 25 and 40 minutes after the stressor in four different age groups and observed a peak response at 25 minutes in 74.5–85.8% of infants depending on the group. This means that for most of the infants, 40 minutes are a sufficient time to initiate the recovery phase of the cortisol stress response. This is unfortunate, as this study missed an opportunity to study maternal PPD associations with the infant cortisol recovery from various stressors at the different ages in addition to cortisol reactivity. Similarly, although two or more post-stressor cortisol samples were measured, only the difference between one post-stress and baseline cortisol levels, i.e., cortisol reactivity or total cortisol secretion (AUC), was analyzed in many studies (Braithwaite et al., 2016; Galbally et al., 2019; Giesbrecht et al., 2017; Stroud et al., 2016).

Three studies included all the collected infant post-stress cortisol measurements and the change from the baseline in their analyses and explored the association of this change with the PPD exposure. Davis et al. (2011) did not find associations between the neonate cortisol stress reactivity (20 min-baseline) or recovery (40 min-baseline), as they defined the cortisol indices, and maternal prenatal depressive or anxiety symptoms or stress. They did not report the neonate cortisol concentrations as such, but the cortisol profiles in the Figure 2 suggest that the average cortisol levels did not change between 20 and 40 minutes. They also reported statistically significant differences between the baseline and both the 20- and 40-minute post-stress cortisol but did not mention anything about the possible difference between the 20- and 40-minute cortisol levels, which thus probably did not exist. It might be that the heel-stick blood draw with median duration of four minutes was such a strong stressor that it elicited a strong and long-lasting cortisol stress response among neonates that recovery did not occur during the time frame in question.

Grant et al. (2009) found a statistically significant interaction between the maternal prenatal anxiety diagnosis and overall pattern of cortisol stress response of the infant. Infants of prenatally anxious mothers had a non-significant and small increase in their cortisol levels between the last two measurements at 25 and 40 minutes after the stressor, whereas the control infants exhibited a significant decrease during that time. Otherwise, the cortisol slopes between the other sampling times or separate cortisol concentrations did not differ between the anxiety-exposed and control infants. For these reasons and because of the difficulty to explain the observations, the authors concluded that the result could be a chance finding. In general, the majority of the infants tend to reach the stress reactivity peak cortisol levels during the first 15–30 minutes after a stressor (Kirschbaum and Hellhammer, 1989; Ramsay and Lewis, 2003), but it can take as long as 40 minutes (Goldberg et al., 2003). In accordance with this, the infants in the study of Grant et al. (2009) reached their peak response at 15 minutes (N = 31), 25 minutes (N = 30) or 40 minutes (N = 27) after the stressor, which means that among 69% of the infants, the cortisol levels were possibly recovering from the stressor during 25–40 minutes after the stressor. From this point of view, the study might have captured the difference in the cortisol recovery slope between the prenatal anxiety-exposed infants and their controls. On the other hand, only 46% of the infants were classified as being a stress responder, and there was no group level cortisol increases after the stressor. Therefore, the sample was not necessarily suitable for the analysis of the recovery from the stressor when there was minimal reactivity to the stressor initially.

Finally, Yong Ping et al. (2015) observed a 40% increase in cortisol levels between baseline and 20 minutes in the post-stress sample and a 63% increase between baseline and 45 minutes in the post-stress sample in 2.5-year-old children during the mother-toddler separation test. As the mean cortisol levels continued to increase through the measurement period, there were no possibilities to analyze the cortisol recovery from the stressor.

Based on the reviewed studies among the infants exposed to the variety of maternal PPD, little is known about the infant cortisol recovery from the acute stressor. Among some studies, the absence of the cortisol stress recovery phase during the cortisol measurement time window, which has been 45 minutes at maximum, has not allowed the analysis of the association between the PPD exposure and cortisol recovery. Still, in some studies, the typical recovery has been observed, but no attention has been paid to that part of the cortisol stress response. The study of Davis et al. (2011) is the only study that mentions the cortisol recovery in their publication, which suggests that the lack of infant cortisol recovery research is not solely due to the lack of appropriate data. Rather, the focus of the research in general has not been in the recovery. Clearly, more research is needed on the topic.

2.5.6 Findings of no association

There are always studies with findings of no association, but fortunately those studies are also published because they contain useful and important information as well. The lack of association can be found if the hypothesis about the association between infant PPD exposure and cortisol stress response is not valid, or when the study design, including power, has not been able to detect the association. In both cases, the information is valuable as it helps to formulate more accurate hypotheses and to conduct research with better quality.

Among the findings of no association in the cortisol stress response of PPD-exposed infant studies is a study by Braithwaite et al. (2016). The reasons for the lack of observed differences in the infant cortisol stress responses between the PPD exposure and the control groups, assuming that there should have been one, were probably the combination of a relatively small final total sample size, unbalanced group sizes with a very small case group from a low-risk community sample and a possibly large variation in baseline sampling. The authors state that based on their power calculations to detect a significant difference between the two infant groups with a p value of less than .05 and with a 90% probability, they would have needed 64 participants. Indeed, the final infant cortisol analyses contained 71 infants but only 13 (18%) from the PPD-exposure case group, i.e., infants of mothers with a greater than 10 EPDS total sum scores prenatally. Power calculations were based on the author's previous study (Murphy et al., 2015) on the saliva cortisol stress responses of the pregnant mothers. It is unlikely that power calculations based on a sample from a pregnant mother could reliably give the needed sample size among the infants. Variation in the infant saliva cortisol levels is larger than among adults (Kiess et al., 1995), therefore it is likely that a larger sample size is needed in infant studies to detect the weak associations commonly reported between PPD exposure and infant cortisol. Ideally, power calculations should be based on approximately similarly aged infants because the variation in the cortisol levels diminishes as the infant ages (Kiess et al., 1995). In addition, HPA axis functioning changes considerably during pregnancy, and if there were the absence of comparable infant studies, non-pregnant adults would be a more reliable group for reference. Lastly, Braithwaite et al. (2016) described that the baseline cortisol samples were collected by the mothers at any time during the day before the inoculation, which was the stressor for the infant. The time of day for the baseline sampling was not controlled for in the analyses, and the possible variation in the collection time was not reported. For any measurements where the change from the baseline is the focus, it would be important to standardize the baseline sampling in the timing, environment, etc. if possible. Particularly, when the measurement is as fluctuating and prone to the effects of the environment as the level of a single cortisol measurement is known to be. Standardizing the cortisol baseline sampling decreases the background noise

from the measurements, which increases the likelihood that the signal, i.e., differences in the cortisol reactivity from the baseline between individuals, will be detected.

Tollenaar et al. (2011) reported an association between maternal prenatal fear of bearing a handicapped child and infant cortisol stress reactivity among the three out of four infant age groups that were studied. They had selected age-appropriate stressors for each age group, such as physical discomfort or mild pain for the younger infants and psychological stressors for the older infants. The stressor for the infants at the age of five months was the Still-Face procedure, being interaction disruption, which did not evoke a cortisol stress response at the group level and only 49 (41%) infants from the group of 119 responded with increased cortisol levels. This might have limited the capacity to observe the possible association between PPD exposure and infant cortisol stress reactivity at the age of 5 months compared to other age groups where the association was observed. Similarly, another study that used the Still-Face as a stressor for seven-month-old infants exposed to prenatal maternal anxiety and their controls did not observe group level cortisol increase to the Still-Face (Grant et al., 2009), and only 46% of infants presented with an increase in their cortisol levels during the Still-Face. This suggests that the Still-Face procedure does not invoke consistent cortisol stress reactivity among the infants of five to seven months of age. This is in line with the recent meta-analysis of cortisol reactivity to psychological stressors in infancy where only the parental separation was found to trigger a consistent cortisol increase among infants compared to the Still-Face procedure, novelty and frustration (Puhakka and Peltola, 2020).

In two studies where the association between maternal PPD and infant cortisol stress reactivity have been measured twice because of the repeated PPD measurements or cortisol stress tests during infancy, only one of the associations has been positive. Galbally et al. (2019) was discussed in Section 2.5.2. Osborne et al. (2018) did not find any differences between the cortisol stress reactivity in two-month-old infants of mothers with prenatal MDD and their healthy controls. Both groups of infants reacted to the vaccination with elevated cortisol at the same magnitude. At the age of 12 months, the MDD-exposed infants again reacted with the elevated cortisol stress response to the vaccination, whereas control infants did not react anymore. In this case, the negative result with no association at 2 months was not necessarily against the hypothesis that PPD associates with increased infant cortisol stress reactivity but might have supported earlier findings of typical infant cortisol stress reactivity development, such as the dampening of the cortisol reactivity to the mild pain during the first year of life among healthy infants (Davis and Granger, 2009; Tollenaar et al., 2010). The results suggest that the PPD exposure might have altered this development, as the postnatal maternal depression and other confounders did not explain the results. On the other hand, other studies have

reported associations between maternal PPD and infant cortisol reactivity to vaccination at the age of two months showing that the fear of bearing a handicapped child predicted decreased infant cortisol stress reactivity (Tollenaar et al., 2011) and that low/high prenatal depressive symptoms predicted higher infant cortisol stress reactivity with an U-shaped association (Fernandes et al., 2015). Differences between the study samples, such as ethnicity and type and severity of the PPD, might explain the differences in the results. There is a chance for false-positive findings among the research as well. It is also possible that in these studies that presented negative findings, the maternal PPD did not predict the infant cortisol stress response. Not all individuals are affected by the PPD exposure.

2.5.7 Protective and risk factors for the influences of PPD exposure

Although not always reported, there might have been resilience factors among the studied samples that have protected the offspring from the influences of the PPD exposure. Likewise, in some cases, the association of the PPD exposure with the infant cortisol stress reactivity has been detectable only when additional vulnerability or risk factors are present. These factors might relate to the characteristics of the mother or the infant, such as in their genetics, epigenetics, low socioeconomical status and temperament or quality of the environment being parenting, breastfeeding and parental relationship and support.

Two studies from Luecken et al. (2013, 2015) give interesting examples of both. In their first study among a low-income, Mexican-American minority population, the researchers showed that higher prenatal subjective stress was experienced when the family income is perceived insufficient and also predicted higher cortisol stress reactivity for six-week-old infants of mothers who reported low partner support during pregnancy relative to infants of mothers reporting higher partner support or lower economic stress. There was no main effect of PPD on infant cortisol reactivity. In their next study on 12-week-old infants, higher prenatal maternal depressive symptoms were associated with higher total cortisol secretion in the AUCg among infants with higher temperamental negativity compared to infants with low temperamental negativity. With low PPD exposure, infant cortisol levels did not differ based on the temperamental negativity. An unfortunate limitation of the later study was that mother-infant interaction tasks, which were used as a stressor, did not evoke a statistically significant cortisol stress response in infants.

Table 1. Studies on cortisol stress response among the infants exposed to maternal prenatal psychological distress.

STUDY	N (CASE/ CNTR)	PPD EXPOSURE MEASUREMENT	PPD TIMING (GWKS)	INFANT AGE	STRESSOR	SALIVA SAMPLING TIMES	CORTISOL INDEX, STATIST.	RESULTS
1. Grant 2009	88 (17/71)	MINI-Plus interview for anxiety	35–39. Interview for last 6 month	7 mo	Still-face procedure	BL, 15, 25, 40 min	Mixed models	Cortisol response at 25-40 min related to maternal prenatal anxiety.
2. Tollenaar 2011	173	STAI (state subscale), Daily Hassles, PRAQ-R, PES	37	5 wks 8 wks 5 mo 12 mo	Bathing, vaccination, still face, maternal separation	BL, 25 and 40 min	20–30 min cortisol-BL	↑ fear of bearing a handicapped child related to ↑ cort reactivity at 5 wks, ↓ cort reactivity at 8 wks and 12 mo. No association at 5 mo.
3. Davis 2011	116	STAI (state subscale), PSS, CES-D	15, 19, 25, 31, 37	24 hours	Heel-stick	BL, 20 and 40 min	20 min-BL, 40 min-BL, Mixed models	No association.
4. Luecken 2013	220	EHS	35	6 wks	Mother-infant interaction tasks	BL, 0, 20 and 40 min	Mixed models	↑ prenatal economic stress associated with ↑ cortisol reactivity only with ↓ partner support.
5. Luecken 2015	322	EPDS	35	12 wks	Mother-infant interaction tasks (did not elicit sig. cortisol reactivity)	BL, 0, 20 and 40 min	AUCg, regression analyses (MPlus)	↑ depressive symptoms predicted ↑cortisol in infants with ↑ temp. negativity, but ↓ cortisol among infants with ↓ temp. negativity.
6. Fernandes 2015	133 (58 at 2 mo)	EPDS, K10	35	2 mo	Vaccination	BL and 20 min	20 min-BL	U-shaped association: low/high EPDS quintiles had ↑ cort reactivity
7. Yong Ping 2015	94	Subjective (IES-R) and objective stress (IF100) to Iowa Floods	1. trim. (26%) 2. trim (39%) 3. trim. (35%)	30 mo	Mother-toddler separation	-45, 0 (BL), 20, 40 min	20 min-BL, 45 min-BL, AUCi, AUCg. Mix model.	↑ Obj. / sub. PPD related to ↑ AUCi in whole group. ↑ Sub. PPD related to ↑ AUCi and cort 40 min-BL in females only.

STUDY	N (CASE/ CNTR)	PPD EXPOSURE MEASUREMENT	PPD TIMING (GWKS)	INFANT AGE	STRESSOR	SALIVA SAMPLING TIMES	CORTISOL INDEX, STATIST.	RESULTS
8. Braithwaite 2016	88 (21/67)	EPDS (>10 scores)	190. day (104.–281.)	68 days	Vaccination	BL, 0, 20 and 40 min after	Repeated ANOVA	No associations
9. Stroud 2016	153 (64/39/ 50)	MDD based on SCID-I/NP: control, prenatal MDD and preconception-only MDD groups.	23, 30, and 36	1 mo	Handling for NICU Network Neuro-behavioral Scale	BL, 0, 20 and 40 min	BL and AUC	Females: prenatal MDD exposed had 51% ↑ BL and 64% ↑ AUC vs. controls, and 75% ↑ AUC vs. preconception-only MDD
10. Giesbrecht 2017	236	short version of the POMS	15 and 33	3 mo	Blood draw	BL, 5, 20 and 40 min	Mixed models	Females exposed to ↑ distress and ↓ CAR or daytime cortisol slope showed ↑ cort responses. Females exposed to ↑ distress and ↑ CAR or daytime cortisol slope showed ↓ cort response.
11. Osborne 2018	106 (49/57)	MDD based on SCID I-CV	25	2 and 12 mo	Vaccination	BL and 20 min.	Mixed design ANOVA	2 mo: no association 12 mo: ↑ cort levels in case infants vs. controls
12. Galbally 2019	241 (51 /190)	SCID-IV, EPDS, STAI (state subscale), stressful life events	< 20 gwks and 3. trimester	12 mo	Mother-infant separation	BL, 20 and 40 min	AUCg, AUCi. Cross-lagged panel models	↑ depressive and anxiety symptoms at 3. trim. associated with ↓ AUCg

CNTR = control; GWK = gestational week; BL = cortisol baseline before a stressor; M.I.N.I. = The Mini International Neuropsychiatric Interview; PES = Pregnancy-specific daily hassles by Pregnancy Experience Scale; EHS = Economic Hardship Scale; PSS = Cohen's Perceived Stress Scale; CES-D = Center for Epidemiological Studies Depression Inventory; STAI = State-Trait Anxiety Inventory; IES-R = Impact of Event Scale-Revised; IF100 = The Iowa Flood 100; K10 = Kessler 10 Scale of Psychological Distress; MDD = major depressive disorder; SCID-I/NP = Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-Patient Edition; SCIDI-CV = Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Clinical version; POMS = Profile of Mood States; EPDS = Edinburgh Postnatal Depression Scale; PRAQ = Pregnancy-Related Anxiety Questionnaire; SCL = Symptom Checklist; CAR = cortisol awaking response.

2.6 Sex differences in the association between PPD exposure and infant cortisol stress response

Compared to human females, male fetuses are more likely to show adverse consequences after a broad range of prenatal adverse exposures, such as environmental toxins, being pesticides, etc. and maternal behavior in their use of opioids and alcohol in many studies on childhood development. Mechanisms behind the sex-dependent vulnerability to or protection from prenatal negative influences are unknown. It is also likely that there is a limit in the protection capacities a sex can offer, and this capacity is probably exposure specific. Strong adversities most likely affect the development of both sexes (DiPietro and Voegtline, 2017).

Animal studies have shown sex differences in the outcomes of offspring after prenatal stress exposures. Exaggerated cardiovascular and HPA axis stress responses and anxious and depressive behavior have been reported in females, but changes in memory and learning among males have been more common (Glover and Hill, 2012). Somewhat similar to animal studies, human females exposed to prenatal stress, such as PPD, stressful life events, cortisol and placental CRH, have more commonly had increased fearful or reactive behavior and alterations in brain structures. Human males have expressed maturational delays and decreased negative emotionality and reactivity (Sandman et al., 2013; Sutherland and Brunwasser, 2018).

Despite an increasing number of neurodevelopmental and behavioral studies on prenatal stress-exposed children are taking sex differences into account, sex differences in the associations between PPD exposure and infant cortisol stress responsiveness are less studied. Carpenter et al. (2017) found higher cortisol stress reactivity in females, but not in males, after prenatal stress exposures, such as asthma, psychosocial stress or glucocorticoid medications or markers of stress exposures, such as low birth weight or preterm birth in 14 out of 23 studies in their systematic review.

From the four studies with PPD exposure, only the study of Yong Ping et al. (2015) had found increased cortisol stress reactivity in PPD-exposed females. Lack of sex-specific associations in the other three studies were suggested to be a result of too broadly defined or mild PPD exposure or that the hormonal response of the pregnant mother to the prenatal stress is needed for sex-specific prenatal programming.

The suggestions by Carpenter et al. might partly explain why only three studies found sex-specific associations out of 12 studies reviewed in the previous Section 2.5. More specifically, sex interaction with a prenatal exposure predicting the infant cortisol stress response was statistically significant in two of the three studies (Yong Ping et al. 2015 and Giesbrecht et al. 2017) suggesting real sex differences. Yong

Ping et al. (2015) found that maternal prenatal PTSD-related symptoms during floods in Iowa associated with a higher cortisol stress response to mother-child separation in females only. Similarly, higher cortisol stress responses were found in female infants of mothers who had reported MDD episodes during pregnancy compared to female infants of mothers who had MDD episodes only before conception and healthy controls (Stroud et al., 2016). Both studies had clearly defined and strong PPD exposures. Further, a higher cortisol stress response was observed in exposed female infants when higher maternal PPD was combined with lower maternal prenatal CAR and a less steep daytime slope. Instead, a blunted female infant cortisol stress response associated with higher maternal PPD, CAR and a steeper diurnal cortisol slope. In male infants, cortisol stress reactivity was independent of the combination of maternal prenatal cortisol and PPD exposure. Interestingly enough, when considering maternal prenatal diurnal cortisol levels only, a smaller CAR and a flatter diurnal slope associated with blunted cortisol stress responses in females but in males, a blunted stress response associated with larger maternal CAR and a steeper diurnal slope (Giesbrecht et al., 2017).

This suggests indeed that maternal PPD and cortisol levels might have independent but also interactive effects on infant cortisol reactivity with different outcomes between sexes. Females seem to be more vulnerable than males to the effects of PPD when the outcome measure is infant cortisol stress reactivity. Still, the number of studies to date is insufficient to make any firm conclusions considering the complexity of the issue.

As there are few publications on PPD exposure and infant cortisol stress recovery in general, the knowledge on possible sex differences in cortisol stress recovery after PPD exposure is also lacking and obviously more research is needed.

Generally, sex differences in various infant developmental outcomes after prenatal stress exposures seem to depend on the type and/or combination of the prenatal adversity and infant outcome in question. The type and combination of risk factors from pregnancy onwards that predict adolescent depression might be sex specific (Maxwell et al., 2018). The prevalence of some psychiatric disorders is not the same among sexes, such as in depression, which is twice as common among females. The difference in this prevalence has been suggested to be partly a result of sex-dependent HPA functioning, which could be programmed differently by PPD depending on sex (Hicks et al., 2019). Partly in line with this suggestion, both female and male adolescents that had been exposed to maternal prenatal anxiety had a higher and flattened cortisol diurnal profile compared to controls but only in females this predicted depressive symptoms (Van Den Bergh et al., 2008). Whether similar associations would exist with the cortisol stress response has not been reported. The developmental trajectory of the cortisol stress recovery during infancy is poorly known compared to better characterized changes in cortisol stress reactivity as

infants age. Finally, the role of age in sex differences in the association between prenatal distress and cortisol stress response among infants is unknown.

2.6.1 Mechanisms

Mechanisms behind the possible sex differences in the associations between PPD exposure and infant cortisol stress response are not known, but placenta and gonadal hormones are two probable explanations with increasing evidence supporting the suggestions. Researchers have observed differences in the regulation and expression of placental genes, proteins and steroids depending on the sex of the fetus. This indicates that the placenta might mediate the maternal stress signals to the developing fetus differently depending on sex (Carpenter et al., 2017; Clifton, 2010).

Further support for the role of the placenta in sex-specific prenatal programming in humans was found by Carpenter et al. (2017) in their systematic review where they reported a lower expression of 11β-HSD2, an increased expression of 11β-HSD1 and alterations in GR expression and localization in female placenta following maternal stress. Maternal prenatal stress was characterized as glucocorticoid medication or asthma, and none of the reviewed studies included the association between maternal PPD and placental outcomes. One small study in humans has shown that maternal depressive symptoms associated with decreased placental 11β-HSD2 expression for the mothers of females only. Decreased 11β-HSD2 expression associated with increased infant cortisol stress reactivity in the whole group. Because cortisol stress reactivities were not reported by sex, it is possible that this smaller study observed sex differences only in placenta samples (Jahnke et al., 2021). Another study was successful in showing associations between decreased placental 11β-HSD2 methylation levels and increased infant baseline cortisol in the prenatal MDD-exposure group. A similar association was not seen in the control group or between the 11β-HSD2 methylation levels and infant cortisol stress reactivity (Stroud et al., 2016). Overall, these findings suggest that glucocorticoid permeability of human placenta increases during maternal prenatal stress, including PPD, in pregnancies with a female fetus. To show that this further associates with altered infants cortisol stress reactivity depending on sex, more research in humans is needed.

2.6.2 Gonadal steroid hormones and the HPA axis

The hypothalamic-pituitary-gonadal (HPG) axis secretes gonadal steroid hormones androgens, estrogens and progesterone, which regulate the functioning of the HPA axis and secretion of glucocorticoids. Similarly, the HPA axis regulates the functioning of the HPG axis and secretion of gonadal steroid hormones. There is an

overlapping distribution of gonadal steroid hormone and glucocorticoid receptors, both GR and MR, in several stress regulating neurons. Glucocorticoid levels increase via activation of estrogen receptor alpha (ERα) but decrease with activated estrogen receptor beta (ERβ) or androgen receptor (AR). In females, estrogen increases CBG levels in blood. Practical examples of the net result of the interactions between the HPA and HPG axes are that glucocorticoids inhibit the HPG axis and suppress reproductive behavior during stress, when the priority is coping from stress and not reproduction (Handa and Weiser, 2014; Oyola and Handa, 2017; Sapolsky et al., 2000). Secondly, cortisol reactivity in healthy adults is dependent on sex, the menstrual cycle, pregnancy, menopause and usage of hormonal contraceptives, which all are situations with altered gonadal steroid hormone levels (Kajantie and Phillips, 2006).

During pregnancy, gonadal steroid hormones might modify the intrauterine environment independently or by interacting with other neuroendocrine hormones, such as glucocorticoids (DiPietro and Voegtline, 2017). After birth, there is a transient surge of gonadal steroid hormones during the first year of infant life (Bae et al., 2019). This includes mini-puberty among infants of 1–3 months of age (Kuiri-Hänninen et al., 2014). At the same time, the infant adrenal cortex experiences other rapid developmental changes. During the first six months, the size of the large fetal adrenal gland decreases because the fetal zone of the adrenal cortex disappears by apoptosis. DHEA and DHEAS secretion decreases and reaches the low levels seen in infants from six months onwards (Kamin and Kertes, 2017). Later in childhood, during adrenarche, DHEA and DHEAS secretion starts again from the developing zona reticularis of the adrenal cortex (Auchus and Rainey, 2004). Effects of DHEA are often opposite to the effects of cortisol, and they both regulate stress responses (Kamin and Kertes, 2017). In addition to that, gonadal steroid hormones, DHEA and glucocorticoids modulate the functioning of each other, and they also influence the development of the brain. In turn, the brain circuits regulate the activity of the HPA and HPG axis as the origin of the both axes are in the hypothalamus (Handa and Weiser, 2014; Oyola and Handa, 2017).

It should be expected that if functioning of this complex neuroendocrinological system is altered by PPD, it would result in alterations in cortisol stress responses. Further, the developmental stage or age would be expected to moderate the association between PPD and cortisol stress response considering the vast development that occurs in the infant HPG and HPA axes, adrenal cortex and brain during the first year of life.

3 Aims

We studied both the role that maternal PPD plays in the development and functioning of the cortisol stress response during infancy and when the infant immune response is challenged due to viral infection. Saliva cortisol was used as a marker for the functioning of the developing HPA axis at the age of 10 weeks, 6 months and 14 months. Maternal PPD was determined by self-reported depressive-, general anxiety- and pregnancy-related anxiety symptoms assessed at gestational weeks 14, 24 and 34. The presence of rhinovirus was a marker for the activated infant immune response.

Specifically, our aims were to study:

1. The association between maternal PPD and infant cortisol stress reactivity, and whether the association is different in the presence of subclinical rhinovirus infection at the age of 10 weeks (Study I).
2. The sex differences in the associations between maternal PPD and infant cortisol stress reactivity and recovery phases of the cortisol stress response at the age of 10 weeks (Study II).
3. The age-dependent changes in the associations between maternal PPD and infant cortisol stress reactivity and recovery phases of the cortisol stress response at the ages of 10 weeks, 6 months and 14 months (Study III).

We hypothesized that PPD exposure is associated with:

1. Altered infant HPA axis reactivity which is especially evident during concurrent subclinical rhinovirus infection (Study I).
2. Increased cortisol reactivity, as a steeper reactivity slope, and/or slower cortisol recovery, with a less steep recovery slope. These associations are moderated by sex, so that the effect is observed only in females (Study II).
3. Increased cortisol stress reactivity to an acute physical stressor independent of age compared to a typical development with reduced cortisol reactivity along with the age. We also hypothesized that sex differences in cortisol recovery found in the Study II would be a stable phenotype across the follow-up (Study III).

4 Materials and Methods

The studies are 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 Southwestern 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).

4.1 Study population and design

The study population comprised 363 10-week-old, 205 six-month-old and 263 14-month-old infants, of which, 157 (43%), 88 (43%) and 100 (38%) were exposed to maternal PPD, respectively. The study population was 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 the 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 (EPDS), overall anxiety (Symptom Checklist-90, SCL-90, anxiety subscale) and pregnancy-specific anxiety symptoms (PRAQ-R2) at gwks 14, 24 and 34 were used for defining maternal PPD. The total sum score cut-off points for 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 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 selective serotonin reuptake inhibitors

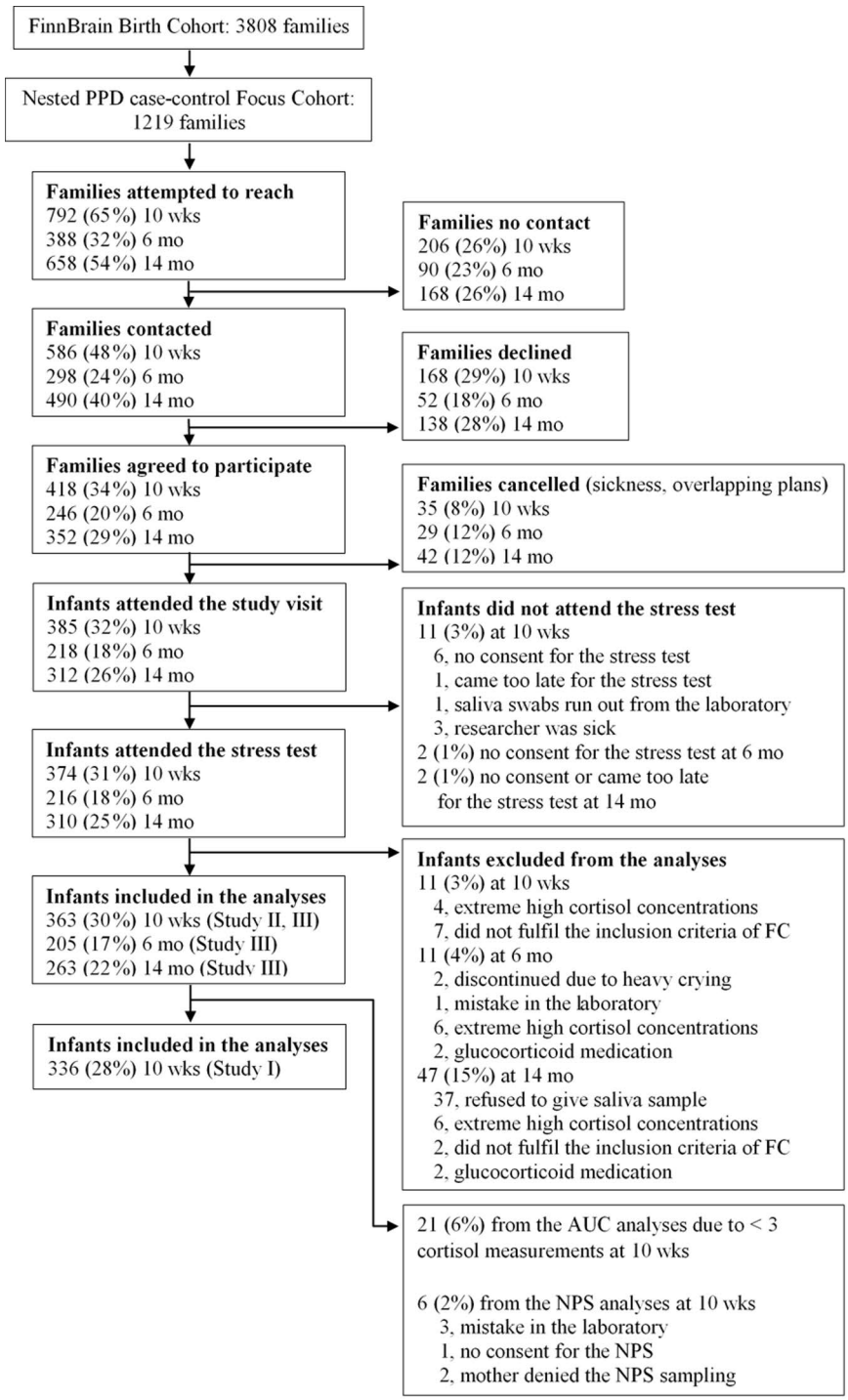

Figure 2. Flowchart for the Studies I–III. PPD = prenatal psychological distress, FC = focus cohort, AUC = area under the curve, NPS = nasopharyngeal swabs.

(SSRIs) during their pregnancy were also included as cases as SSRI usage is one of the specific exposures of interest in the cohort. In the Studies I–III, the SSRI status was a parameter in the sensitivity analyses because the PPD exposure was of special interest. After the collection of the pregnancy data of the whole cohort, the potential PPD case target group was 20% and the control group was 27% of the pregnant women in the Cohort (Karlsson et al., 2018).

Mothers' age at expected date of delivery, education, parity, 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 the 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. 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, 6 months and 14 months were inquired from the mothers at the beginning of each 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 hours and any medications taken by the mothers were 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 in the questionnaire any smoking during the three months after birth or at the time of study visit. Breastfeeding was categorized based on the type of breastfeeding at the time of the study visit.

To recruit the infants for the study visits that included a stress test to measure saliva cortisol stress response profiles for an acute stressor, research personnel contacted the families from the Focus Cohort in the order of the infant age within each age group. Due to project logistics, we could not attempt to contact all the 1219 families from the Focus Cohort (see details in Figure 2). 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, respectively, attended the stress test. Of those infants, some were excluded from the cortisol analyses for having extremely high cortisol concentrations (> 1000 nmol/l in Study I and II and > 400 nmol/l in Study III) (Bae et al., 2019), for not fulfilling the Focus Cohort criteria for high or low maternal PPD exposure, for refusing to keep the saliva sampling device in their mouth or due to glucocorticoid medication. Finally, the analyses included 363 (30%) infants at 10 weeks (Study II and III), 205 (17%) infants at six months (Study III) and 263

(22%) infants at 14 months of age (Study III). In Study III, 101 (22%) infants out of 462 in total participated in all three stress tests at three different ages. In addition, Study I included 336 (28%) infants at the age of 10 weeks after removing those infants without sufficient cortisol data for the AUCi and the nasopharyngeal sample.

4.2 Prenatal psychological distress exposure

The PPD case group was comprised of mothers with a variety of combinations of different levels in total scores among the three types of PPD that were measured. In Study I, where the aim was to investigate the combined infant PPD exposure and rhinovirus status in the associations with the infant cortisol stress response, the case and control groups were used in the analyses. In Studies II–III, continuous scores were used instead of binary exposure categories to assess in more detail what amount of exposure, in scores, was needed for a certain amount of change in the cortisol stress reactivity and recovery. In addition, in Study II and III, pregnancy-related anxiety symptoms were studied separately from the other symptoms, as these have been suggested to be a distinct aspect of anxiety that is specific for a pregnancy (Bayrampour et al., 2016). These symptoms associate with health outcomes of children and pregnant women separate from general anxiety and depression (Acosta et al., 2019; Kataja et al., 2017). The following questionnaires were used:

4.2.1 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).

4.2.2 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.

4.2.3 Pregnancy-Related Anxiety Questionnaire, revised 2 (PRAQ-R2)

The PRAQ-R2, a version of the PRAQ-R suitable for both nulliparous and parous women (Huizink et al., 2016), was used in the study. It is a 10-item self-report that is a shortened version of the 34-item PRAQ (Huizink et al., 2004; Van den Bergh, 1990). Scores on each item ranged from 1, being definitely not true, to 5, being definitely true. The items of PRAQ-R2 can be divided into three subscales being 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 PRAQ-R2 at gwk 14 was included in the study protocol later than the other questionnaire measurement time points. Consequently, the study sample comprised only 82 mothers at 10 weeks, 76 mothers at six months and 89 mothers at 14 months in the PRAQ-R2 gwk 14 measurement and, for this reason, this measurement was omitted from the analyses.

4.3 Saliva cortisol sampling during the stress test

Infant cortisol samples were collected during the Focus Cohort infant stress test study visit at the research facilities. The study visits were carried out between October 2012–February 2016 for the 10-week-olds, October 2013–May 2016 for the six-month-olds and October 2013–January 2017 for the 14-month-olds. The study visit days started at 8–8:30 a.m. and lasted until 4–6 p.m. depending on the age group with maximum of five infant stress tests per day. During the visits, research personnel 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 minutes for the infants in order to normalize the cortisol baseline among all the infants. During this time, research personnel interviewed the mother for their background health information and asked for written informed consent, while the infant was resting on their mother's lap, in a child seat or on the blanket on the floor next to the mother. The cortisol baseline sample was taken after 15 minutes of rest just before the next phase of the study visit.

For the 10-week-old and 14-month-old infants, the stress test study visit included a pediatrician examination, whereas for the 6-month-old infants, the family met the nurse specialized for infectious diseases in children. 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. The nurse discussed with the parents, provided information about the common illnesses among the infants and gave instructions for the care of the infant in case of these illnesses.

At the end of the pediatrician and nurse visits, 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. At the age of 10 weeks, she/he was also given glucose to pacify the pain. Consequently, these measures can be considered as a mild physical discomfort. The procedures were done in the same order for all infants.

The saliva samples at 0, 15, 25 and 35 minutes after the stressor were collected for cortisol reactivity and recovery. 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.

4.4 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. The manufacturing of the previous assay version ended before the data collection was finished. As the available kit version changed during the data collection, samples from 54%, 92% and 94% of infants at the age groups of 10 weeks, 6 and 14 months, respectively, were measured using the newer kit RE62111. All 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.

4.4.1 Immunoassay validation with LC-MS/MS analysis

Comparability across the two immunoassay versions was validated by analyzing a set of same samples with both assays and liquid chromatography-tandem mass spectrometry (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.

A sample of 50 µL of saliva was taken for the LC-MS/MS analysis. Deuterated cortisol (IsoSciences, King of Prussia, PA, USA) was used as an internal standard. 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.

4.5 Virus testing (Study I)

The nasal swab specimen for virus assessment was taken from front nostril and stored at −80°C before the analysis. Swabs were suspended in phosphate-buffered saline, and nucleic acids were extracted by NucliSense easyMag (BioMerieux, Boxtel, the Netherlands) or a MagnaPure 96 (Roche, Penzberg, Germany) automated extractor. PCR for adenovirus, bocavirus, coronaviruses, enteroviruses, metapneumovirus, influenza A and B viruses, respiratory syncytial virus A and B, rhinovirus and parainfluenza virus types 1–4 was performed using a commercial multiplex test kit (Anyplex™RV16, Seegene, Seoul, Korea). Virus analyses were carried out in the Department of Clinical Virology, Division of Microbiology and Genetics at Turku University Hospital, Turku, Finland. The participating infants were without signs or symptoms of acute febrile respiratory infection as determined and systematically documented by a pediatrician during the study visit.

4.6 Statistical analyses

4.6.1 Study I

Area under the cortisol stress response curve with respect to the baseline (AUCi) was used for the measure of HPA axis reactivity. The AUCi differences within the PPD exposure status (PD vs. control), rhinovirus status (+ vs. -) and infant sex were assessed using the t-tests. The main hypothesis that the HPA axis reactivity is altered in the PPD/rhinovirus+ group compared to the rest of the infants was tested with a linear regression model where infant sex was controlled for. Education and smoking of the mothers were used as covariates for the adjusted model, and they were selected based on statistical and clinical relevance.

Analyses included all the infants with a baseline cortisol value and ≥2 measured/available cortisol values after the acute stressor. The missing cortisol values were imputed by using the predictive mean matching multiple imputation technique where 50 complete datasets were constructed, so that all included subjects had all four (0, 15, 25 and 35 min) cortisol values in addition to the baseline value (Little, 1988). The missing values were predicted using the other cortisol values,

their measurement time points (after the acute stressor) and the cortisol measurement kit as the predictors. After imputation, the area under the cortisol curve (between 0 and 35 minutes) above/below baseline (AUCi) was calculated for each subject separately in each 50 completed datasets. AUCi was defined as:

$$AUC_i = \int_0^{35} [f_{PW}(t) - cort_{BL}]dt$$

where $f_{PW}(t)$ is the piecewise linear approximation of the cortisol curve and $cort_{BL}$ is the baseline cortisol value. To test our main hypothesis, the model of standard linear regression was used to analyze the differences in the AUCi:

AUCi = PPD exposure + rhinovirus + PPD exposure × rhinovirus + sex,

was used with such contrast coding for PPD exposure and rhinovirus that we were able to test the null hypothesis:

$$H_0: \mu_{PPD/+} = (\mu_{PPD/-} + \mu_{control/+} + \mu_{control/-}) / 3$$

where $\mu_{x/x}$ is the mean AUC_i in group x/x. The analyses were first made separately for each imputed dataset. The final results were then obtained by pooling the results using Rubin's rules (Rubin, 1987). The R package mice was used for multiple imputation (van Buuren and Oudshoorn, 2011). Natural logarithm transformed cortisol values were used in the analyses. Sensitivity analyses were performed without corticosteroids or SSRI-medicated mothers as SSRI exposure might alter infant HPA axis functioning (Oberlander et al., 2008; Pawluski et al., 2012). In addition, the prenatal SSRI medication was one of the inclusion criteria for the Focus Cohort. Thus, SSRI-medicated mothers are over-presented in the case group and not randomly presented in both case and control groups.

4.6.2 Study II and III

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. Covariates were chosen based on the earlier literature (Egliston et al., 2007; Kudielka et al., 2009) and the characteristics of our sample.

Mixed models were used to analyze the associations between different types of PPD exposures and infant cortisol reactivity and recovery. Models were fitted separately for each age group using all the available cortisol data.

Fixed effects:
Sex + PPD + TimeTerms + Sex×PPD + Sex×TimeTerms + PPD×TimeTerms + Sex×PPD×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 the EPDS and the SCL-90 anxiety subscale sum scores or the PRAQ-R2 sum scores. In the former case, the variable was computed by first calculating the means of the EPDS and the 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 the PRAQ-R2 sum scores at gwks 24 and 34. In the case of missing values at some gwks, the means were based on those values that were observed leading to N = 636 and N = 630 (10 weeks), N = 205 and N = 204 (6 months) and N = 263 and N = 262 (14 months) in the EPDS/SCL and PRAQ measures, respectively.

  In addition to the reasoning discussed above for the separate analyses with the PRAQ-R2 from the EDPS and the SCL, the EPDS and SCL scores were also combined as they are known to correlate strongly. Indeed, correlations in our sample between the PRAQ-R2 and the EPDS (r = .437–.521) or between the PRAQ-R2 and the SCL anxiety subscale (r = .421–.561) total scores at gwks 14–34 were somewhat smaller compared to associations between the EPDS and the SCL anxiety subscales (r = .551–.718) among the mothers of infants in the ten weeks age group. Similarly, correlations between the EPDS and the SCL anxiety subscale total scores (r = .551–.709) were higher compared to corresponding correlations between the EDPS and the PRAQ-R2 (r = .452–.539) or between the SCL and the PRAQ-R2 (r = .454–.492) among mothers of 6-month-old infants. Among mothers of 14-month-old infants, corresponding correlations between the EPDS and the SCL anxiety subscale total scores (r = .511–.697) were also higher compared to correlations between the EDPS and the PRAQ-R2 (r = .426–.491) or between the SCL and the PRAQ-R2 (r = .381–.500).
- *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 minutes (baseline), 0 minutes and 15 minutes 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 between infants. However, to avoid an overly complex random effects structure, the breakpoint at -30 minutes was omitted from the random effects.

- *Feeding* was a binary variable indicating whether the infant was fed before each cortisol measurement during the study visits.
- *Kit*, a binary variable indicating which cortisol EIA kit version was used, either the RE62011 (older) or RE62111 (newer), 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 minutes and 100 minutes 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. In addition, in Study III, breastfeeding was included in the analyses because the ratio of different breastfeeding styles changed considerably during the follow-up. Breastfeeding was omitted from the models in Study II because 91% of the mothers reported breastfeeding at 10 weeks. Maternal postnatal smoking was also included in Study III.
- When analyzing the associations in all infants independently of sex, all the terms in the model that included interaction with sex were omitted (Study II).

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.
- 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 the associations between PPD and cortisol reactivity/recovery were formed as suitable linear combinations of the regression parameter estimates from the mixed models. 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. The separate estimates for males and females were extracted from the interaction model.

Statistical analyses were performed using R 3.5.1 (R Core Team 2018) and IBM SPSS Statistics version 26 in Study I, R 3.6.2 (R Core Team 2019) and IBM SPSS Statistics version 26 in study II and R 4.0.5 (R Core Team, 2021) and SPSS Statistics version 26–27 in Study III. A two-sided p-value < .05 was considered statistically significant. Raw p values are reported.

Sensitivity analyses were performed by re-running each analysis without SSRI-medicated mothers, as SSRI exposure might alter infant HPA axis functioning (Oberlander et al., 2008; Pawluski et al., 2012). In addition, the prenatal SSRI medication was one of the inclusion criteria for the Focus Cohort. Thus, SSRI-medicated mothers are over-presented in the case group instead of being randomly presented in both case and control groups (i.e., high vs. low PPD exposure).

4.7 Ethical considerations

The studies were approved by the Ethics Committee of the Hospital District of Southwest Finland. The decisions for the study visits for the 10-week-olds were given in 10.9.2012, § 213 and for the study visits for 6- and 14-month-olds in 21.5.2013, § 187 with a reference number 57/180/2011. Separate 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. Parents were offered a free infant examination by a pediatrician at 10 weeks and 14 months of infant age and an opportunity to discuss with a nurse specialized for infectious diseases in the children at six months of infant age. Parents could choose whether they wished to participate in the infant stress test for saliva cortisol stress response studies as a part of the study visit or not, and which infant biological samples, blood and/or nasopharyngeal, if any, were allowed to be taken for the purposes of the FinnBrain Birth Cohort Study. Parents were told that they can discontinue the study visits at any time during the visit with no need to state the reason. At the beginning of each study visit, a local topical anesthetic cream was applied to the infant skin in the antecubital fossa area to alleviate the pain during the venipuncture. In addition, at the age of ten weeks, infants were given liquid oral sucrose from a syringe during the venipuncture to reduce the pain further. If necessary and with parents' approval, a paediatrician could refer the family for further clinical examination to the local health care provider. All biological samples, filled paper record forms and saved

electronic data were ID coded and stored in accordance with the good research practices and Finnish legislation to maintain participant confidentiality. FinnBrain Birth Cohort research personnel and students have signed a confidentiality agreement.

5 Results

The majority of the infants in all age groups responded to the stress test with increased cortisol levels. With the classification criteria of >15.5% baseline-to-peak increase (Miller et al., 2013), 71%, 63% and 66% of infants in the age groups of 10 weeks, 6 months and 14 months were classified as responders. Cortisol levels varied considerable within the age groups as was expected (Table 2).

All saliva cortisol concentrations measured with both older and newer versions of the immunoassay and LC–MS/MS correlated consistently with each other (r = .754–.955).

5.1 Cortisol stress reactivity in rhinovirus-positive, PPD-exposed infants (Study I)

In Study I, we explored the association between the PPD exposure and the cortisol stress reactivity among the infants with and without a positive rhinovirus finding in their nasopharyngeal sample. The study population included 336 10-week-old infants of whom 177 (53%) were males, 148 (44%) were exposed to PPD and 76 (23%) were rhinovirus-positive. Other viruses that were tested were found in a maximum of 12 infants per virus. The PPD-exposure group did not differ significantly from the control group in the number of rhinovirus-positive infants (21% vs. 24%, p = .50). Mothers in the PPD group had a lower education level and were more likely to report smoking during their pregnancy but other selected background characteristics did not differ between PPD and control groups.

Infant cortisol stress response (AUCi) did not differ based on the PPD exposure or rhinovirus status independently. Females had higher cortisol stress responses compared to males (Figure 3). When comparing the group of PPD/rhinovirus+ infants to the control/rhinovirus+, PPD/rhinovirus- and control/rhinovirus- groups of infants, we observed a lower AUCi in the PPD/rhinovirus+ group when controlling for infant sex (Figure 4A and 4B). The difference between PPD/rhinovirus+ group and the mean of the combined three other groups was 14.7 (95% CI 3.8–25.6) ln [nmol/L] × min, p = .008. Controlling for maternal education level and smoking did not alter the results [15.3 (95% CI 4.1–26.4) ln (nmol/L) × min, p = .007].

Table 2. 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 minutes after the stressor.

	10 weeks			6 months			14 months		
	Median (Q25–Q75)	RANGE	N	Median (Q25–Q75)	RANGE	N	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

In addition, the AUCi was statistically significantly lower only in PPD/rhinovirus+ males, but not in females when separately tested for both sexes (Figure 4C). Finally, within the PPD-exposure group, the rhinovirus+ infants had 14.9 (95% CI 3.3–27.3) ln [nmol/L] × min lower AUCi compared to PPD-exposed infants without rhinovirus (p = .01).

Excluding infants with maternal use of SSRI (N = 48) or corticosteroids during pregnancy (N = 35) did not alter the results concerning the difference between PPD/rhinovirus+ and the average of control/rhinovirus+, PPD/rhinovirus- and control/rhinovirus- groups.

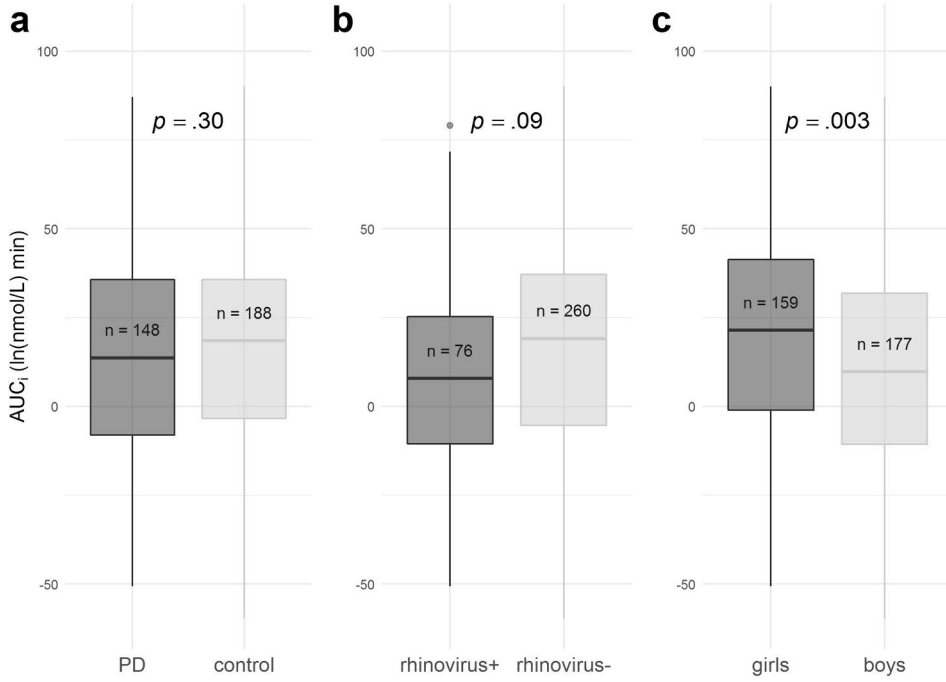

Figure 3. Infant cortisol stress responses (AUCi) compared between the groups of: **a)** PPD and control, **b)** rhinovirus- and rhinovirus+ and **c)** girls and boys. The standard boxplots demonstrate the AUCi in the groups. AUCi: area under the curve above/below baseline; PD: prenatal psychological distress.

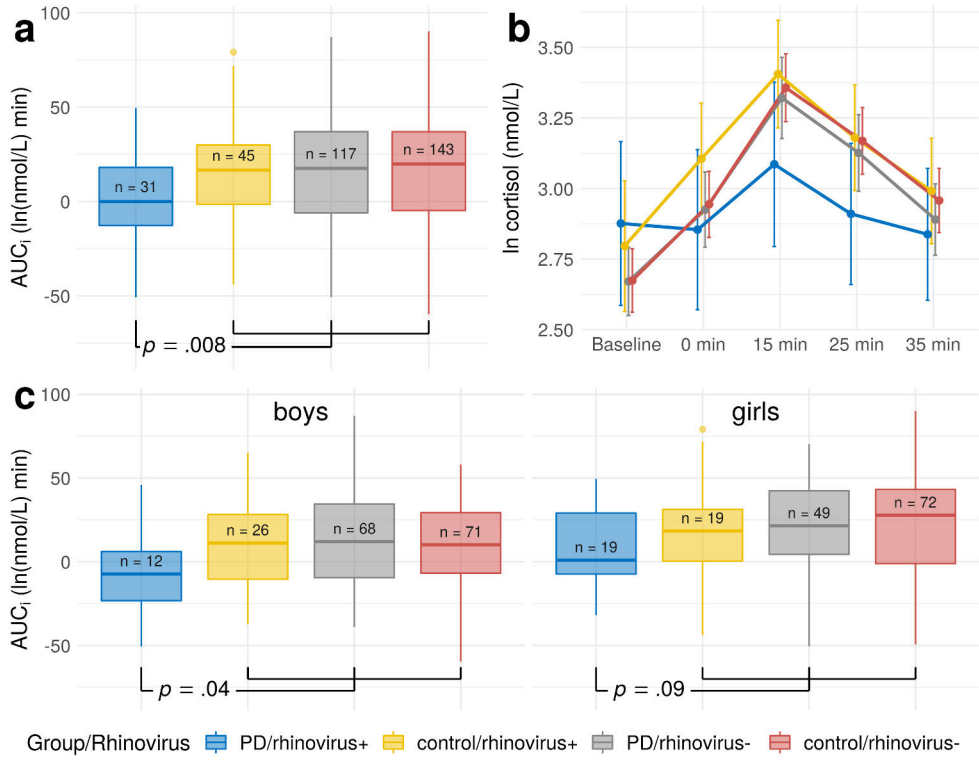

Figure 4. Infant cortisol stress responses during the stress test at the age of 10 weeks. The boxplots demonstrate the AUCi in the groups. **A)** AUCi compared between the PD/rhinovirus+ group and the average of control / rhinovirus+, PD / rhinovirus- and control / rhinovirus- groups. **B)** Mean cortisol values at five time points: baseline before the stressor and 0 min, 15 min, 25 min and 35 min after the stressor among the four groups. **C)** AUCi compared between the groups separately in girls and boys. AUCi: area under the curve above/below baseline, PD: exposure to prenatal psychological distress.

5.2 Sex and age differences in the associations between PPD exposure and cortisol stress response during infancy

In Studies II and III, we further explored the development of the infant cortisol stress response in the context of PPD exposure by examining the associations separately with cortisol reactivity and recovery instead of combining them in the measure of AUCi.

Characteristics of the mothers and infants per age group are described in Table 3 and Table 4. The most common recent medication and other products in use for the infants at the age of 10 weeks, six months and 14 months were vitamin D (82% / 92% / 88%), probiotics (42% / 42% / 33%), simethicone/dimethicone (11% / 1% / 1%), antibiotics (1% / 2% / 4%) and paracetamol or nonsteroidal anti-inflammatory

drugs (0.6% / 2% / 5%). Relatively few mothers from the three infant age groups reported any use of glucocorticoids or thyroxine medication (3–11%), SSRIs (7–14%) or hormonal contraceptives (19–28%) during their pregnancy and/or postpartum (10 weeks–14 months), which could have possible relevance to the saliva cortisol levels of the breastfed infants. There were no illicit drug users among the mothers. Mothers of male infants in each age group reported more commonly postpartum alcohol consumption compared to mothers of female infants: 68% vs. 57%, $p = .035$ (10 weeks), 57% vs. 43%, $p = .048$ (six months) and 59% vs. 41%, $p = .029$ (14 months). There were no sex differences in the maternal and child medication use or type of breastfeeding in any age group.

Table 3. Characteristics of the mothers. Values are mean (standard deviation) for continuous variables and number of cases (%) for discrete variables.

Mothers		10 wks			6 mo			14 mo		
		N[1]	mean (SD) or N (%)	Range	N[2]	mean (SD) or N (%)	Range	N[3]	mean (SD) or N (%)	Range
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)	
Smoking	gwks 14–34	355	48 (13)			22 (11)			23 (9)	
Smoking	3 mo		36 (10)		202	23 (11)		256	11 (4)	
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)	

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

Table 4. Characteristics of the infants. Values are mean (standard deviation) for continuous variables and number of cases (%) for discrete variables.

INFANTS		10 wks			6 mo			14 mo		
		N[1]	mean (SD) or N (%)	Range	N[2]	mean (SD) or N (%)	Range	N[3]	mean (SD) or N (%)	Range
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; wks = weeks; mo = month; 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).

5.2.1 Infant cortisol stress reactivity and recovery at 10 weeks of age (Study II)

In Study II, we first explored the association between the PPD exposure and infant cortisol stress response at 10 weeks of age in all infants independently of sex. All the estimates were low and statistically non-significant.

Next, we conducted the same analyses with sex interactions. We did not find convincing evidence for sex differences in the association between either combined EPDS + SCL scores or PRAQ scores and cortisol reactivity. In females, a 1 SD higher EPDS + SCL score was estimated to associate with a 14% less steep reactivity slope (95% CI = -28–4%), but the result was not statistically significant. In males, the corresponding estimate was close to zero (95% CI = -11–19%).

Instead, infant cortisol stress recovery clearly associated differently with EPDS + SCL scores depending on sex (18%, 95% CI = 4–33%, p = .008). In females, a 1 SD increase in EPDS + SCL score associated with a 10% less steep recovery slope (95% CI = -18–0%, p = .040), while interestingly in males, the association was suggested to be in the opposite direction (6%, 95% CI =-1–14%, p = .089). The evidence for sex interaction in the case of PRAQ was much weaker, and the estimate was clearly smaller, but the direction was similar than in case of EPDS + SCL (Table 5, Figure 5).

5.2.2 Infant cortisol stress reactivity and recovery at six months of age (Study III)

In Study III, we followed up further the associations between the PPD exposures and infant cortisol stress responses during infancy. We did not observe any associations between the PPD exposures and infant cortisol stress reactivity or recovery among either sex at the age of six months (Table 5, Figure 5).

5.2.3 Infant cortisol stress reactivity and recovery at 14 months of age (Study III)

Finally, at the age of 14 months, the PPD exposures did not predict the infant cortisol stress reactivity either, but we observed sex differences in the association between the PPD exposures and cortisol recovery. In females, a higher EPDS + SCL and PRAQ scores predicted an 11% enhanced recovery with 95% CI 1.01–1.23 with EPDS+SCL and 1.02–1.21 with PRAQ. In males, we did not observe any association between PPD exposure and cortisol recovery (Table 5, Figure 5).

5.2.4 Sensitivity analyses

Although the sex difference in the association between EPDS +SCL sum score and cortisol recovery in 10-week-olds remain essentially the same (est = 1.20, 95% CI = 1.01–1.43, p = .037), the association between the EPDS + SCL sum score and cortisol recovery in 10-week-old females (est = 0.89, 95% CI = 0.77–1.02, p = .089) did not remain significant after removing the SSRI-medicated mothers from the analyses. In addition, at the age of 14 months, the sex interaction in the association between EPDS+SCL scores and cortisol recovery did not remain statistically significant (est = 0.86, 95% CI = .72–1.03, p = .100) without the SSRI-medicated mothers in the sample. Both exposures, i.e., combined depressive and general anxiety symptoms as well as pregnancy-related anxiety symptoms still predicted enhanced female cortisol recovery at 14 months.

The changes in the results may be due to a decreased sample size as 29% of the PPD case group mothers at 10 weeks, and 30% at 14 months had SSRI medication during their pregnancy and/or postpartum.

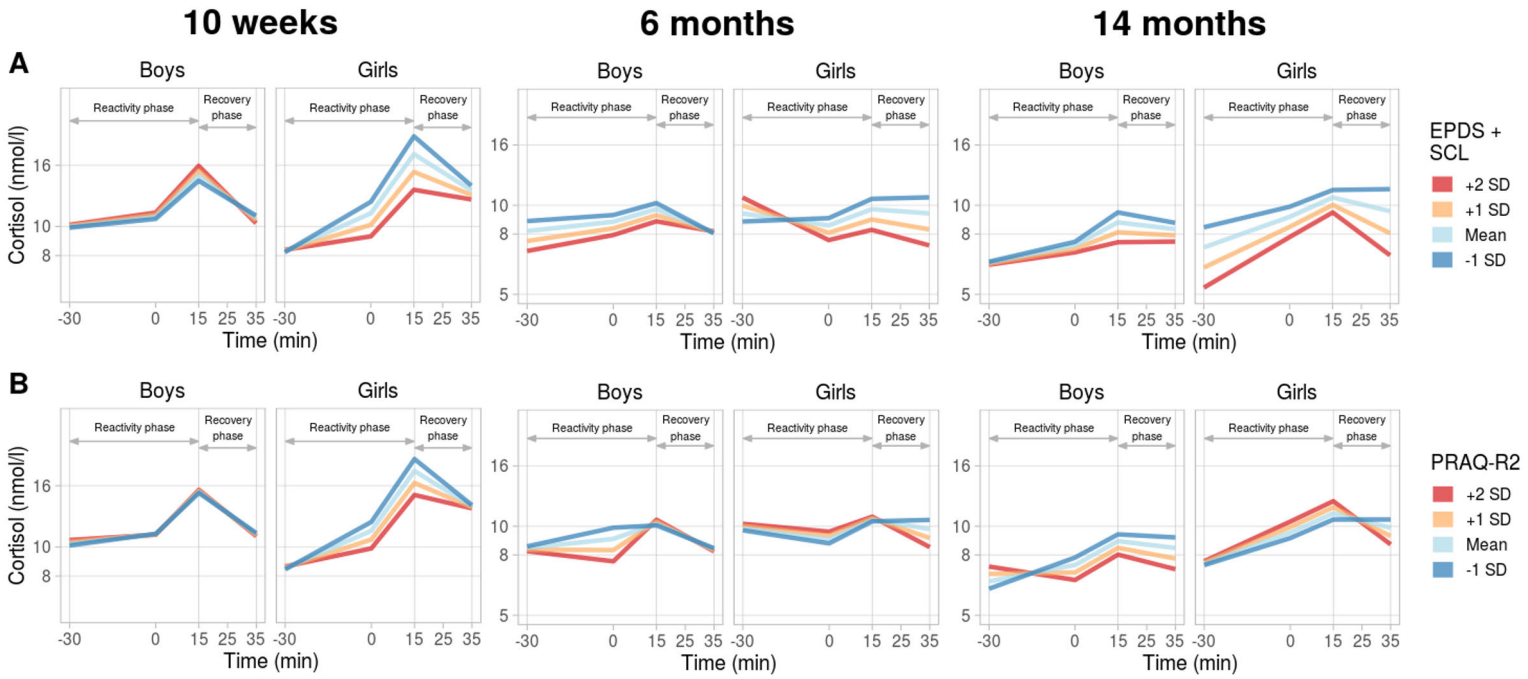

Figure 5. Estimated average infant cortisol stress responses based on the mixed models drawn for different levels of maternal prenatal stress. Symptom total scores were modelled as continuous variables, but for the illustration, the estimates are presented for four selected values (mean – 1 SD, mean, 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, 6 months and 14 months. EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Checklist-90, anxiety subscale; PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised 2.

Table 5. 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 (Study II) and 6 and 14 months (Study III). Estimates present 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.

10 WEEKS	**Reactivity**			**Recovery**		
EPDS+SCL	est	p	95% CI	est	p	95% CI
females	0.86	.124	0.72–1.04	**0.90**	**.040**	**0.82–1.00**
males	1.03	.693	0.89–1.19	1.06	.089	0.99–1.14
interaction	1.19	.143	0.94–1.51	**1.18**	**.008**	**1.04–1.33**
PRAQ-R2						
females	0.90	.242	0.77–1.07	0.92	.144	0.82–1.03
males	0.99	.927	0.85–1.16	1.02	.692	0.94–1.10
interaction	1.10	.421	0.88–1.37	1.11	.159	0.96–1.27
6 MONTHS						
EPDS+SCL						
females	0.88	.256	0.70–1.10	1.04	.555	0.91–1.19
males	1.03	.768	0.86–1.23	0.95	.149	0.88–1.02
interaction	1.17	.286	0.87–1.57	0.91	.229	0.79–1.06
PRAQ-R2						
females	0.99	.923	0.81–1.21	1.08	.165	0.97–1.22
males	1.03	.775	0.83–1.28	1.02	.641	0.93–1.12
interaction	1.04	.787	0.77–1.40	0.94	.422	0.81–1.09
14 MONTHS						
EPDS+SCL						
females	1.10	.321	0.91–1.34	**1.11**	**.034**	**1.01–1.23**
males	0.93	.313	0.82–1.07	0.96	.414	0.88–1.05
interaction	0.85	.159	0.67–1.07	**0.86**	**.034**	**0.76–0.99**
PRAQ-R2						
females	1.03	.776	0.83–1.28	**1.11**	**.014**	**1.02–1.21**
males	0.89	.113	0.78–1.03	1.03	.622	0.92–1.16
interaction	0.87	.282	0.67–1.12	0.93	.289	0.80–1.07

EPDS+SCL combines both questionnaires at gwks 14–34 (z-score), N = 363 (10 weeks), N = 205 (6 months) and N = 263 (14 months). PRAQ-R2 combines PRAQ-R2 at gwks 24–34 (z-score), N = 360 (10 weeks), N = 204 (6 months) and N = 262 (14 months). Models were adjusted for mother's age, education, smoking, breastfeeding (Study III), 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. EPDS = Edinburgh Postnatal Depression Scale; SCL = Symptom Check List-90 (anxiety subscale); PRAQ-R2 = Pregnancy-Related Anxiety Questionnaire, revised 2; gwks = gestational weeks.

6 Discussion

Our three studies found evidence for:

1) A blunted cortisol stress response during a subclinical rhinovirus infection in 10-week-old infants exposed to the maternal prenatal psychological distress (Study I).
2) Decreased cortisol stress recovery among the 10-week-old females exposed to higher maternal prenatal depressive and anxiety symptoms (Study II).
3) Increased cortisol stress recovery among the 14-month-old females exposed to higher maternal prenatal depressive, general anxiety and pregnancy-related anxiety symptoms (Study III).

The results suggest age- and sex-dependent associations between the PPD exposure and cortisol stress recovery during infancy and sex-dependent associations between the infant PPD exposure, anti-viral immune system and cortisol stress response.

In line with our hypotheses, PPD exposure was associated with altered infant cortisol stress response during a concurrent subclinical rhinovirus infection. A blunted cortisol stress response was more evident among PPD/rhino+ males indicating a possible sex-dependent interaction between the immune system and HPA axis functioning during early infancy after the PPD exposure. In addition, in accordance with our hypotheses, PPD exposure was associated with slower cortisol stress recovery, and this was observed only in females at the age of ten weeks. Partly in line with our hypothesis, we found evidence for a stable sex difference in the cortisol stress recovery during the follow-up in that altered cortisol recovery was observed only in exposed females also at the age of 14 months. Interestingly, the cortisol stress recovery itself was not stable as the direction of the association changed along the infant age. A decreased cortisol recovery in 10-week-old exposed females had developed into increased cortisol recovery during the following year. Contrary to our expectations, we did not observe altered cortisol stress reactivity among the PPD-exposed infants at any age group independent of or depending on sex.

Observed associations were weak on average, and the minimum differences in cortisol stress responses between the exposure groups or associations between the PPD and cortisol variables were close to zero. The variation in cortisol stress response profiles between infants was substantial, and the PPD exposure explained only a small proportion of that variation. On the other hand, one would not expect to see a strong association between the relatively low level of maternal PPD and cortisol stress response of healthy infants in the sample from a Western general population with a reasonably high level of socioeconomical status and well-being.

An overall low level of maternal PPD might partly explain the absence of association between the PPD exposure and infant cortisol stress reactivity in our sample. We were still able to demonstrate the possible changes in the cortisol stress recovery among the exposed female infants. This could indicate that the negative feedback mechanism of the HPA axis, where cortisol regulates its own recovery back to a baseline state, could be more sensitive to the influences of the PPD compared to cortisol reactivity. It is possible that with stronger PPD exposure, we could have observed altered infant cortisol stress reactivity as well. In that case, the association between cortisol recovery could have been even stronger.

Based on our results and the studies of others, both maternal prenatal depressive and general anxiety symptoms as well as pregnancy-related anxiety symptoms have a potential to influence infant HPA axis functioning. Pregnancy-related anxiety has been associated with decreased cortisol reactivity to vaccination among eight-week-old infants in a study similar to ours (Tollenaar et al., 2011). Prenatal maternal depressive symptoms, based on EPDS scores, had an U-shaped association with increased infant cortisol stress reactivity to vaccination at 2 months (Fernandes et al., 2015). Higher general anxiety, through STAI, and depressive symptoms, through EPDS, have been associated with a decreased cortisol stress response in the AUCg to mother-infant separation at 12 months (Galbally et al., 2019). Two studies that used maternal MDD that were based on a structured clinical interview, have reported increased infant cortisol stress reactivity (Osborne et al., 2018; Stroud et al., 2016).

Our results support the earlier findings (see 2.5.2 Timing of PPD exposure) that there is likely not any specific vulnerable time window during pregnancy for the maternal PPD to influence the HPA axis development of the child. A pregnancy, as such, can be a sensitive period for HPA axis development. We ended up combining the total scores of the depression and anxiety symptom questionnaires from all measurement time points (gwks 14, 24 and 34) in our analyses because the correlation of total scores between the time points was high and most of the scores from different time points associated similarly with infant cortisol when analyzed separately (data not published). This also emphasizes the difficulty in separating the possible influence of different timing of PPD during pregnancy on infant cortisol stress response in the type of study design mostly used, like ours.

In accordance with a few previous studies, we found support for the sex-dependent association between the PPD and the infant cortisol stress response. Giesbrecht et al. (2017), Stroud et al. (2016) and Yong Ping et al. (2015) have reported altered cortisol stress reactivity among the PPD-exposed female infants, whereas our results in Studies II and III suggested that also female cortisol stress recovery might be more prone to the effects of PPD exposure compared to males. In addition, our result from Study I, where rhinovirus-positive, PPD-exposed male infants had a blunted cortisol stress response compared to rhinovirus negative, non-exposed males and only PPD-exposed males and only rhinovirus positive males suggests that sexually dimorphic associations between the PPD and HPA axis functioning might depend on the state of the other biological systems. To our knowledge, there are no previous studies on human infants showing a statistically significant association between PPD exposure and cortisol stress response among males instead of females.

One possible mechanism behind the observed sex differences in the HPA axis functioning in PPD-exposed infants is dimorphic functioning of the placenta during pregnancy as reviewed in Section 2.6.1. Our previous study from the FinnBrain Birth Cohort showed a positive association between chronically high maternal prenatal depression trajectory and maternal hair cortisol concentrations during pregnancy (Mustonen et al., 2019). The altered cortisol stress recovery observed only in females could have resulted from higher maternal prenatal cortisol exposure in females compared to males due to a diminished protective 11β-HSD2 barrier in the placenta. On the other hand, the blunted cortisol stress response in rhinovirus-positive, PPD-exposed infants, where the association was more clearly seen in males compared to females, is more difficult to explain. Knowledge on the prenatal programming of the immune system, HPA axis and HPG axis as well as interactions between gonadal hormones, cortisol and antiviral immune system functioning and development during infancy is limited. More research is needed especially in humans.

The mechanism behind the changes in the association between higher PPD exposure and cortisol stress recovery rates during the follow-up, where decreased recovery at 10 weeks of age was followed by increased recovery at 14 months of age in females, could relate to the major developmental changes in the adrenal cortex, secretion of gonadal hormones and the brain and their interactions during early infancy as discussed in Section 2.6.2. Speculatively, one possible explanation could be that the originally slower cortisol stress recovery in younger infants increased their exposure to cortisol during the following months that most likely included stressful events as infants learned new skills and encountered novel situations. About one year later at the age of 14 months, a negative feedback mechanism could have developed toward a more hypersensitive mode, producing faster cortisol stress recovery from acute stressor to compensate the earlier effect of PPD on cortisol stress

recovery. Increased exposure to cortisol during that year could have altered the interactions between the developing HPA axis, the brain and HPG axis via altered sensitivities of the glucocorticoid receptors and hence changed the sensitivity of the negative feedback mechanism.

Furthermore, infants express varying and increasing levels of stranger fear around six months forward, which might have moderated the experienced stressfulness of the study visit and recovery from it (Brooker et al., 2013). Although a similar stressor was used in each age group for each infant, the infant is not the same at a later timepoint because they have reached a different level of maturity.

Our result of a blunted cortisol stress response to acute stress during subclinical rhinovirus infection among PPD-exposed infants, which was more clearly seen in males, is interesting considering that the most commonly increased, not blunted, cortisol stress reactivity has been associated with PPD exposure and associations between PPD and altered cortisol stress reactivity has emphasized female infants. Rhinovirus infection itself is a stressor for the body that activates the HPA axis along with the immune system, and, together, these two systems function in a coordinated manner in fighting against infections. Our results suggest that in PPD-exposed infants, the interaction between the activated immune system and HPA axis is altered as the HPA axis, which already may had been activated by a rhinovirus-activated immune system, did not respond to a subsequent acute stressor, being the study visit, with the same intensity than it would have without preceding rhinovirus and/or PPD exposure.

If a blunted cortisol stress response to an acute stressor is interpreted as having less adequate capability to respond to encountered challenges, our results could indicate that infants exposed to PPD could be less tolerant to everyday life stressors during infections compared to non-PPD-exposed ill infants and to occasions when PPD-exposed infants are healthy. As PPD exposure has been associated with an increased risk for concurrent respiratory infections (Korhonen et al., 2019), PPD exposure could increase the time in early infancy when PPD-exposed infants are equipped with inadequate stress responsiveness compared to an infant with only occasional infections. Even if PPD exposure alone would not significantly alter infant cortisol stress responsiveness, an additional burden, “a second hit,” from recurrent infections could increase the altered cortisol stress responsiveness during infancy and make these infants more vulnerable to stress. Based on our study, it is not possible to determine if other viruses than rhinoviruses would have associated similarly with a blunted cortisol response in a PPD-exposed infant. The number of infants with other viruses present in their nasopharynx was too low.

In addition, more research is needed to assess if the observed blunted cortisol stress responsiveness associates with later disease development such as asthma, atopic disorders and wheezing. Rhinoviruses are very common in the general

population, especially among young children, and are the typical cause for respiratory infections, although symptoms are mild and often asymptomatic (Monto and Sullivan, 1993). A rhinovirus-induced wheezing illness during early childhood (< 3 years) has been associated with the later development of wheezing/asthma in a recent meta-analysis (Liu et al., 2017). There is some evidence that the association holds also in low-risk children without pre-existing increased risks for asthma (de Winter et al., 2015). Rhinovirus, as such, does not cause asthma, but rhinovirus infection-induced wheezing is considered as a marker for an altered immune response in susceptible individuals (Kelly and Busse, 2008). The origins of these immunological alterations are not well understood, and PPD exposure could be among the related factors.

6.1 Strengths of the studies

Compared to the other studies in the field of prenatal programming of the infant cortisol stress responses, our studies have several strengths. Although there are previous studies with overlapping strengths, in contrast to those studies, we have been able to combine several of those strengths into the studies here.

Our definition for maternal prenatal psychological distress exposure comprised longitudinal measures of three types of common mental health symptoms repeated three times from early pregnancy at gestational week 14 until late pregnancy at gestational week 34. This is one of the most comprehensive PPD exposure characterizations among the existing studies, which typically have concentrated on the end of a pregnancy (Braithwaite et al., 2016; Fernandes et al., 2015; Tollenaar et al., 2011). The physiology of the mother and the fetus changes along with the normal progress of a pregnancy, and maternal mood is not likely to be constant during the whole pregnancy in all individuals. Depending on the timing of experienced PPD during pregnancy, PPD might have different effects on the physiology of the mother and hence the fetus. It is also possible that, in the earlier studies where PPD exposure measures have been centered on the end of a pregnancy, the programming effects on the fetal HPA axis development have actually occurred already during early pregnancy. That said, we did not observe any of our single PPD measurement time point to be more influential than the other and ended up combining them.

A second clear strength of our studies is the repeated measures of the infant cortisol stress responses to a similar stressor used during the early ages when the infant development is rapid. This increases the confidence that observed changes in the associations between the PPD exposure and cortisol stress responses during the follow-up were due to age and not the stressor type. There are only few earlier longitudinal studies of cortisol stress responses among the PPD-exposed infants (Osborne et al., 2018; Tollenaar et al., 2011). In the study of Tollenaar et al., the

stressors were different in every infant age group, which makes it difficult to distinguish between the effect of age and stressor. Osborne et al. do not mention the sex of the infants, and it is not known whether their results can be generalized to both sexes or not.

Third, our sample size of 462 infants is among the largest population studied as it is common to have about a hundred participants. Cortisol levels have wide inter-individual variation and especially during infancy when the HPA system is maturing rapidly. A large sample is needed to detect associations with other factors against this background noise. Our findings suggest very low effect sizes in the association between PPD exposure and infant cortisol stress response, and our large sample size increases the reliability of the findings in that the weak associations should not be due to lack of power. In addition, the large sample size made it possible to examine the sex differences using sex by PPD interaction modelling.

Fourth, to our knowledge, our studies are the first to consider cortisol recovery as a separate factor in the analyses. Overall, cortisol research is stress-reactivity oriented. Even the studies that have collected more than just the two pre- and post-stressor cortisol samples and thus could have also explored the recovery phase of the stress response tend to analyse and report only reactivity (Braithwaite et al., 2016; Giesbrecht et al., 2017; Stroud et al., 2016; Tollenaar et al., 2011). The area under the curve is also a common and a good index for the whole cortisol stress response, which we also utilized in our Study I, but it does not capture the recovery separately from reactivity.

Fifth, in comparison to other studies, we were able to check and control for many possible confounders, such as prenatal and postnatal medications and smoking of the mothers and birth outcomes and medications of the infants. These confounders and many other pieces of background information were drawn from the numerous prospective questionnaires that the families had filled in three times during their pregnancies and at the infant ages of three, six and 12 months. In addition, we were able to complement this information from the Medical Birth Register of National Institute for Health and Welfare, such as prenatal glucocorticoid treatments. A versatile background information was also helpful in determining whether the observed higher cortisol levels (> 3 SD) among some infants could be due to infant sickness, medication or other similar characteristics. As there were no indication of those, we ended up excluding only the very high cortisol levels (> 400 nmol/l in our sample) in order to allow the normal variation of the cortisol levels to be as high as it seems to be among the studied age groups based on Bae et al. (2019), who reported infant basal serum cortisol levels to vary almost ten-fold between individuals (46.7–424.6 nmol/l, 2.5–97.5 percentile).

Sixth, research personnel followed and completed a detailed protocol record form for each mother-infant pair during the study visits to ensure the comparability

between the visits during the several years that our prospective data collection lasted. Information about the infant teething, medication usage, time and duration of the sleeping and feedings before the cortisol baseline measurement and during the visit as well as recent medications, smoking and caffeine and nicotine consumption of the mother were also recorded. These factors might interfere with the breastfed infant cortisol levels or the analyses of the saliva samples.

Seventh, the infant cortisol baseline sample was collected after 15 minutes of rest instead of being taken soon after the arrival to the laboratory. This should minimize the effects of previous daily hassles on infant cortisol baseline. It should also give enough time for the infant to adjust to the new environment, which might be perceived as stressful at first. A reliable baseline measurement is crucial to the cortisol reactivity measure as the following samples are compared to the baseline. With the AUCi, the reliable cortisol baseline is even more important as all the other cortisol levels are considered with respect to the baseline.

Eighth, our study sample was drawn from the general population instead of being a clinical sample. It also included equally both sexes in all age groups such that the results can be generalized to the normal population. Although it is very important to study clinical populations as well, the notion that also the levels of maternal PPD below the clinical threshold might associate with infant HPA axis development is significant.

Ninth, we used mixed model analysis for repeated cortisol measurements instead of repeated measures of ANOVA (Hoffman and Stawski, 2009; Hruschka et al., 2005). A mixed model approach is recommended when the aim is to differentiate between the within-individual and between-individuals variation in longitudinal design. It allows random individual intercepts and slopes, as random effects, and correlated residuals, as dependent errors. It is not sensitive to missing values or variation in the time points of measurements. All these characteristics are needed in cortisol measurements and study design presented here. Some saliva samples can be dry or the infant gets too restless to give a sample during the visit leading to missing values and different amount of observations among infants. Variation in cortisol levels is high between individuals, but it also changes within individuals as infants age (Tollenaar et al., 2010). Further, cortisol kinetics, i.e., the steepness of the cortisol slopes, differ between individuals and again, within individuals during the development (Jansen et al., 2010).

Tenth, more than a half of the infants, 71%, 63% and 66% at the age of 10 weeks, 6 months and 14 months, respectively, were qualified as a cortisol responder for the stress test. A challenge in human infant studies is to perform a strong enough but ethically valid stressor to induce a cortisol stress response. The amount of responders is seldom reported but, for example, only 46% of infants were reported to be a

responder in the study of Grant et al. (2009) and only half of the infants in the one of the age groups in the study of Tollenaar et al., 2011.

Eleventh, a novel focus was on the infant HPA axis reactivity during subclinical virus infection.

Finally, the immunoassay measurements with two different EIA kit versions were validated against the LC-MS/MS measurements, which is considered as a gold-standard method.

6.2 Limitations

The results from our studies should be considered with the following limitations.

The maternal prenatal symptom measurements differed from each other in that the PRAQ-R2 was implemented later than the EPDS and the SCL-90 anxiety subscale to be a part of the questionnaires at gwk 14. Following the limited number of mothers with PRAQ-R2 data at gwk 14, it was excluded from the analyses in Studies II and III.

An infant's baseline cortisol level was based on the single sample taken before the paediatric examination. Baseline measurements collected at home in a familiar environment at the same time of the day than the study visit could have provided a more accurate measure of the infant baseline cortisol level. Several baseline samples per infant could also have improved the baseline estimation. On the other hand, this would have been more demanding for the families and might have led to a decreased number of participants.

We used AUCi for the cortisol stress response in Study I instead of mixed models as AUC was the recommended indicator for the cortisol stress response at the time (Fekedulegn et al., 2007; Khoury et al., 2015; Pruessner et al., 2003). The AUC does not always differentiate between different cortisol stress response patterns precisely (Atkinson et al., 2016). In addition, comparing the results from Study I to Studies II and III is not straightforward, because, in Study I, we did not look at the reactivity and recovery separately or calculated the AUC in Studies II and III.

Some infants were breastfed during the study visits. We instructed the mothers to schedule the feeding before the study visit to avoid possible effects of feeding to the cortisol levels and quality of samples. We could not or wanted to prohibit the mothers from feeding their children for ethical reasons, therefore, we recorded the feedings during the study visit and statistically controlled for them in the analyses.

SSRI medication of the mothers might partly explain our results in Studies II and III as SSRI exposure might alter infant HPA axis functioning (Oberlander et al., 2008; Pawluski et al., 2012). When prenatal and postnatal SSRI-medicated mothers were excluded from the analyses, the association between PPD exposure and infant cortisol reactivity at the age of ten weeks diminished. On the other hand, SSRI-

medicated mothers experienced higher levels of PPD than the rest of the mothers in the study, and the observed reduction in the associations was also due to lower level of PPD exposure. In addition, the sensitivity analyses had a smaller sample size and hence less power to detect the associations.

We used depressive and anxiety symptoms as a prenatal stress exposure for the infants, and for this reason, our results cannot be generalized to other types of stress exposures during pregnancy such as daily hassles, low social or partner support, relationship satisfaction, a low socioeconomic status or natural disasters, although many of these factors might associate with each other depending on the quality of the population.

A stressor used at six months of age differed slightly from the other age groups in that we did not have paediatric examinations for the infants but only a nurse visit. The nurse did not examine the infant as the paediatrist did but discussed with the parents instead. Physical examination and removing the clothes are considered as a mild stressor for the young infants, and it might be that the experienced stressfulness of the study visit was somewhat diminished at the age of six months compared to other ages.

Only 101 (22%) infants out of the total 462 participated in all three study visits. Thirty-six percent of the infants participated in two study visits and 41% in only one visit. For this reason, we performed a separate analysis for each age group instead of combining all infants in the same longitudinal data analysis in Study III. This is not the ideal statistical method for the longitudinal developmental analysis, but, as the associations between PPD exposure and infant cortisol stress responses are reportedly weak, the statistical power would not have been sufficient to detect possible associations among the 101 infants only.

We did not measure any inflammatory biomarkers, such as cytokines, for immune system activation in Study I to better characterize the status of the immune system among the infant with and without rhinovirus and PPD exposure and to demonstrate possible differences in the activation of the immune system caused by the rhinovirus between the groups.

6.3 Suggestions for future studies

Cortisol recovery from stress has been strongly underrepresented among the cortisol studies during infancy and childhood. To gain a better understanding of the development and functioning of the whole cortisol stress response, future studies should include the recovery phase as part of their analyses. The duration of the study visits needs to be longer for the detection of the recovery, which is sometimes a challenge, especially with children. Still, to model the rate of the recovery, one does not need to wait until the cortisol levels are back exactly at the basal level, which

might take as long as an hour. As long as the follow-up after the onset of stress is long enough to detect that the cortisol levels have started to decrease, the steepness of the recovery slope can be calculated. Some previous studies have reported cortisol measures until 40 minutes after the stress, during which they have observed the recovery of the cortisol levels, but study questions and hypotheses have focused on cortisol reactivity only. This suggests that the length of the study visit may not be the limitation but rather the way researchers tend to interpret their data.

Typically, researchers focus on one organ, system and/or disease, which is understandable as that alone is often challenging enough. The amount of data that can be collected by a single researcher or research group has its limits. Still, the HPA axis regulates and interacts with so many other physiological systems that broader research on system-level interactions could promote the understanding of the mechanism behind well-being and diseases. Research on HPA axis development and functioning and its role in the development of diseases would benefit from more integrative research methods where other biomarkers from the immune system, the gonadal axis and/or brain imaging would have been taken into account and used to further explain the observed variation in cortisol stress responses in PPD-exposed and healthy children and between sexes.

As increasingly has been noted, sex differences should always be taken into account in analyses as well as ensuring that the sample size will allow sex interactions and sub-group comparisons between and within the sexes.

One challenge in interpreting the results from various cortisol studies and explaining the difference between them is the various cortisol indicators and statistical analyses that have been used. Even when the confounders and other covariates would be the same by name, such as socioeconomical status, breastfeeding, time since eating and feeding prior the cortisol sampling etc., they can be different in how they have been categorized and utilized in the models. Combining collected cortisol data from various research groups and reanalyzing the data with standardized methods could show how different or related the results are. At the same time, the smaller sample sizes that have failed to detect possible associations could be put into good use.

Child developmental studies of stress should include a stress test that would evoke the sufficient cortisol stress response in children of different ages. Among adults and older children, there are several modified versions of the Trier Social Stress test, and Puhakka et al. (2020) have suggested age-appropriate versions of the parental separation test for a standardized psychological stressor across infancy. A standardized pediatrician examination combined with sampling that was used successfully in our studies to evoke a cortisol stress response in infants with a broad age range from 10 weeks to 14 months could be another possibility for a physically mild pain stressor. An advantage of the blood and nasopharyngeal sampling is that,

while it is a stressor that is relatively easy to standardize, it provides the means to collect other biomarkers that are important in cortisol research. A downside is that a study visit like this requires resources that not every research group has, such as a professional for blood sampling and collection. Another limitation is that families do not necessary participate willingly in the study visit because of the mildly painful sampling. In our study, we have noticed that providing a free doctor or nurse visit with a specialist for infants and giving feedback about blood samples, such as a basic cell count and iron concentration, could result in a satisfactory participation level.

In addition to the acute cortisol stress response, the role of the HPA axis functioning during a longer period in child development and in the health of an individual is also an important aspect for research. The hair cortisol concentration (HCC) has been used as a measure for the average cortisol output of the past few months in children, adults and during pregnancy. Most commonly, HCC has been interpreted as a marker for chronic stress (Bryson et al., 2021; Greff et al., 2019; Khoury et al., 2019; Mustonen et al., 2019). In addition, a possibility to analyze broader adrenal and gonadal steroid hormone profiles and their metabolites from a single hair sample could enhance the means to study the role of the hormone pathways, including their metabolic enzymes, in the development and health (Gomez-Gomez and Pozo, 2020). Hair, as a non-invasive sample that can be collected also at home by the study subjects themselves, is a promising tool for both clinical and research purposes. As being a relative new biomarker, there is still a need for a better consensus for the protocols and interpretation of the results in order to obtain reproducible and reliable results (Greff et al., 2019).

7 Summary/Conclusions

Our studies support the earlier findings that maternal PPD associates with the altered infant cortisol stress responsiveness. Previous research has found evidence for altered infant cortisol stress reactivity after exposure to maternal PPD, and our results further suggest that also the recovery phase of the cortisol stress response is potentially vulnerable to the influences of maternal PPD. The alterations in infant cortisol stress recovery were age- and sex-dependent, which has not been reported earlier. Ten-week-old females were more likely to show slower cortisol stress recovery after exposure to higher maternal PPD, but when they reached the age of 14 months, the rate of cortisol recovery was increased in females with higher PPD exposure. Our results suggest that even if the level of experienced psychological distress during pregnancy would be so mild that it does not associate with infant cortisol stress reactivity, it might still associate with the rate of cortisol recovery from the stressor as these two related aspects of the cortisol stress response are partly regulated via different mechanisms. This suggests that the recovery phase might be more vulnerable to the PPD exposure. In addition, PPD exposure does not necessary influence the infant cortisol stress responsiveness independently as such, but its influence on HPA axis functioning might become apparent during specific situations as when the immune systems is activated by a viral infection. We did not observe an association between maternal PPD and infant cortisol stress reactivity or the whole cortisol stress response. Instead, 10-week-old, PPD-exposed infants with subclinical rhinovirus infection during the acute stress test had a blunted cortisol stress response. PPD- or rhinovirus-exposure alone did not associate with the cortisol stress response. Follow-up is needed to evaluate whether this is more apparent among very young infants only or if the association is observable later as well. The blunted cortisol stress response was more evident among rhinovirus/PPD-exposed males than females, which further suggests that the sex-dimorphic associations between PPD exposure and infant cortisol stress responsiveness are not that straightforward. It might be that not just one of the two sexes is more vulnerable to the effect of PPD exposure, but that sex specificity might depend on the context. Further studies are needed to investigate whether an altered cortisol stress response during infancy predicts child psychosocial development and later health outcomes, such as

depression and anxiety or other stress-related diseases. The well-being of the mothers should be supported by finding the ways to decrease the experienced distress already during pregnancy that would promote the well-being of the offspring as well.

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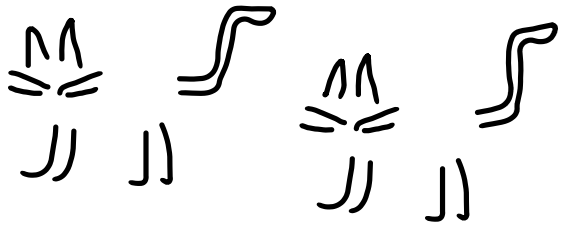
Turku, 3rd of December 2021
Susanna Kortesluoma



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