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Biological aspects of postpartum mental health: prevention opportunities and mother's perceptions

PhD Thesis

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Stinne Høgh
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Abbreviations

ACTH	Adrenocorticotrophin hormone
AUCi	Area Under the Curve with respect to increase
BMI	Body Mass Index
CAR	Cortisol Awakening Response
Cohen PSS	Perceived Stress Scale
cGDF15	Growth Differentiation Factor 15 in cerebrospinal fluid
CRH	Corticotrophin-releasing hormone
CSF	Cerebrospinal fluid
C-section	Cesarean section
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
E1	Estrone
E2	Estradiol
E3	Estriol
EPDS	Edinburgh Postnatal Depression Scale
GDF15	Growth Differentiation Factor 15
HPA axis	Hypothalamic-Pituitary-Adrenal axis
Hs-CRP	High sensitivity C-reactive protein
ICD-10	International Classification of Diseases 10th Revision
MAMA Trial	Maternal Mental Health Trial
MIC-1	Macrophage inhibitory cytokine-1
M.I.N.I.	Mini-International Neuropsychiatric Interview
pGDF15	Growth Differentiation Factor 15 in plasma
POMS TMD	Profile of Mood States, Total Mood Disturbance
PSQI	Pittsburgh Sleep Quality Index
RCT	Randomized controlled clinical trial
SSRI	Selective serotonin reuptake inhibitors
WHO-5	World Health Organization Well-Being Index

List of manuscripts included in the thesis

PAPER I Høgh S, Lange EØ, Høgsted E, Larsen K, Hegaard HK, Borgsted C, Frokjaer VG. The Cortisol Awakening Response is blunted in healthy women early postpartum. Psychoneuroendocrinology. 2024:107048.
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PAPER II Høgh S, Borgsted C, Hegaard HK, Renault KM, Bruzzone SEP, Clemmensen C, Klein AB, Frokjaer VG. GDF15 levels are not associated with Cortisol Awakening Response or mental health across the perinatal transition.
Manuscript in preparation.

PAPER III Høgh S, Hegaard HK, Renault KM, Cvetanovska E, Kjærbye-Thygesen A, Juul A, Borgsted C, Bjertrup AJ, Miskowiak KW, Væver MS, Stenbæk DS, Dam VH, Binder E, Ozenne B, Mehta D, Frokjaer VG. Short-term oestrogen as a strategy to prevent postpartum depression in high-risk women: protocol for the double-blind, randomised, placebo-controlled MAMA clinical trial. BMJ Open 2021;11:e052922. doi: 10.1136/bmjopen-2021-052922

PAPER IV Høgh S, Hegaard HK, Renault KM, Svendsen MN, Navne LE, Frokjaer VG. Women's perceptions of biological causes and potentials of genomic risk markers in postpartum depression.
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English summary

The peripartum period is a particularly vulnerable period in a woman's life due to biological, social, and emotional changes. Managing life stress, acquiring new skills, providing nurturing care, and maintaining productivity become pivotal in navigating the demanding physiological and emotional aspects of pregnancy and motherhood. Most women transition smoothly across the peripartum; however, approximately 40% experience maternity blues, and 10-14% are affected by postpartum depression, alongside others facing various levels of subclinical mental distress. Postpartum depression is a disabling disorder with a risk of negative consequences for the entire family, including infant development and future health. Several lines of evidence suggest that postpartum mental health is influenced by the rapid and substantial changes in sex hormone levels, transitioning from high levels of placenta-produced sex steroids during pregnancy to subsequent hormonal withdrawal in the postpartum phase. Women who develop postpartum depression have been proposed to be particularly sensitive to these fluctuations at a genomic level, which points to a distinct underlying pathophysiology. Consequently, the immediate and early postpartum period likely presents a unique opportunity for targeted interventions aimed at protecting maternal mental health.

This thesis aimed to contribute to the existing evidence of biological transitions during the perinatal period and the impact on maternal mental health in the early postpartum period while possibly identifying potential prevention targets.

In Paper I, we showed that mentally healthy postpartum women had a blunted Cortisol Awakening Response (CAR), significantly different from healthy non-perinatal women. The absolute evening cortisol levels were significantly lower in postpartum women than non-perinatal women. We also found a significant association between lower CAR and higher well-being scores in the postpartum group analyses. These findings suggest that a blunted CAR might serve as a healthy adaption to early motherhood.

In Paper II, we found a correlation between pregnancy levels of Growth Differentiation Factor 15 (GDF15) in cerebrospinal fluid (cGDF15) and GDF15 in plasma (pGDF15). We showed that cGDF15 was not associated with postpartum mental well-being scores and CAR. Further, we demonstrated a significant association between pregnancy pGDF15 and estradiol (E2) and estriol (E3). Our novel results highlight the distinctive hormonal dynamics during the perinatal period, including GDF15, which appear to be not correlated with mental health scores.

Paper III is a protocol for a double-blind, randomized, and placebo-controlled trial. In this study, we aim to evaluate if three weeks of estradiol treatment immediately postpartum can prevent depressive episodes in high-risk women defined by a history of perinatal depression. We further aim to assess if specific genomic biomarkers can be applied to women in this cohort, thereby offering insights for future personalized prevention strategies. This study is ongoing.

In Paper IV, we explored women's perspectives on testing for genomic risk markers that could potentially identify those at high risk of postpartum depression, employing qualitative semi-structured interviews. Our findings revealed a sense of ambiguity surrounding the idea of testing for genomic risk markers. While some women perceived it as having the potential to prevent postpartum depression and diminish stigma, others perceived it as potentially heightening awareness of depressive symptoms, thus possibly manifesting as a self-fulfilling prophecy.

Taken together, the results of the papers included in this thesis indicate that the transition from high cortisol and GDF15 levels during pregnancy to low levels postpartum presents different effects on early postpartum mental health. The results suggest that dysregulation of the HPA axis might serve as a mentally adaptive response, while GDF15 seems to have no direct relation to mental health outcomes. Finally, it is essential to consider the ambiguity demonstrated by women regarding the testing for genomic risk markers linked to hormonal sensitivity and postpartum depression before introducing new genomic risk marker technologies.

Dansk resumé

Overgangen fra graviditet til moderskab er en periode med mange biologiske, sociale og emotionelle forandringer. Evnen til at overkomme stress, drage omsorg samt være produktiv bliver afgørende, når man skal navigere i de krævende fysiologiske og emotionelle aspekter af moderskabet. De fleste kvinder gennemgår denne transition uden problemer, men omkring 40% oplever ”maternity blues” og 10-14% kvinder udvikler postpartum depression (også kaldet fødselsdepression af lægpersoner). Derudover oplever mange flere kvinder subkliniske depressive symptomer uden at have en klinisk depression. Postpartum depression har en negativ indflydelse på hele familien. Dette inkluderer barnets sundhed, herunder trivsel, kognitiv udvikling og mentalt helbred. Forskning peger på, at mental sundhed efter fødslen er påvirket af ændringer i kønshormonsniveauer, når produktionen af de placenta-producerede kønshormoner pludseligt stopper, og der dermed sker et drastisk fald post partum. Derudover er kvinder, der udvikler post partum depression muligvis særligt følsomme overfor disse udsving i kønshormoner på et genomisk niveau, hvilket tyder på, at postpartum depression har en særlig patofysiologi. Således kan der være unikke muligheder for målrettede interventioner, der kan beskytte den mentale sundhed post partum.

Formålet med denne afhandling er at bidrage til forståelsen af det komplekse samspil mellem hormonelle udsving fra graviditet til post partum og mental sundhed i den tidlige post partum periode.

I artikel 1 fandt vi, at kvinder post partum har et dæmpet kortisolresponset på opvågning (Cortisol Awakening Response (CAR)), og at både CAR og aftenkortisol var reduceret relativt til ikke-perinatale kvinder. Derudover fandt vi, i gruppen af post partum kvinder, en association mellem lavere CAR og højere mentalt velbefindende. Disse fund peger på, at et reduceret CAR er en del af en sund mental tilpasning til moderskabet.

I artikel 2 fandt vi, at Growth Differentiation Factor 15 (GDF15) i cerebrospinal væske (cGDF15) og GDF15 i plasma (pGDF15) var korrelerede. Vi viste, at GDF15 ikke var associeret med post partum mental velbefindende og CAR. Vi fandt derudover en signifikant sammenhæng mellem pGDF15 og østrogenerne; østradiol (E2) og østriol (E3). Vores resultater fremhæver de særlige hormonelle dynamikker i den perinatal transition, herunder GDF15, som tilsyneladende ikke er associeret med scores for mental sundhed.

I artikel 3, som er en protokolartikel, bygger vi på tidligere genereret viden om sammenhængen mellem østrogen og mental sundhed i et dobbeltblindet, randomiseret og placebo-kontrolleret lægemiddelforsøg. Der indsamles fortsat data til dette studie. I dette studie ønsker vi at undersøge den forebyggende effekt af tre ugers behandling med transdermal østradiol umiddelbart efter fødslen på depressive episoder hos kvinder, der er i høj risiko på baggrund af tidligere perinatal depression. Derudover undersøger vi, om specifikke genomiske biomarkører kan identificere kvinder, som har størst gavn af denne behandling. Dette vil bidrage med vigtig viden i forhold til fremtidige forebyggende strategier.

I artikel 4 undersøgte vi, gennem et kvalitativt interviewstudie, kvinders forestillinger og forventninger til screening for hormonfølsomhed i relation til risiko for at udvikle postpartum depression. Vores analyse viste, at kvinderne fandt testning for hormonfølsomhed modsætningsfyldt. På den ene side fandt de håb og tro på, at det kunne anvendes som led i forebyggelse af postpartum depression samt reducere stigmatisering. Samtidig påpegede de et potentiale for øget opmærksomhed på depressive symptomer, og at det dermed kunne blive en selvopfyldende profeti.

Samlet set indikerer resultaterne i denne afhandling, at overgangen fra høje niveauer af cortisol og GDF15 i graviditeten til lave niveauer post partum er associeret forskelligt med den maternelle mentale sundhed. Dette fremhæver kompleksiteten af de biologiske aspekter af mental sundhed post partum og antyder, at den biologiske transition også kan fungere som en del af en sund tilpasning til moderskabet. Derudover viser vores resultater, at det er vigtigt at inkludere i en strategi til eventuel fremtidig introduktion af genomiske teknologier i relation til postpartum depression, at de involverede kvinder muligvis finder disse screeningsmetoder modsætningsfyldte.

INTRODUCTION

Preface

> *"I wasn't suicidal, but I was like, I can't handle this. I really just felt like... How am I going to get out of this? How am I... I really tried to find a way out, but this baby, it was there, and it was just a huge burden. I mean, really. I wished with all my heart that he had never been born. It was truly the worst idea we had ever had, to have a baby" (Ida, interview from Paper IV)*

Entering motherhood is surrounded by expectations of a natural awakening of maternal instincts and endless love for the new life one has brought into the world. Yet, as Ida's quote suggests, this idyllic vision may not always align with reality, and postpartum depression has been aptly described as *"the thief that steals motherhood"* (1).

Over the past decades, the mechanisms influencing postpartum mental health have been intensively studied. Significant efforts have been directed toward understanding the etiology and developing strategies for treating and protecting mental well-being during a period that is supposed to be associated with happiness and good health.

The endeavor of this thesis aims to contribute to the understanding of the complex biology underlying postpartum mental health. The thesis comprises a general introduction to the concept of postpartum mental health and an introduction to biological factors of relevance for mental health across the perinatal transition used in this thesis, i.e., the Hypothalamic-Pituitary-Adrenal (HPA) axis dynamics postpartum, the Growth Differentiation Factor 15 (GDF15) and estrogens. Subsequently, the four papers included in the thesis will be presented and discussed (Figure 1). In addition to the quantitative studies of biology and postpartum mental health, this thesis aims to give room for women's voices to illuminate the subjective experience of biology and postpartum depression and the potentiality of testing for genomic risk markers.

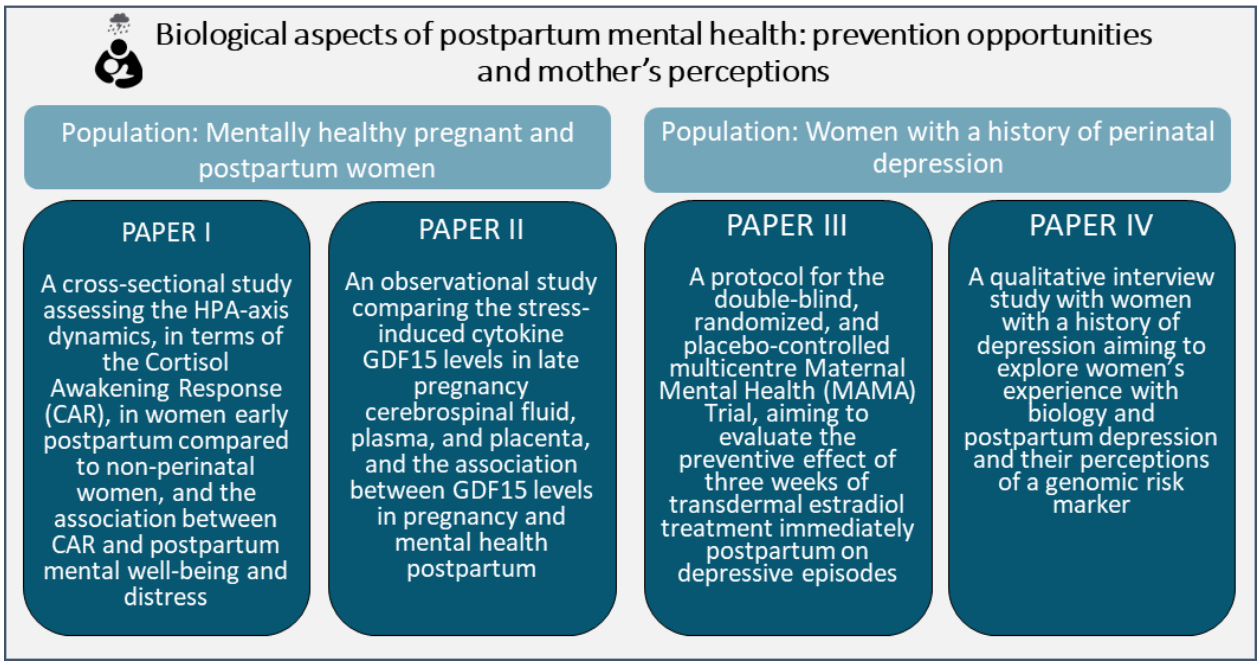

Figure 1 | The four papers of the thesis presented by aim, design, and study population.

Postpartum mental health

Mental health is defined by the World Health Organization as *"a state of mental well-being that enables people to cope with the stresses of life, realize their abilities, learn well and work well, and contribute to their community. It is an integral component of health and well-being that underpins our individual and collective abilities to make decisions, build relationships and shape the world we live in"* (2). This essential aspect of well-being shapes emotional, psychological, and overall health, thus playing a significant role in emotional experiences and functioning. The postpartum period is particularly vulnerable due to biological, social, and emotional changes. Being able to cope with life stress, make decisions, learn, and work well is crucial when dealing with the physical and emotional demands of parenthood. While most women transition healthily, approximately 40% experience maternity blues, and 10-14% experience postpartum depression, with additional individuals experiencing mental distress (3, 4). The prevalence of perinatal depression shows global variation, with an estimated rate of around 11.4% in high-income countries, with Denmark reporting the lowest prevalence among the Nordic countries at 6.48% (3, 5, 6).

Perinatal depression, as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), is a depressive episode marked by the onset of mood symptoms during

pregnancy or within the first four weeks postpartum (7). In research contexts and as defined by the World Health Organization, the postpartum period is commonly extended to encompass the first year after childbirth (8, 9).

Postpartum depressive symptoms often manifest in the immediate postpartum period, with the highest risk occurring 10-19 days postpartum (10). Despite the relatively high prevalence of postpartum depression, it is essential to recognize that many new mothers also experience mental distress symptoms such as anxiety and sleep disturbances (11-14). Notably, there is a substantial risk of recurrence in subsequent pregnancies or postpartum periods, estimated at approximately 40% (15).

Hence, postpartum depression and mental distress present a significant global public health challenge, leading to adverse consequences for the well-being of both mothers and their offspring.

Consequences of maternal postpartum depression

Experiencing depression or mental distress during the months after birth not only affects the mother but, especially if left untreated, can impose consequences on the entire family.

Women who have experienced postpartum depression are more prone to developing depressive and anxiety symptoms later in life, exhibiting lower social functioning, and facing challenges in relationships and sexual function (16). Ultimately, there is an elevated risk of suicide among women experiencing postpartum depression, with suicide identified as the leading cause of death during the period between 6 weeks and 12 months postpartum (17, 18). Partners of women experiencing postpartum depression are more likely to experience depression themselves during the postpartum period (19).

A review found that children born to mothers with postpartum depression face a higher risk of developing internalizing disorders, exhibiting poor social competence during their school years, and experiencing an increased risk of depression in adolescence (20). Furthermore, there is an elevated risk of behavioral (externalizing) difficulties, including attention deficit hyperactivity disorder and oppositional defiant disorder (20, 21). Consistently, maternal postpartum depression has been associated with cognitive outcomes in their offspring, affecting the infant's learning abilities, achievement of developmental milestones, language, and social withdrawal (16, 20, 22, 23). However, these effects do not appear to persist when evaluating the long-term outcomes of maternal depression (20). Shared genetic paths presumably contribute, at least to some extent, to the correlation between maternal postpartum depression and mental health outcomes in offspring.

However, a significant environmental contribution to the etiology of mental disorders has been observed (24), suggesting a gene-by-environment relationship. Accordingly, epigenetic changes (i.e., modifications in gene expression that do not involve alterations to the underlying DNA sequence) have been proposed to serve as a mediating factor in the long-lasting effects of early life experiences (20). Furthermore, research shows that the quality of parenting in the early years of life, which can be affected by suboptimal mental health, is an essential factor when assessing the association between maternal depression and offspring outcomes (20).

Experiences of postpartum depression

Postpartum depression has often been described with the notions of *loss* (25-27). Women who experience postpartum depression describe feelings of *loss* of their former life, *loss* of self-reliance, *loss* of personal space, *loss* of control, and even a *loss* of sense of self (25-29). The feelings of loss of control and identity have been described as negatively impacting self-esteem and self-image (27, 30). Women with postpartum depression have often described how their feelings of loneliness were a result of self-isolation related to the stigma they felt related to their illness (31). The stigma has been related to fear of judgment by others as a bad mother and self-stigmatization in not living up to the self-made ideal of "the perfect mother" (26, 27, 30, 31). Women experiencing postpartum depression have expressed difficulties in bonding with their newborns, and some have had thoughts about giving the infant up for adoption. Also, women have described not finding joy in caring for their infants, instead fulfilling their needs mechanically (30). The significance of support from families and friends in coping with postpartum depression has been highlighted, alongside the crucial role of professional support in ensuring the well-being of both the mother and her baby (27).

Women experiencing postpartum depression often attribute their depressive symptoms to personal vulnerability rather than perceiving them as part of an illness (28). The feelings of stigmatization and fear of being judged by others have been found to hold women back from seeking help from professionals (27).

Risk factors for postpartum depression and mental distress

The etiology of postpartum depression is multifaceted, and previous studies and systematic reviews have identified several psychosocial-, pregnancy-related-, and biological factors for

developing postpartum depression (32-37). The psychosocial risk factors for postpartum depression include stressful life events, history of perinatal depression, lack of social support, violence, history of depression, family history of psychiatric illnesses, and age (33-37). The pregnancy-related risk factors for postpartum depression comprise gestational diabetes, cesarean section, poor sleep quality, unintended pregnancy, hyperemesis gravidarum, and preeclampsia (32, 34, 36). Regarding the biological risk factors, results from a comprehensive umbrella review of systematic reviews and meta-analyses of observational studies have highlighted the serotonin-transporter-linked polymorphic region (5-HTTLPR) polymorphism and vitamin D deficiency. Nevertheless, the observed associations were modest in strength (36). Another systematic review of biological factors found hypothalamic-pituitary-adrenal dysregulation, inflammatory processes, and genetic vulnerabilities as the most substantial risk factors (35). However, as concluded by the two reviews, more research is needed on the influence of biology on postpartum depression (35, 36).

Preventing postpartum depression

Given the profound impact that postpartum depression can have on both mothers and their families, it is essential to prioritize efforts aiming to prevent it. Counseling interventions, particularly cognitive behavioral therapy and interpersonal therapy, have emerged as effective strategies for mitigating the risk of postpartum depression (38).

In addition to counseling, specific exercise programs have shown promise in reducing the incidence of perinatal depression; however, only minor effects have been found on the preventive effect (38, 39). Moreover, a meta-analysis conducted in 2021 examining the effectiveness of antidepressant treatment for postpartum depression showed that selective serotonin reuptake inhibitors (SSRIs) were associated with a reduction in depressive symptoms during the 5 to 12-week follow-up period (40). Additional research is essential to gain a deeper understanding of the effectiveness of preventive measures for postpartum depression (40, 41).

Biological transitions and postpartum mental health

Throughout pregnancy and the postpartum period, significant shifts occur within the endocrine system. While these changes might be deemed pathological in non-pregnant individuals, during

pregnancy, they function to safeguard and sustain the developing fetus. These alterations encompass a spectrum of hormones and cytokines, pivotal in mental health and disease. Interestingly, across the reproductive age, women exhibit a twofold higher likelihood of experiencing depression compared to men, with the risk notably altered during hormonal transition phases like menarche, pregnancy, postpartum, and menopause (42-45). Epidemiological studies indicate that the peak risk for severe psychiatric disorders during the pregnancy-postpartum phase occurs in the immediate postpartum period, specifically within the first three weeks after childbirth (10).

Moreover, a study by Putnam et al. found that depression with onset within the first eight weeks postpartum was categorized as severe in more than 20%, which was four times higher than depression with onset during pregnancy (antenatal depression) (46). The study further found that women with onset of depression during the first four weeks postpartum were more likely to have an anxious, anhedonia subtype of depression (46). This timing coincides with the large hormonal decline postpartum and adds to the hypothesis that there may exist noteworthy conceptual and biological distinctions influencing the severity and nature of depression between women with symptom onset during pregnancy versus those experiencing it postpartum. Moreover, fathers do not demonstrate an elevated risk of severe psychiatric disorders in the initial year postpartum (10), thus providing additional support for hormonal influences on maternal postpartum mental health.

Estrogens and postpartum mental health

During pregnancy, sex steroid hormones increase rapidly to support the growing fetus (47). Estrogens, i.e., estrone (E1), estradiol (E2), and estriol (E3), increase up to 300 times the levels of a non-pregnant state during pregnancy, with estriol undergoing the most substantial increase from nearly non-detectable levels (48-50).

E2 is primarily produced by the placenta, and all estrogens contribute to maintaining the pregnancy and growing the fetus (47, 48, 51). Upon the delivery of the placenta, the synthesis of placenta-derived estrogens stops abruptly, leading to an immediate decline in E1, E2, and E3 (49).

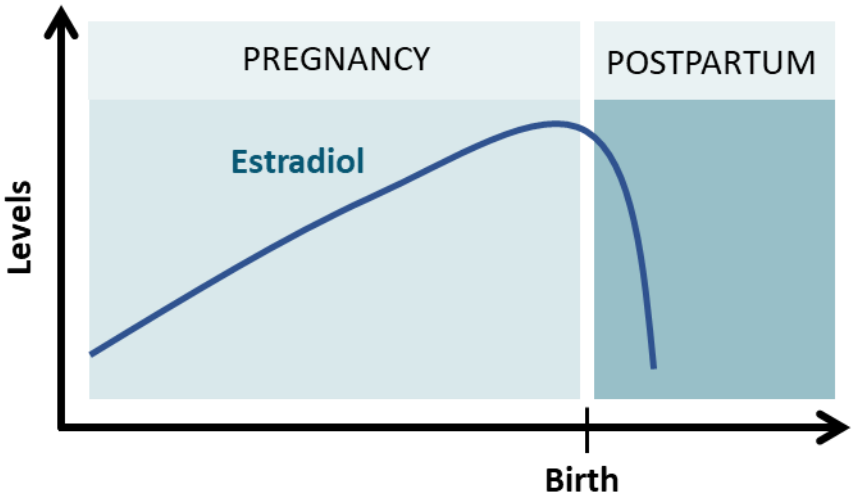

Figure 2 | Schematic illustration of Estradiol (E2) concentrations during the perinatal transition.

Studies assessing the influences of estrogens on maternal mental health have primarily focused on Estradiol (E2). Interestingly, a study has presented evidence indicating that manipulation of sex hormones, which were induced by pharmacological manipulation using a gonadotrophin-releasing hormone agonist, could induce subclinical depressive symptoms in approximately 12% of healthy non-perinatal volunteers. This phenomenon was associated with changes in estradiol levels (52). Estradiol is known to influence important domains and key brain regions that are known to be impaired in women with major depressive disorder (53, 54).

Some studies assessing the more direct association between E2 and postpartum depression did not find an association between levels of E2 and postpartum depression (55-57), while others have found that higher E2 during pregnancy or postpartum was associated with more depressive symptoms postpartum (58-60). Additionally, one study found that a larger decrease in E2 from pregnancy to postpartum was associated with fewer postpartum depressive symptoms (60). Conversely, one study found that lower E2 postpartum was associated with depressive symptoms (61). The disparities might be due to differences in methods, such as sample size, timing of blood sampling, assessment of depressive symptoms and study population. Additionally, transdermal estradiol seems to be useful in preventing depressive symptoms in perimenopausal women, which is another group of women who experience a hormonal transition (62).

Factors like estrogen sensitivity, i.e., that certain women may display an increased sensitivity to fluctuations in sex steroid hormones, particularly estradiol, have been suggested as leading to subsequent neurobiological effects on mood and behavior (63). This is supported by recent

research that identifies markers of genomic estrogen sensitivity, specifically DNA methylation and gene transcription profiles, associated with the risk of developing perinatal depression (64-66). Thus, postpartum likely has a specific pathophysiology, offering a unique opportunity to protect maternal mental health and ideally offering prevention to women at high risk.

The Hypothalamic-Pituitary-Adrenal axis and postpartum mental health

The Hypothalamic-Pituitary-Adrenal (HPA) axis is essential in regulating the stress response. When exposed to a stressor, the hypothalamus releases Corticotrophin-releasing hormone (CRH), which, in turn, stimulates the release of adrenocorticotrophin hormone (ACTH) from the pituitary gland. ACTH then prompts the release of cortisol from the adrenal glands. In a self-regulating mechanism, cortisol provides feedback to glucocorticoid and mineralocorticoid receptors in the pituitary and hypothalamus to control its secretion (67).

Stress is a state of physical or mental tension induced by factors that can change a social condition. A "stressor" is any physical or psychological stimulus that induces stress. In the early stages of a stressor, cortisol promotes an adaptive response by triggering behaviors crucial for survival, such as increased alertness, vigilance, arousal, and heightened awareness (68). In response to infection or severe tissue damage, the HPA axis can be activated by proinflammatory cytokines (reviewed in (69)).

The HPA axis undergoes significant changes during pregnancy and postpartum. Increased levels of estradiol and CRH produced by the placenta result in a substantial thousand-fold rise in cortisol levels (70-72). The high levels of CRH during pregnancy contribute to the implantation process and protect the fetus from the maternal immune system (72). In the postpartum period, the HPA axis undergoes an acute withdrawal of placenta-produced CRH, leading to a decline in cortisol levels (67, 70).

The secretion from the HPA axis follows a diurnal pattern, reaching its highest values in the morning and lowest values during the nighttime. The cortisol awakening response (CAR) is commonly used as a measurement to evaluate the stress response. The CAR entails measuring the dynamics of the HPA axis, including the increase in cortisol levels and the downregulating phase within the initial hour after waking up (73-75). Due to the response to awakening, which is closely tied to the circadian rhythm and easy to measure, the CAR is a favorable marker of the HPA axis dynamics. It offers insights into an individual's ability to adapt to daily demands (74, 76-79). The dynamic capacity of the hypothalamic-pituitary-adrenal (HPA) axis is crucial for adaptive

responses to stress and plays a pivotal role in maintaining mental health (74, 77, 78). There is a high correlation between cortisol in saliva and unbound free cortisol levels in blood, making it a relevant indicator for assessing, e.g., stress responses (71, 80).

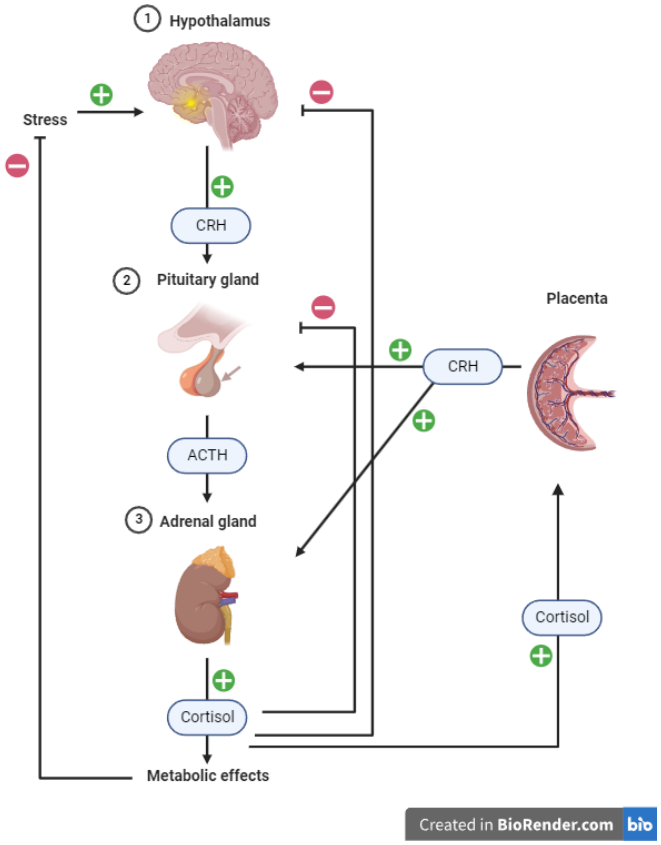

Figure 3 | Schematic representation of the maternal-placental hypothalamic-pituitary-adrenal axis during human pregnancy. CRH, Corticotrophin-releasing hormone; ATCH, Adrenocorticotropic hormone. Adapted from "Hypothalamic-Pituitary-Adrenal Axis", by BioRender.com (2024).
Retrieved from https://app.biorender.com/biorender-templates.

Only two studies have evaluated CAR in postpartum women compared to non-perinatal women, with conflicting findings. One study discovered a significant decrease in CAR among healthy women six months postpartum (N=51) compared to non-perinatal women. Conversely, another study observed no discrepancy in CAR between healthy women at 6-8 weeks postpartum (N=79) and non-perinatal women (81, 82). However, these two studies solely captured the rising phase of the CAR by measuring at only two time points.

Studies indicate that the HPA axis may be involved in the dysregulation of perinatal mental health; however, the results are conflicting (81-85). Four studies have evaluated the association between CAR and maternal postpartum mental health, also with conflicting results. Two studies found a

lower CAR in postpartum women than non-perinatal women (81, 82), while another two found no association (85, 86). All the studies assessed CAR based on two saliva samples, hence not capturing the downregulating phase of the response (75, 87).

Growth Differentiation Factor 15 and postpartum mental health

The (GDF15) cytokine, initially identified as macrophage inhibitory cytokine-1 (MIC-1), is part of the transforming growth factor β (TGF-β) superfamily and was first identified in 1997 by Bootcov and colleagues (88). GDF15 is an autocrine regulator of macrophage activation and is triggered in response to stimuli inducing cell stress (88, 89).

GDF15 functions via a recently identified receptor known as glial-derived neurotrophic factor receptor alpha-like in the hindbrain, which initiates signaling pathways through the tyrosine kinase receptor Ret (90). In humans, GDF15 circulating levels increase with factors such as age, smoking, and drug use (91, 92). Notably, GDF15 is significantly elevated by tissue damage and has been primarily linked to appetite regulation and exercise-induced weight loss (91, 93, 94). Notably, research has shown elevated GDF15 levels in individuals experiencing psychosis, those with a history of ischemic stroke followed by depression, and individuals diagnosed with depression (95-97).

The GDF15 is expressed at high levels in the placenta (98). During pregnancy, levels of GDF15 increase significantly as pregnancy progresses, reaching an almost 200-fold increase in serum and more than 3-fold increase in cerebrospinal fluid (CSF) compared with levels in a non-pregnant state (99-101). Notably, the rise in GDF15 levels is more prominent in humans than in mice and rats, indicating a potentially vital role for GDF15 in human pregnancy (99). It has been suggested that the increased levels of GDF15 during pregnancy have a protective role in maintaining the pregnancy and protecting the rapidly growing fetus by suppressing the production of proinflammatory cytokines (101, 102). Despite limited knowledge of the role of GDF15, it has been suggested that GDF15 may act as a sentinel, preventing systemic exposure to toxins by introducing nausea, vomiting, and conditioned aversion (89).

In pregnancy, high levels of GDF15 have been associated with nausea and vomiting and with the diagnosis of hyperemesis gravidarum (103-106). Moreover, GDF15 has been proposed as a potential biomarker for pregnancy complications, showing associations with conditions such as preeclampsia, gestational diabetes in the third trimester of pregnancy, and miscarriage (107-110).

Intriguingly, research indicates that women carrying a female fetus exhibit higher serum levels of GDF15 than those carrying a male fetus (100, 111).

The association between GDF15 in late pregnancy and postpartum mental health has not yet been evaluated. However, it is widely acknowledged that cytokines play a role in major depression, as evidenced by notably higher concentrations of tumor necrosis factor-alpha and Interleukin-6 in depressed individuals (112). In the postpartum period, cytokines have also been associated with depressive symptoms (113, 114).

GDF-15 is triggered in response to stressors (89, 91). It has recently been associated with activating the endocrine response to stress through the Hypothalamic-Pituitary-Adrenal (HPA) axis in non-perinatal mice and rats (115). Notably, the study revealed that while GDF15 was not necessary for HPA axis activation in response to infection-related stress, it was essential for toxin-related stress. This suggests that GDF15 functions as the organism's mechanism to cope with and respond to significant external threats or stress (115). Rodents are usually good models of human conditions, reflecting physiological processes, which is also the case with the HPA axis (116). However, a recent study found that levels of circulating GDF15 increased up to 100-fold in human pregnancies but only twofold in rodents, suggesting that rodents might not be applicable as models when studying GDF15 and pregnancy (99).

Potentials of genomic risk markers and postpartum mental health

Identifying genomic risk markers for enhanced sensitivity to estrogen signaling and postpartum depressive symptoms is currently in its early stages (64, 66, 117). In the field of perinatal depression research involving genomic risk markers, there is an expectation that these markers possess a force waiting to fulfill its potential in the future. Genetic researchers are optimistic that the discovery of these genomic risk markers holds the potential to protect maternal mental health in the future (64, 66, 117, 118). The articulation around genomic markers for perinatal depression is characterized by hopeful expressions such as *"enhance the clinical management of psychiatric treatment during the course of pregnancy."* (66), "*providing avenues of improved diagnostics and treatment"* (117), and *"Identification of potential genetic and epigenetic biomarkers may potentially enhance the accuracy of current predictive models, facilitating early intervention to improve outcomes for mothers and infants"* (118).

Using the notion of *"potential"* or *"potentiality"* for hormonal sensitivity and postpartum depression via genomic risk markers creates positive expectations of something powerful to

happen. According to Taussig and colleagues, potentiality is a central concept within precision medicine and represents a temporal complexity about something that does not (yet and may never) exist (119). In contrast to notions of possibility, promises, and expectations, all of which denote something understandable and definitive, potentiality is often depicted with more ambiguity (119). serves to describe human capacities, envision human futures, and illustrate undesirable outcomes. Consequently, potentiality can be employed to articulate both optimistic perspectives and concerns. The concept of potentiality introduces numerous "unknowns" and can be viewed as the counterpart to risk within biomedicine (119).

In biomedicine, potentiality is typically infused with hope and visions of the positive impacts of novel medical interventions and assurances of disrupting unfavorable or undesired outcomes (119). In biomedicine, potentiality is disconnected from what will definitively occur but instead holds optimistic expectations for a favorable future. Following Taussig and colleagues, potentiality in anthropology is characterized by three distinct meanings: firstly, it pertains to the evident transformation in the future, whether or not interventions take place; it involves transformations that have the possibility to manifest in various forms; and thirdly, it encompasses transformations that can be influenced by human intervention to evolve into something other than it is (119).

The discourse surrounding genetic testing and precision medicine is frequently presented positively, emphasizing hope, potential, capability, and power (119, 120). Yet, framing genes as imbued with potential simultaneously elicits desire and fear, necessitating a nuanced engagement with hope and risk (120). In biomedicine, the concept of potentiality has been applied in various contexts, including direct-to-consumer personal genomic tests, translational medicine, newborn screening, cancer genetics, and human embryonic stem cell research (120-125).

OBJECTIVES

The overall aim was to contribute valuable insights into the intricate interplay between hormonal changes and maternal mental well-being, with hopes of fostering a more comprehensive understanding and potential paths for prevention.

Paper I

In this study, we aimed 1) to assess if the HPA-axis dynamics in terms of the Cortisol Awakening Response (CAR) in women early postpartum differ from non-perinatal women; 2) to evaluate if absolute evening cortisol levels in women early postpartum differ from non-perinatal women; and 3) in the postpartum women group, evaluate the association between HPA-axis dynamics in terms of the Cortisol Awakening Response (CAR) and maternal mental well-being.

Paper II

In this study, we aimed to 1) evaluate the association between pregnancy cGDF15 levels and cortisol early postpartum, 2) evaluate the association between pregnancy cGDF15 levels and mental health in pregnancy, and postpartum and 4) evaluate the association between pGDF15 and estrogens and high-sensitivity C-reactive protein (hs-CRP) in late pregnancy.

Paper III

Paper III is a protocol for the Maternal Mental Health (MAMA) Trial. Currently, the trial is ongoing. The primary aim of the MAMA trial is to evaluate if transdermal estradiol treatment for three weeks immediately postpartum can prevent depressive episodes in women who are at particularly high risk due to a history of perinatal depression.

Paper IV

Paper IV presents the first result from the MAMA Trial. This study aimed to explore perceptions of testing for genomic risk markers that potentially identify women at risk for developing postpartum depression among women with a history of postpartum depression.

METHODS

Paper I

Participants and design

We included women from The Center for Integrated Molecular Brain Imaging (Cimbi) database (126). This database has a unique dataset of HPA-axis dynamics and absolute cortisol levels from healthy volunteers and postpartum women, including women from a recently completed study (ClinicalTrials.gov Identifier: NCT03795688).

In the study, we included women aged 18-45 years who were approximately five weeks postpartum and natural cycling healthy non-perinatal women, all with concurrent data on cortisol dynamics (CAR). Women who had a Body Mass Index (BMI)≥35 kg/m2, if there was no status on contraceptives, or if they reported the use of corticosteroids, were excluded. See Figure 4 for an overview of the study design.

Our analysis involved a total of 141 women. Specifically, we compared 50 postpartum women with 91 non-perinatal women in terms of CAR. We also compared the absolute evening cortisol levels of 50 postpartum women with 93 perinatal women. Lastly, we conducted a within postpartum cohort analysis of CAR and postpartum mental health, which included 42 postpartum women.

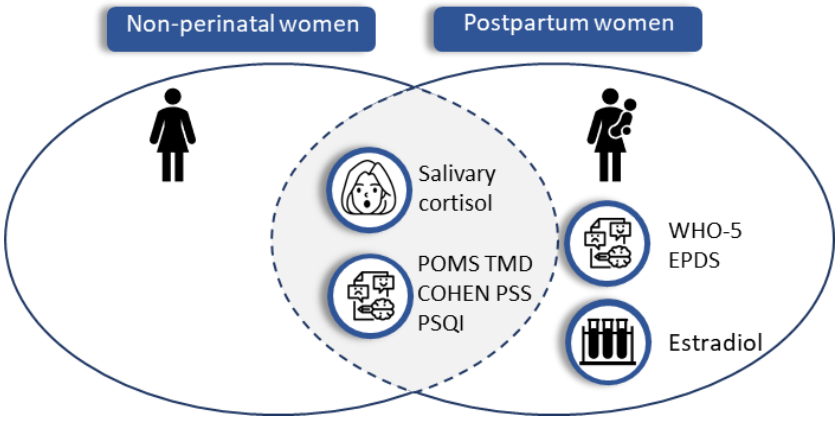

Figure 4 | Overview of the study design for Paper I. WHO-5, World Health Organization Well-Being Index; EPDS, Edinburgh Postnatal Depression Scale; POMS, Profile of Mood States Total Mood Disturbance; Cohen PSS, Perceived Stress Scale; PSQI, Pittsburgh Sleep Quality Index.

Cortisol measurements

The participants collected serial saliva samples at home immediately upon awakening in the morning, again after 15, 30, 45, and 60 minutes, and finally before sleep. They were instructed not to brush their teeth, eat, drink (except for water), or smoke before or after completing the 5th sample. To assess compliance, participants noted the time of each sample on a self-reported form. The saliva samples were stored at a maximum of 5˚C and subsequently at -80˚C after being delivered to the laboratory by mail or in person. For assessing the Cortisol Awakening Response, we used the area under the curve with respect to increase (AUCi) using five consecutive saliva samples collected over 0-60 minutes from awakening (75, 87).

Psychometrics

To assess the mental health of the postpartum women, we used self-reported questionnaires. We used the World Health Organization Well-Being Index (WHO-5), the Edinburgh Postnatal Depression Scale (EPDS), the Profile of Mood States (POMS), and the Perceived Stress Scale (Cohen PSS) (127-130). We used the Mini-International Neuropsychiatric Interview (M.I.N.I.) to ensure that none of the postpartum women had undiagnosed severe psychiatric disorders (131). Additionally, we used the Pittsburgh Sleep Quality Index (PSQI) Global score in a sensitivity analysis to evaluate whether the CAR was affected by sleep quality (87, 132).

World Health Organization Well-Being Index

The WHO-5 was used to assess the subjective mental well-being over the past two weeks. The WHO-5 contains five positively phrased items, which the respondent is asked to rate on a Likert scale ranging from 1 to 5, with higher scores indicating better well-being. Each item's score is multiplied by 4 to get the percentage score (0 to 100). A lower total score represents worse mental well-being (127).

Edinburgh Postnatal Depression Scale

The EPDS is designed to screen for mental distress and possible depression in new mothers (133). It covers depressive symptoms during the previous seven days. The scores range from 0 to 30, and higher scores indicate worse symptoms. A cut-off score of ≥11 in a Danish setting is considered

the optimal score for depression according to DSM-V and the International Classification of Diseases 10th Revision (ICD-10) criteria (134).

Profile of Mood States

The POMS assesses short-term moods, which are thought to be more fluctuating and transient. The Total Mood Disturbance (TMD) assesses the overall mental state, and scores range from -32 to 200, with higher scores indicating worse mental state (128).

Perceived Stress Scale

The Cohen PSS measures the degree to which an individual perceives their life as stressful during the past month. It specifically assesses unpredictability, a sense of control, and overwhelming demands. This 14-item scale generates a score ranging from 0 to 40, with higher scores indicating higher perceived stress (130).

Mini-International Neuropsychiatric Interview

The M.I.N.I. is a structured diagnostic interview designed to evaluate psychiatric disorders in alignment with both DSM-V and ICD-10 classifications. It is structured to be consistent with the time frame for DSM-V and is also compatible with the ICD-10. It covers 19 psychiatric diagnoses, capturing information related to current, past, or lifetime occurrences (131).

Pittsburgh Sleep Quality Index

The PSQI was used to assess sleep quality during the past month and is reliable for use in a sample of pregnant women (135). It contains 19 items related to sleep quality. The Global scores global PSQI score ranging from 0 to 21 assesses the overall sleep quality. Higher global scores indicate worse sleep (132).

Statistical analysis

We used a multiple linear regression model to compare CAR and absolute evening cortisol data from women in the early postpartum period with that of non-perinatal women. The model was adjusted for age and cortisol levels at awakening (i.e., sample #1). We conducted a sensitivity analysis employing the "Area (nmol/L*min) under the cortisol curve above baseline" to restrict our evaluations on positive contributions to the CAR. Additionally, to account for the potential

impacts of batch discrepancies and differences in assay types regarding cortisol measurements, we employed the Generalized Least Squares (GLS) test. We also included variables that potentially could affect the CAR, i.e., sleep quality and perceived stress scores in a sensitivity model.

The absolute evening cortisol levels were below the lower detection limit of 0.55 nmol/L in 40 out of 154 samples (36 postpartum and 4 non-perinatal women). Therefore, we imputed these values as 0.54 nmol/L.

In the postpartum cohort, we evaluated the association between CAR and mental health in the early postpartum period (WHO-5, EPDS, POMS TMD, and Cohen PSS) using multiple linear regression models. We included estradiol (E2) levels and parity in the model as covariates.

We applied the Bonferroni-Holm correction to account for multiple comparisons in the exploratory analyses.

The statistical software RStudio, version 4.2.2 was used to analyze all data (136). P-values <0.05 were considered statistically significant.

Paper II

Participants and design

Participants in Paper II were included under the same study protocol as the postpartum group in Paper I (ClinicalTrials.gov Identifier: NCT03795688).

In Paper II, we leveraged the longitudinal study design by incorporating data from three study visits. In this study, 95 healthy pregnant women, aged between 18 and 40 years, scheduled for planned cesarean section (C-section) were included in late pregnancy (typically the day before the planned C-section). Reasons for having the planned C-section were fetal breech position, previous C-section, previous myomectomy, obstructing fibroid, previous rupture of the anal sphincter, and placenta previa. We excluded women with a history of severe somatic or psychiatric illness, pre-pregnancy BMI below 18 or above 35, severe postpartum hemorrhage (>1000 ml), significant neonatal morbidity, use of antidepressants, substance abuse, or non-fluency in Danish.

On the day of the C-section, 0.5-1 ml of cerebrospinal fluid (CSF) was obtained as part of the anesthetic procedure for spinal anesthesia during the C-section. The CSF was promptly transferred to dry ice and stored at -80°C until GDF15 analysis. Placenta biopsies were extracted from healthy cotyledons on the maternal side within 30 minutes of birth, immersed in PBS Eppendorf, and

submerged in 1 ml RNAlater. The biopsies were stored at 4°C for 24-48 hours, then drained of RNAlater and stored at -80°C until analysis.

At five weeks postpartum, blood samples were collected from the women, and they completed mental health questionnaires. The postpartum women were provided with a saliva sample kit for at-home collection, specifically designed for serial cortisol determination. See Figure 5 for an overview of the study design.

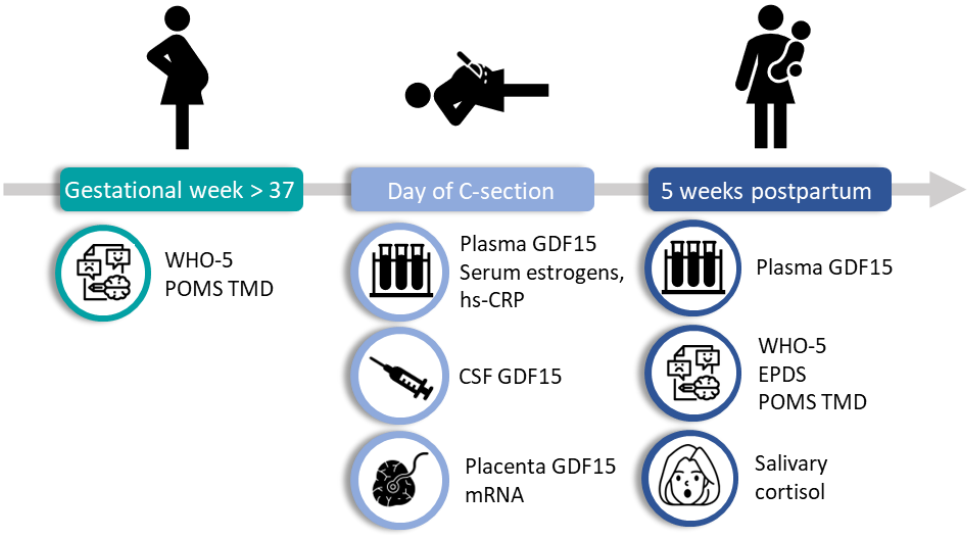

Figure 5 | Overview of the study design for Paper II. GDF15, Growth Differentiation Factor 15; hs-CRP, high sensitivity C-reactive protein; CSF, cerebrospinal fluid; WHO-5, World Health Organization Well-Being Index; EPDS, Edinburgh Postnatal Depression Scale; POMS, Profile of Mood States Total Mood Disturbance.

GDF15 measurements

GDF15 in CSF and plasma

GDF15 was measured in plasma and CSF using the Quantikine ELISA Human GDF-15 Immunoassay (ELISA, R&D systems, catalog no. DGD150). A collaborating laboratory performed the ELISA assays, which were done in accordance with the manufacturer's protocol.

RNA extraction & cDNA synthesis

A collaborating laboratory did the RNA extraction and cDNA analysis. Briefly, placenta biopsies were obtained from healthy cotyledons on the maternal side within 30 minutes of birth, utilizing an 8 mm sterile punch biopsy. The placental tissue was subsequently homogenized in Trizol reagent (QIAzol Lysis Reagent, Qiagen) with a stainless-steel bead (Qiagen) and a TissueLyser LT (Qiagen), operating for 3 minutes at 20 Hz. Quantification of placenta mRNA was conducted

using the reference gene Rpl13a. For a more comprehensive understanding of the methodologies employed, please refer to the detailed description provided by Klein et al. (99).

Estrogens

Estrone (E1), estradiol (E2), and estriol (E3) concentrations in serum samples were determined through liquid chromatography-tandem mass spectrometry (LC-MS/MS) following liquid-liquid extraction, as outlined in the methodology provided by Frederiksen et al. (137). E3 was exclusively analyzed during pregnancy since levels are typically below the limit of detection (12.3 pmol/L) in non-pregnant states (49, 137).

High sensitivity C-reactive protein

High-sensitivity C-reactive protein (hs-CRP) was assessed using serum stored at -20 degrees Celsius and analyzed on a Cobas 8000 with a c502 module by a latex particle-based immunoassay (LIA) turbidimetry method.

Cortisol measurements and psychometrics

To assess the HPA-axis dynamics, we used the Cortisol Awakening Response (CAR) described in the methods of Paper I. Further, we used the absolute cortisol levels at awakening for exploratory analysis.

To assess the mental health of the postpartum women, we used the self-reported questionnaires WHO-5, POMS, and EPDS. The methods of Paper I provide a detailed description of the psychometric instruments.

Statistical analysis

We evaluated the correlation between cGDF15 and pGDF15, as well as placenta *GDF15* mRNA, using Pearson correlation coefficients and 95% confidence intervals (CI). Furthermore, we performed Pearson correlation coefficient analyses between cGDF15 and pGDF15 and placenta *GDF15* mRNA multiplied by placental weight.

The association between cGDF15 and mental health or cortisol outcomes was evaluated in multiple linear regression models adjusted for age, E2, and hs-CRP. The WHO-5 outcome and the CAR were considered our primary outcomes in the analysis.

Finally, a linear regression model was used to analyze the association between pGDF15, estrogens (E1, E2, E3), and hs-CRP.

We used the Bonferroni-Holm correction method in our exploratory analyses to account for multiple comparisons. The statistical analysis was conducted with RStudio, version 4.2.2 (136). Results were considered statistically significant at the 5% level.

Paper III

Participants and design

The Maternal Mental Health (MAMA) Trial is a double-blind, 1:1 randomized, placebo-controlled multicenter trial, adhering to the guidelines outlined by the Consolidated Standards of Reporting Trials recommendation for randomized controlled clinical trials (RCTs) (138). The MAMA trial involves four obstetrics departments organized under Copenhagen University Hospital. The protocol is registered on ClinicalTrials.gov Identifier: NCT04685148.

Inclusion criteria for participating in the MAMA trial are a history of perinatal depression (onset during pregnancy or within the first six months postpartum), 18 to 45 years of age, and singleton pregnant with gestational age ≥34+0 weeks. Women experiencing moderate to severe depression in the current pregnancy, who have severe psychiatric disorders, a history of suicide attempts unrelated to depressive episodes, neurological disorders, significant somatic illness, risk factors for thromboembolic disorders, hypertension or preeclampsia, contraindications for estrogenic treatment, current use of psychotropic drugs, non-proficiency in Danish, a pre-pregnancy body mass index >35 kg/m2, ongoing alcohol or drug abuse are not eligible for participation. In case of severe postpartum hemorrhage (>1500 mL) or perinatal death of the infant, women are excluded from continuing in the trial. See Figure 6 for an overview of the study design.

Intervention

Participants are randomly assigned to receive either estradiol patches (200 µg/day) or placebo patches within 48 hours of childbirth. The participants self-administer the patches twice weekly for three weeks. A dedicated phone line is available for participants to seek assistance with any questions regarding patch administration or the trial in general. Compliance with the trial protocol

is assessed through a form completed by participants during each patch change. Noncompliance is failure to administer the patches for four or more days within three weeks.

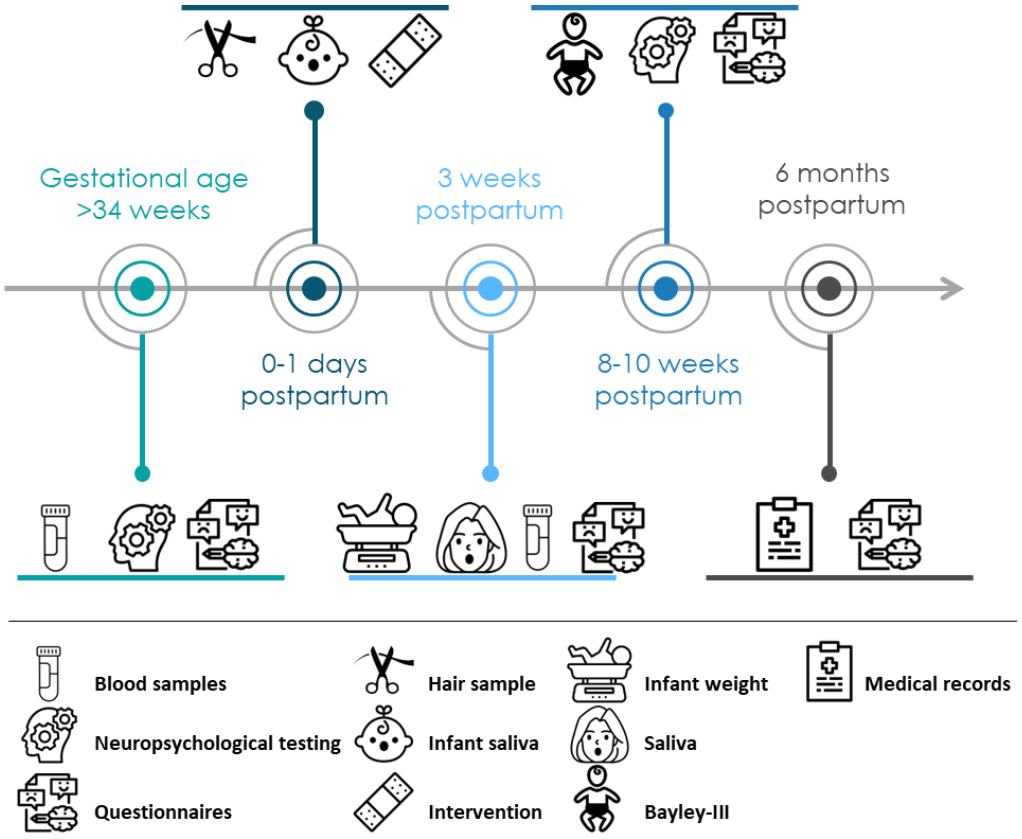

Figure 6 | Overview of the study design for The Maternal Mental Health (MAMA) Trial (Paper III).

Paper IV

Participants and design

The interview study was conducted in conjunction with the MAMA clinical trial and comprised women from the Capital Region of Denmark. Women who had enrolled in the MAMA Trial between January and April 2022 were invited to participate in the interview study. They received an information letter and in-person details about the study, covering its objectives, voluntary participation, and confidentiality. Women with a self-reported history of postpartum depression, verified by a psychiatrist, were eligible to participate in the study. By using a purposive sampling strategy, we ensured that the women varied in background and that both women experiencing and not experiencing a recurrence of postpartum depression were represented (139).

We performed individual, semi-structured interviews with the women in their homes. Most interviews took place in the presence of their 3-5-month-old infant, either sleeping or awake during the interview. The interviews lasted one hour on average (ranging from 45 minutes to one hour and 45 minutes).

For the interviews, the transdisciplinary group of clinicians and researchers developed an interview guide encompassing key themes related to women's experience of postpartum depression. This guide covered aspects such as their experiences, reflections on triggering factors, and perspectives on the biological aspects of postpartum depression. Notably, the theme guide also covered the women's view on hormone sensitivity testing to assess the risk of postpartum depression (Table 1).

SH conducted all interviews, and for quality assurance, LEN, an experienced interviewer, participated in one interview to provide supervision and guidance. The interviews were audiotaped and subsequently transcribed ad verbatim. SH transcribed six interviews, and student assistants transcribed the remaining interviews.

Table 1 | Interview theme guide. Reprinted from Paper IV.

Introduction	The aim of the study, the possibility of withdrawal, audio recording, anonymity, and the way interview data will be disclosed.
Opening question	Please tell us your story about the previous birth, where you think it begins…
Guiding interview themes	• Expectations to motherhood • Experience with postpartum depression • Social interactions and relationships – *Healthcare professionals* – *Partner* – *Other relatives* • Experience with being pregnant again and thoughts of recurrence and prevention. • Reflections about triggering factors in relation to postpartum depression. • The value of genomic risk marker testing in mental illness and postpartum depression. • Perceptions of testing for genomic risk markers related to enhanced sensitivity to estradiol signaling and postpartum depression. • Perceptions of prevention and treatment of postpartum depression. • Retrospective rationalizations. Looking back on the postpartum depression. and creating explanations. • Thoughts about the future – future pregnancies and births • Questions and was there something we forgot to talk about • *Thank you*

Data analysis

We drew on a phenomenological tradition analyzing the interviews. In phenomenology, a philosophical approach founded by Edmund Husserl, the researcher explores consciousness and the structures of lived experience without causal explanations or preconceptions (140). Central to phenomenology is the notion of studying phenomena as they manifest in immediate subjective expertise and using the lifeworld of the subjects as a research field (140).

In research, phenomenology involves achieving transcendental subjectivity. In this stage, the researcher aims to stand apart from their subjectivity and not influence the object of study, allowing for a pure exploration of participants' experiences transcending beyond mere objective observations (140). This process often requires a series of reductions, such as the transcendental stage, which involves transcending everyday attitudes through "epoché" or bracketing (140). By suspending assumptions and focusing on what is inherent in consciousness, researchers can pre-reflectively access participants' experiences to grasp the essence of a phenomenon (140).

We analyzed the interviews using thematic analysis described by Braun and Clarke using NVivo software (141, 142). The six phases of thematic analysis encompassed the first thoroughly examining the interviews, making note of the initial meanings observed. Next, we systematically reviewed the data, identifying and extracting relevant areas aligned with our research aim. Subsequently, we conducted a cross-referencing of codes to identify emerging themes. These themes were then critically reviewed about the coded data, culminating in creating a thematic map illustrating the analysis. Finally, the findings were synthesized and documented (142).

In the analysis process, we ensured comprehensive data reflections by engaging researchers from diverse fields, including midwifery, obstetrics, psychiatry, and anthropology. Data redundancy identification after 12 interviews signaled data saturation, and vi decided to end the study after the 13th interview. All participants were assigned fictional names.

ETHICAL APPROVALS

All studies included in Paper I were approved by the Capital Region's Committee on Health Research Ethics in Denmark (VEK H-2-2010-108, VEK H-6-2014-057, VEK H-4-2012-105, VEK (KF)01-2006-20, VEK H-15004506, VEK H-15017713, VEK H-18029563, VEK H-2-2014-070, and VEK H-4-2011-103) (126).

Paper II was approved by the Capital Region's Committee on Health Research Ethics in Denmark (VEK H-18029563).

Papers III and IV are registered under the same trial protocol and approved by the Danish Medicines Agency (EudraCT: 2020-001592-33), the Regional Committees on Health Research Ethics in the Capital Region of Denmark (H-20036213), and the Knowledge Centre on Data Protection Compliance in the Capital Region of Denmark (P-2020-712). The manufacturers of estradiol patches and placebo patches have not played a role in developing the protocol, nor have they provided any financial support for the trial; however, they have been informed about the trial, as per the standard procedure of the Danish Medicines Agency.

Additionally, all studies included in this thesis were conducted in strict accordance with the principles outlined in the Declaration of Helsinki (143), and all participants voluntarily provided written consent before participating.

RESULTS

In the Results section, the findings of Papers I, II, and IV will be presented. For Paper III, being a protocol paper for a clinical trial, an update on the trial status and outcomes of interest will be presented.

Paper I

Cortisol samples were obtained from postpartum women, on average, 38 days postpartum (SD 11 days, range 15-69 days), while the questionnaires were completed, on average, 34 days postpartum (SD 10 days, range 16-63 days). In the initial cortisol sample collected upon waking, postpartum women displayed significantly lower absolute cortisol levels than non-perinatal women, with a mean of 6.0 nmol/L (SD 2.7) and 11.8 nmol/L (SD 5.6) (Table 1 in Paper I manuscript).

Cortisol awakening response (CAR) and absolute evening cortisol levels early postpartum compared to healthy non-perinatal women

The postpartum women showed a mean CAR (AUCi) of 38.2 nmol/L*minutes, 84% lower than non-perinatal women, who had a mean CAR of 238 nmol/L*minutes. The postpartum women had a significantly lower CAR than the non-perinatal women in a model adjusted for age and cortisol levels at awakening (β = -303, 95% CI [-429; -178], $p<0.001$) (Table 2 and Figure 7).

The evening cortisol levels for postpartum women were 80% lower than that for non-perinatal women, with a mean of 0.9 nmol/L compared to 4.5 nmol/L, respectively. Moreover, we found a significant difference in the multiple linear regression model adjusted for age (β = -3.46, 95% CI [-4.64; -2.28], $p < 0.001$) (Table 2 and Figure 7).

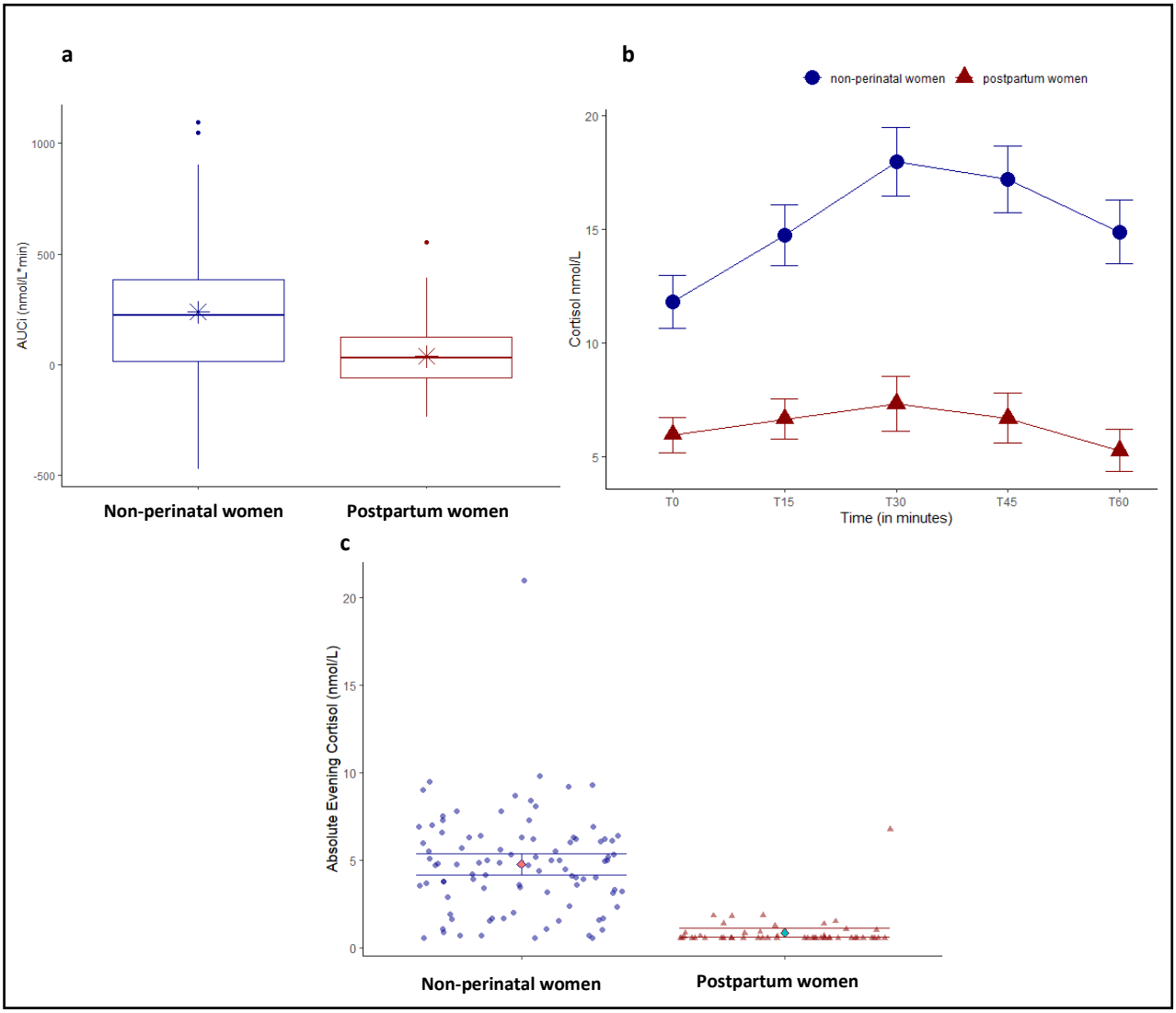

FIGURE 7 | a) Boxplot of the Cortisol Awakening Response (AUCi) in postpartum women (n=50) and non-perinatal women (n=91), b) Saliva cortisol concentrations during the first hour after awakening in postpartum women (n=50) and non-perinatal women (n=91). Error bars represent a 95% confidence interval for the corresponding mean, and c) Absolute evening cortisol in postpartum women (n=50) and non-perinatal women (n=93). Absolute evening cortisol values is shown for each individual. Error bars represent a 95% confidence interval for the corresponding mean. Reprinted from Paper I, Høgh et al., Psychoneuroendocrinology, 2024. DOI: 10.1016/j.psyneuen.2024.107048 (144).

TABLE 2 | Cortisol awakening response (AUCi) and absolute evening cortisol in women early postpartum compared to healthy non-perinatal women. Reprinted from Paper I, Høgh et al., Psychoneuroendocrinology, 2024. DOI: 10.1016/j.psyneuen.2024.107048 (144). This work is licensed under CC BY-NC 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by-nc/4.0/

Cortisol awakening response (AUCi)*			
Covariates for adjustment	**Effect (nmol/L*minutes)**	**95% CI**	**p-value**
Adjusted for age and cortisol at awakening	-303	[-429; -178]	<0.001
Crude analysis	*-200*	*[-292; -108]*	*<0.001*
Absolute evening cortisol			
Covariates for adjustment	**Effect (nmol/L)**	**95% CI**	**p-value**
Adjusted for age	-3.46	[-4.64; -2.28]	<0.001
Crude analysis	*-3.92*	*[-4.76; -3.08]*	*<0.001*

*Time corrected AUCi: AUCi divided by the actual sampling time in minutes multiplied by 60 minutes.
Cortisol awakening response: N=50 postpartum women and N=91 non-perinatal women.
Absolute evening cortisol: N=50 postpartum women and N=93 non-perinatal women.

Cortisol awakening response (CAR) and maternal mental well-being

In a model adjusting for parity and E2, we observed a statistically significant association between CAR (AUCi) and WHO-5 total score (β = -0.04, 95% CI [-0.07; -0.00], p = 0.048). Similarly, we found that CAR was significantly positively associated with levels of mental distress (EPDS score) (β = 0.007, 95% CI [0.002; 0.013], p(corrected) = 0.04). The results indicate that lower CAR postpartum might be associated with higher well-being in healthy women (Figure 8). We did not find a statistically significant association between CAR and POMS TMD or Cohen PSS.

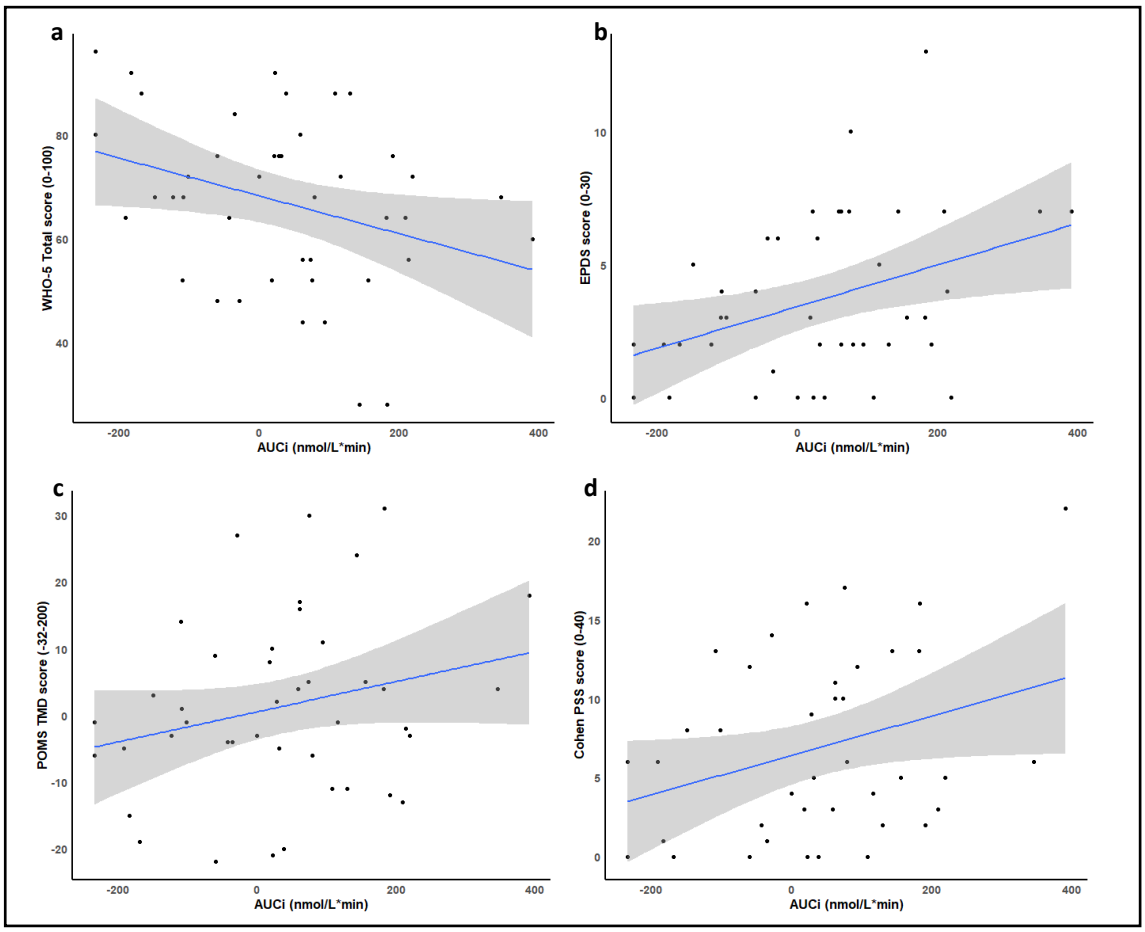

Figure 8 | Scatter plot of the correlation between the cortisol awakening response (AUCi) and a) WHO-5, World Health Organization Well-Being Index total score, b) EPDS, Edinburgh Postnatal Depression Scale Sum Score, c) POMS, Profile of Mood States Total Mood Disturbance score, d) Cohen PSS, Perceived Stress Scale score.

Paper II

The 95 women in the study had a mean age of 33.9 years, 38% was nulliparous, and C-sections were conducted at an average gestational age of 39+0, ranging from 37+2 to 41+2. The women's mental distress and well-being scores were within the range considered normal (127, 134) (Table 1 in Paper II manuscript).

PGDF15 levels substantially decreased from 85.9 ng/mL during pregnancy to 0.502 ng/mL postpartum, reflecting a 99.42% decline (Figure 9).

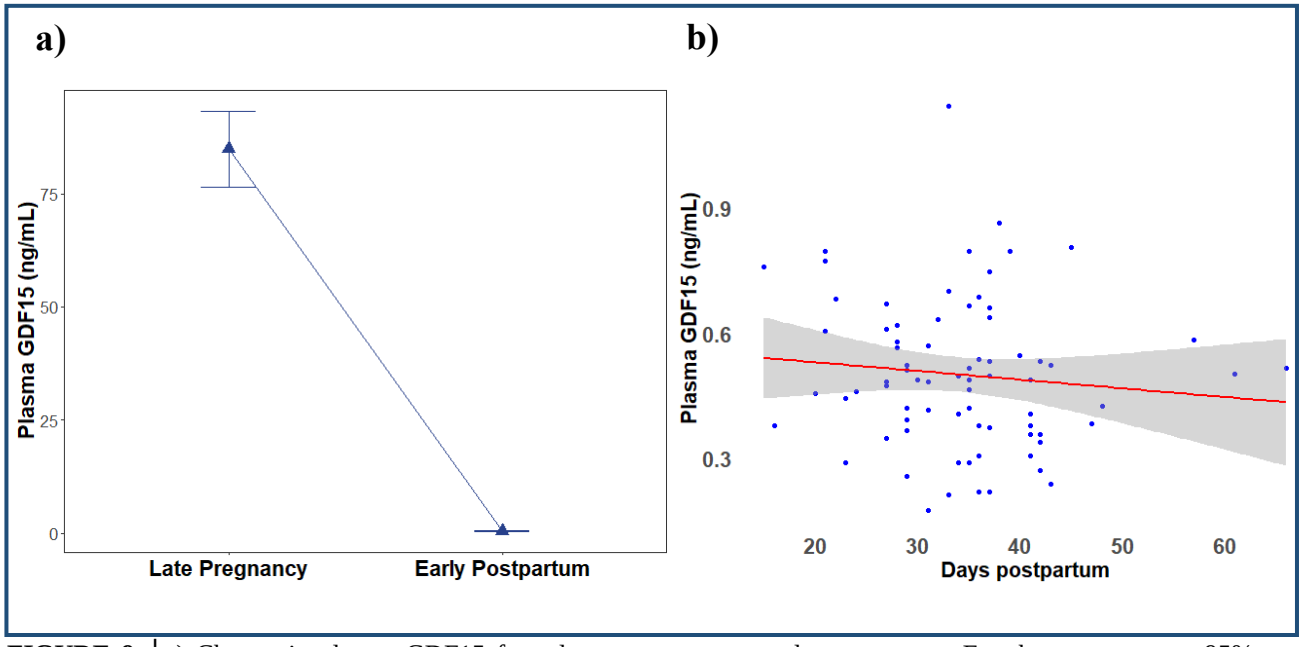

FIGURE 9 | a) Change in plasma GDF15 from late pregnancy to early postpartum. Errorbars represent a 95% confidence interval for the corresponding mean, b) Scatterplot of the relationship between days postpartum and Plasma GDF15 levels. Reprinted from Paper II.

Placental GDF15 mRNA and pregnancy GDF15 levels in CSF and serum

We demonstrated that cGDF15 levels and pGDF15 levels correlated significantly (r=0.52; p<0.001) (Figure 10). Interestingly, cGDF15 and placental *GDF15* mRNA correlated significantly (r=0.43, p=0.01), and we found a trend-level correlation between pGDF15 and placental *GDF15* mRNA (r=0.30, p=0.09). When including placenta weight in the correlation analysis (placental *GDF15* mRNA * placenta weight), we observed a statistically significant correlation between pGDF15 and placenta *GDF15* mRNA * placental weight (r=0.44, p=0.008) (Figure 10).

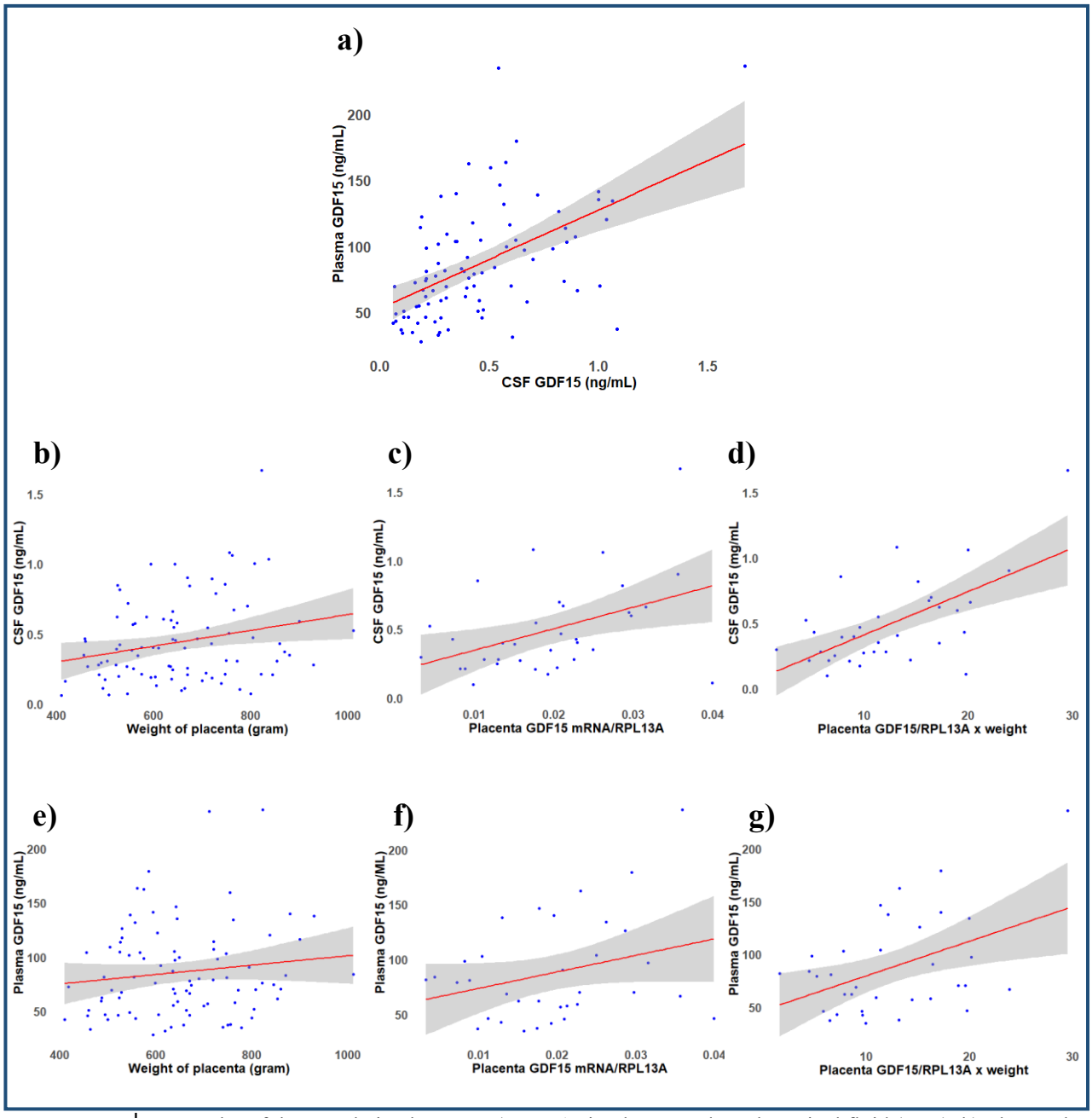

FIGURE 10 | Scatterplot of the correlation between a) GDF15 in plasma and cerebrospinal fluid (CSF), b) Placental weight and CSF GDF15, c) Placenta *GDF15* mRNA and CSF GDF15, d) Placenta *GDF15* mRNA * placenta weight and CSF GDF15, e) Placental weight and plasma GDF15, f) Placenta *GDF15* mRNA and Plasma GDF15, g) Placenta *GDF15* mRNA * placenta weight and Plasma GDF15. Reprinted from Paper II.

Pregnancy CSF GDF15 levels and mental well-being

Neither did we find an association between pregnancy cGDF15 levels and mental well-being, as assessed by the WHO-5 (p=0.24) and the POMS TMD (p=0.31) (Table 3). Similarly, no significant association was observed between pregnancy cGDF15 levels and postpartum mental well-being in both crude and adjusted models (Table 3).

Pregnancy CSF GDF15 levels and Cortisol Awakening response (CAR)/cortisol at awakening

We did not find an association between pregnancy cGDF15 levels and postpartum CAR or cortisol levels at awakening (p=0.70 and p(corrected)=0.38, respectively) (Table 3).

TABLE 3 | Associations for placenta, plasma and CSF GDF15 in late pregnancy. Reprinted from Paper II.

	Crude Model*				Model A**			
	β	**95% CI**	**p**	**p (corrected)**	**β**	**95% CI**	**p**	**p (corrected)**
CSF GDF15 (ng/mL)								
Pregnancy								
WHO-5	-5.9	[-15.7; 4.0]	0.24		-5.1	[-15.4; 5.3]	0.33	*Primary*
POMS TMD	6.5	[-6.2; 19.2]	0.31		6.7	[-6.9; 20.3]	0.33	0.66
Postpartum								
WHO-5	-3.0	[-15.6; 9.7]	0.64		-4.1	[-17.2; 9.1]	0.54	*Primary*
POMS TMD	0.3	[-11.2; 11.9]	0.96		0.4	[-11.9; 12.7]	0.95	1.0
EPDS	0.3	[-2.2; 2.8]	0.82		0.2	[-2.5; 2.9]	0.89	1.0
CAR (AUCi)	44.3	[-123.9; 212.6]	0.60		34.9	[-144.6; 214.5]	0.70	*Primary*
Cortisol at awakening	-1.8	[-4.6; 1.0]	0.20		-1.8	[-4.5, 0.9]	0.19	0.38
Plasma GDF15 (ng/mL)								
Pregnancy								
s-Estradiol (E2)	195.7	[71.1; 320.3]	**0.002**	*Primary*				
s-hs-CRP	-0.01	[-0.03; 0.007]	0.24	0.48				
s-Estrone (E1)	-49.7	[-139.5; 40.2]	0.28	0.48				
s-Estriol (E3)	177.6	[96.6; 258.7]	<0.001	**<0.001**				

CSF, cerebrospinal fluid; CAR, Cortisol Awakening Response; AUCi, area under the curve with respect to increase from baseline at awakening. WHO-5, WHO-5 Well-being index; EPDS, Edinburgh Postnatal Depression Scale; POMS, Profile Of Mood States "Total Mood Disturbance score".

*Linear regression analysis with blood serum, mental health, and cortisol variables as dependent variables and GDF15 measurements as independent variables. β depicts the standardized beta coefficient for GDF15.

**Model A is adjusted for maternal age, hs-CRP and E2. Bonferroni-Holm correction applied through p-value adjustment.

Pregnancy pGDF15 levels, hs-CRP and estrogens (E1, E2, E3)

During the transition from pregnancy to postpartum, E1 and E2 levels decreased to 0.36% and 0.07% of their respective pregnancy levels (Table 1 in paper manuscript). Hs-CRP also decreased from pregnancy to postpartum, albeit not as dramatically as the estrogen levels. Plasma GDF15 levels showed significant associations with both E2 and E3 levels during late pregnancy (E2: β=195.7, 95% CI [71.1; 320.3], p=0.002; E3: β=177.6, 95% CI [96.6; 258.7], p (corrected)<0.001)

(Table 3). Our sensitivity analysis indicated that for CSF-derived GDF15, only E3 was significantly associated with GDF15 (n=95, Table A.1 in Paper II manuscript).

Paper III

Trial status

Recruitment for the MAMA trial began on 4 January 2021, followed by the first baseline visit on 3 February 2021. As of 15 April 2024, we have included 127 participants in the trial (i.e., randomized for the intervention). The trial is expected to be completed by December 2026 (145).

Outcomes of interest in the MAMA Trial

The primary outcome of this study is clinical depression, diagnosed by a medical doctor specialized in psychiatry and based on the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) for Major Depressive Disorder.

Secondary outcomes include, but are not limited to, postpartum subclinical depressive symptoms, maternal care capacity, exclusive breastfeeding, cortisol dynamics, molecular biomarkers of perinatal depression, cognitive performance, and developmental functioning of the infants. A detailed description of the secondary outcomes is provided in Table 2 in the paper manuscript. Table 4 describes the time schedule of enrolment, intervention, and outcome measures of the MAMA Trial.

Table 4. Time schedule of enrollment, intervention and outcome measures of the MAMA Trial. Reprinted from Paper III, Høgh et al. BMJ Open 2021. DOI: 10.1136/bmjopen-2021-052922 (145). This work is licensed under CC BY-NC 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by-nc/4.0/

	STUDY PERIOD						
	Gestational week <34	**Gestational week ≥34+0**	**0-1 day postpartum**	**1 week postpartum**	**3 weeks postpartum**	**8-10 weeks postpartum**	**6 months postpartum**
TIMEPOINT		Baseline			T1	T2	T3
Face-to-face meeting		X	X		X	X	
Enrollment							
Eligibility screening	X						
Informed consent		X					
Allocation			X				
Intervention							
Transdermal estradiol/placebo patch			◆	———	◆		

Assessment of side effects				X	X	X	

Table 4 continued

	STUDY PERIOD						
	Gestational week <34	**Gestational week ≥34+0**	**0-1 day postpartum**	**1 week postpartum**	**3 weeks postpartum**	**8-10 weeks postpartum**	**6 months postpartum**
Medical history		X					
Obstetric history		X					
Socio-demography/ lifestyle		X					
Blood samples		X			X		
Blood pressure		X	X		X		
Saliva sample mother (cortisol)					X		
Saliva sample infant (DNA)			X				
Hair sample mother			X				
HAMD-6		X				X	
STAI-AD		X					
CATS		X					
OS-FHAM short		X					
PBI (mother)		X					
PBI (father)		X					
NEO-P-IR		X					
EPDS		X			X	X	X
WHO-5		X			X	X	X
SHAPS		X			X	X	X
MDI		X			X	X	X
Cohen PSS		X			X	X	X
RRS		X			X	X	X
STAI		X			X	X	X
PSQI		X			X	X	X
OCI		X			X	X	X
MAAS		X					
PSS					X	X	X
PCOS					X	X	X
PRFQ							X
Breast feeding					X	X	X
ASQ:SE-2						X	
Bayley-III						X	
Neuropsychological tests		X				X	

Psychological trait questionnaires: STAI-AD, State Trait Anxiety Inventory-Trait; CATS, Child Abuse and Trauma Scale; OS-FHAM short, substance abuse and family history; PBI, Parental Bonding Instrument; NEO-PI-R, Revised NEO Personality Inventory

Psychological state questionnaires: EPDS, Edinburgh Postnatal depression Scale; WHO-5, WHO-5 Well-being index; SHAPS, Snaith-Hamilton Pleasure Scale; MDI, Major Depression Inventory; Cohen PSS, Perceived Stress Scale; RRS, Rumination Response Scale; STAI, State-Trait Anxiety Inventory - State; PSQI, Pittsburgh Sleep Quality Index; OCI, Obsessive-Compulsive Inventory; MAAS, maternal Antenatal Attachment Scale; PSS, Parental Stress Scale; PCOS, Parents' Sense of Competence Scale;

PRFQ, Parental Reflective Functioning Questionnaire; ASQ:SE-2, Ages and Stages Questionnaire; Bayley-III, Bayley Scales of Infant and Toddler Development-third edition

Paper IV

We conducted 13 interviews with women who had previously experienced postpartum depression, typically occurring 2-3 years ago, except for one who experienced postpartum depression 11 years ago. These women had subsequently undergone pregnancy and childbirth again, and at the time of the interview, all of them had infants aged between 3-5 months.

The majority of the interviewed women were native Danish, aged between 30-35 years, and had higher educational degrees. Notably, three of them had a recurrence of postpartum depression (Table 2 in the Paper IV manuscript).

We identified three key themes of the interviews: 1) Biology as a contributing factor to postpartum depression, 2) The role of external events in making sense of postpartum depression, and 3) The ambiguous potentiality of testing for genomic risk markers of postpartum depression.

Biology as a contributing factor to postpartum depression

For most of the interviewed women, biology was not the first factor that came to mind when considering the reasons for their postpartum depression. Their reflections centered around a lack of support from relatives and healthcare professionals, traumatic birth experiences, and challenges with breastfeeding. However, when prompted to contemplate the influence of biology, the participants mostly regarded biology as encompassing genes and heredity, almost always closely intertwined with other external factors such as life conditions or experiences.

> *"I thought it was me. That I have a flaw that makes me highly anxious. And since I've been depressed before, I think that I'm at risk. Then of course I thought about how difficult the birth was and that I was totally deprived of sleep, not to mention the immense shock of giving birth for the first time. Being responsible for a tiny baby and, well, for a long time I thought the reason was that, with Emil, I was terribly stressed because I breast fed and he had to eat. But it wasn't like that this time; it wasn't something like that making me feel frazzled in the beginning this time, so I can't quite (…) So, but yeah, I definitely believe that there's something about me, genetically, that makes me prone to it."*
>
> (Ida, postpartum depression with her first child and anxiety with her second child)

The role of external events in making sense of postpartum depression

As noted in the initial theme, the women strongly connected their postpartum depression to external events. All of them sought an explanation for why they had to have depression during what was expected to be the happiest time of their lives. For a couple of the women, the onset of their depression was associated with the challenges of breastfeeding. They had linked their maternal identity to succeeding in breastfeeding. The disparity between the ideals of motherhood and the reality they faced left the women with overwhelming feelings of failure in motherhood, adversely affecting their self-perception and exacerbating their sense of guilt.

Moreover, as illustrated in the quote by Lise, adverse events during childbirth were perceived as affecting the attachment to the newborn.

> *"Of course, it's been a combination of many things, but I had a really complicated birth with Esther the first time (...). So, my initiation into motherhood was, uhm, totally ... I got off to a completely bad start, I'd say. I was incredibly drained mentally and physically. Not just the birth but the cesarian section. I didn't get to hold her immediately when she came out. They had to help her, which meant Jens got her. I got her when I woke up in recovery. if you've ever experienced having so much medicine in your body, that needs to be expelled. You're oddly wide awake. Then they give you the child as though it was any old child. Yeah, now you have to breastfeed and everything. I was like; please get this baby away from me. I couldn't cope at all."*
>
> (Lise, no recurrence of postpartum depression)

The ambiguous potentiality of testing for genomic risk markers of postpartum depression

Upon introduction during the interview, certain women envisioned genomic risk marker testing as holding the potential to prevent depression and decrease stigma. However, simultaneously, being aware of their risk score was seen as having the power to increase awareness of depressive symptoms and having the potential to become a self-fulfilling prophecy.

> *"I think that it [genetic screening] can be both positive and negative. Because it's not always, I believe, positive to know what can potentially happen. Because, on the one hand, you could say that it's terribly smart in terms of prevention, but I also*

can't help but think that, well, it can also be a contributing factor in terms of being prone to something happening."

(Lise, no recurrence of depression)

Some of the women spontaneously mentioned the stigma of postpartum depression. A few anticipated tests for risk markers could reduce guilt, stigma, and shame. The quote from Karen illustrates that attributing postpartum depression to biology would relieve her from responsibility, accepting it as a life circumstance. On the other hand, to Karen testing for risk markers represented knowledge with an inevitable future manifestation, implying a lack of control or ability to change the risk conveyed by the genomic test.

"I think it would also reassure me if I could explain it to myself, well, that I'm not crazy, that it has something to do with my body. It's chemistry; it's biology. Something is happening inside me, and we don't know what it is (…). It would give tremendous peace of mind to know, well, that that's what your DNA is like; it has nothing to do with me or, well, it's me, it's my DNA, but I couldn't have done anything differently. It's just a condition in life that I have. It's not really my fault or something I should have done or not done like that; it's the way it is."

(Karen, recurrence of postpartum depression)

To others, the test for risk markers was perceived as rendering the future open and offering the opportunity to comprehend, be aware of, and potentially address postpartum depression.

DISCUSSION

In the Discussion section, the main findings of Papers I, II, and IV will be discussed in light of existing evidence. Additionally, the protocol development for Paper III will be integrated within the broader context of the discussion across papers. In the "Methodological Considerations" subsection, methods employed in Papers I and II are evaluated, identifying areas for enhancement to ensure optimal data quality in Paper III. The strengths and limitations of the specific study can be found in the related papers.

Overall findings

In **Paper I**, we demonstrated that healthy women in the early postpartum period had significantly lower HPA-axis dynamics, corresponding to an 84% reduction in CAR compared to healthy non-perinatal women. Additionally, postpartum women displayed 80% lower absolute evening cortisol levels than non-perinatal women. Notably, within the postpartum group, we found a statistically significant correlation between lower CAR and higher well-being (WHO-5) and reduced mental distress (EPDS). However, we did not observe any association between CAR and more temporary mood states (POMS TMD) or perceived stress levels (Cohen PSS).

In **Paper II**, we demonstrated that cGDF15 and pGDF15 were highly correlated, as well as correlated with the product of placenta *GDF15* mRNA and placental weight (*GDF15* mRNA*placental weight). Additionally, we showed an association between pGDF15 levels and E2 and E3 during pregnancy. We found no association between cGDF15 and mental well-being or cortisol measures, i.e., CAR and absolute cortisol at awakening.

In **Paper IV,** we found that biology played a minor role in the women's stories of postpartum depression. The majority of the women perceived their postpartum depression as primarily influenced by external factors rather than biological factors. Only a few women considered postpartum depression to be associated with sensitivity to hormonal fluctuations. Secondly, the interviewed women envisioned testing for genomic risk markers with ambiguity. On one hand, they perceived it as holding the potential to prevent postpartum depression and reduce stigma, and at the same time, having the potential to become a self-fulfilling prophecy.

Interpretation of results in light of other evidence

Paper I

We found that healthy women had a blunted CAR in the early postpartum period relative to non-perinatal women. This indicates that a blunted CAR is part of a normal physiological transition from pregnancy to postpartum, supported by a study by de Rezende et al., who observed a reduced CAR in euthymic women six months postpartum (81). Our findings of a blunted CAR in healthy women postpartum may thus result from CRH receptor desensitization, which persists at least six weeks postpartum (146). During pregnancy, high levels of CRH are secreted from the placenta, creating a positive feedback loop, unlike outside normal pregnancy. Elevated CRH levels during pregnancy desensitize CRH receptors in the hypothalamus, resulting in reduced CRH secretion and mild adrenal suppression in the postpartum period (70, 146, 147). Further, the blunted CAR may partly result from the demands of parenting, leading to fatigue and exhaustion. These factors have been associated with a decreased CAR in a meta-analysis conducted by Chida and Steptoe (77). In contrast to our findings, Taylor et al. reported that euthymic women at 7.5 weeks postpartum exhibited a CAR similar to that of non-perinatal women (82). The discrepancies in the results could potentially come from measuring cortisol at different time points postpartum and that Taylor et al. only used two saliva cortisol samples to calculate the CAR, not capturing the entire CAR, in particular not the downregulatory phase (75, 79, 82).

Moreover, we found significantly lower absolute evening cortisol levels in postpartum women compared to non-perinatal women, again reflecting the overall CRH-induced suppression (70, 146, 147). Our finding of a mean absolute evening cortisol level of 0.9 nmol/L corresponds to the results reported by Iliadis et al. (148).

In the postpartum group, we found a significant association between lower CAR and higher well-being scores (WHO-5) and lower mental distress scores (EPDS), a trend supported by Corwin et al., who also found that euthymic women exhibited lower total salivary output compared to depressed women postpartum (84). Our results align with the blunted CAR observed in healthy postpartum women compared to non-perinatal women, highlighting that HPA-axis "dysregulation" might be a physiological adaption that protects maternal mental health. However, the landscape becomes less clear when considering studies on postpartum depression. While two studies found a decreased CAR in depressed women compared to euthymic postpartum women (81, 82), two

other studies yielded results indicating no association (85, 86). This suggests the existence of potentially distinct risk profiles between healthy and at-risk women.

Paper II

We found that healthy women had late pregnancy cGDF15 levels comparable to those reported by Andersson-Hall et al. (100). Our study adds to the body of evidence by demonstrating a significant decline in pGDF15 levels from pregnancy to five weeks postpartum. This decline aligns with earlier observations, although from different and much later postpartum time points: the third trimester and six months postpartum in one study and five years postpartum in another (100, 111). However, data concerning the immediate postpartum decline in GDF15 are currently unavailable. We found a correlation between cGDF15 and pGDF15. Further, we found a correlation between cGDF15 and placenta *GDF15* mRNA. Notably, even stronger correlations were observed when also considering placenta weight. This aligns with previous research indicating that placental *GDF15* mRNA is produced throughout the placenta, as demonstrated by Moore et al. and Turco et al. (101, 149). Additionally, in line with our results, Andersson-Hall et al. reported a robust correlation between cGDF15 and serum GDF15 during pregnancy; however, at five years postpartum, they found no correlation (100). These findings suggest that cGDF15 may serve as a more precise indicator of placental-derived *GDF15* mRNA and highlight the potential significance of GDF15 during pregnancy.

We hypothesized that the influence of GDF15 on nausea, vomiting, and hyperemesis gravidarum might also extend to mental health status during pregnancy or postpartum. A meta-analysis from 2016 found that women with hyperemesis gravidarum have an increased risk of mental health issues (150). Additionally, Cimino et al. demonstrated in rats that GDF15 activation of the HPA-axis with chronic infusion of GDF15 led to higher levels of corticosterone (115). However, our study showed no association between GDF15 and maternal mental well-being or cortisol measures, such as CAR or absolute cortisol levels at awakening. Hence, it appears that the causes of the impaired mental health linked to hyperemesis gravidarum, nausea and vomiting are distinct from those of GDF15.

Paper IV

Our analysis depicted a nuanced illustration of the stigma surrounding postpartum depression. Stigma, according to Goffman, is an attribute that reflects devalued stereotypes and prevents a person from being accepted by society (151). Self-stigma is a subset of stigma that results from internalizing public stigmatization beliefs, which have negative impacts on one's self-esteem and self-image (152). Some of the interviewed women believed that the genetic risk marker could give them "biological proof" of their depressive symptoms, thus having the potential to change their self-image and feel less stigmatized. The potential of mitigating guilt through biogenetic explanations was similarly identified in a meta-analysis by Kvaale et al. (153). Conversely, other studies have indicated that genetic information regarding psychiatric conditions might heighten stigma and discrimination among asymptomatic individuals (154, 155).

The potentials of genomic risk markers and testing for hormone sensitivity were generally perceived with ambiguity. To the women, relating postpartum depression to biology at a molecular level seemed to represent something more deterministic. Some women highlighted the possible implications of understanding their risk of postpartum depression as a constraint on their ability to navigate and shape their future. By ascribing the depression to genetic factors, these women might have felt forced to acknowledge the possibility of its recurrence in future pregnancies, consequently diminishing their sense of hope. In line with the perspective of medical anthropologist Monica Konrad, who suggests that learning about a genetic risk can lead individuals to be perceived as ill before the onset of actual illness, the women also highlighted the potential downside of heightened focus on depressive symptoms, which could inadvertently amplify these symptoms, thus manifesting as a self-fulfilling prophecy (156). Consistent with our analysis, previous studies have demonstrated that awareness of genetic factors contributing to depression heightened individuals' prognostic pessimism (153, 157).

On the other hand, other research showed that genetic testing created hope for future prevention among people suffering from depression but also among unaffected people (119, 158, 159). Postpartum depression is distinct from major depression outside the peripartum period in that it is intricately linked to a specific event (childbirth), which is likely to reoccur. This difference in recurrence patterns may lead to a perception of genetic testing's plasticity and potential that differs significantly between postpartum depression and general depression. Specifically, attributing depression to genetic factors may evoke a greater sense of hopelessness and fear of recurrence among women experiencing postpartum depression compared to those with general depression.

However, the interviewed women also pointed out the potential of preventive interventions and reducing stigma if such a marker was introduced.

Methodological considerations

Conducting clinical research requires significant effort in collecting data and ensuring compliance with the study protocol. Data for Papers I and II were partly collected during the Severe Acute Respiratory Syndrome Coronavirus-2 (COVID-19) pandemic, which posed compliance challenges and may account for some missing data.

Successful research heavily relies on participants' commitment and engagement. Saliva samples for cortisol analyses, used in Papers I and II, were collected in the participants' homes and mailed or delivered personally to the research unit. It is possible that some of the missing cortisol data could be attributed to participants forgetting to go to the post office with the samples. In the MAMA Trial (Paper III), we incorporated lessons from this experience. In this study, participants collect saliva samples at home and bring them to the research unit at their subsequent visit, mitigating the risk of data loss. Further, in Papers I and II, we did not have access to data on smoking, breastfeeding, or pre-pregnancy BMI. In the protocol for the MAMA Trial, we have included these data to enhance future study quality.

The advantage of collecting data from women undergoing planned C-sections is a strength of both Papers I and II. This method facilitated the acquisition of blood samples, CSF, and placenta biopsies from all participants included in the study, enabling thorough analysis of GDF15. In contrast, in clinical trials involving laboring women, obtaining placenta biopsies can present challenges due to the unpredictable timing of childbirth throughout the day. Consequently, data collection from placenta biopsies was not feasible within the MAMA Trial cohort (Paper III).

Papers I and II included a mentally healthy pregnant/postpartum population, primarily focusing on assessing mental well-being. Substantial evidence suggests that well-being and illness are not merely opposing poles. Well-being is argued to be not solely the absence of illness (160-162). Mental well-being encompasses a broader concept beyond the mere absence of mental illness, focusing on achieving a positive state of mind in various aspects of life, including emotional, social, and psychological domains (160). Thus, the results of Papers I and II do not automatically map onto the biology of ill-being. However, in Papers I and II, we also evaluated mental distress and found the same trend as with mental well-being.

Participants for Paper IV had all participated in the MAMA Trial (Paper III), which might have heightened their familiarity with genomic risk markers for postpartum depression. Yet, during the interviews, we found that biological factors played a minor role in their understanding of postpartum depression. Finally, the participants in Paper IV were asked about hypothetical genomic risk markers, raising the possibility of different outcomes had such a marker already existed.

Discussion across papers

The findings from the interview study (Paper IV) and other research demonstrate that postpartum depression has profound effects on people's lives (25, 27, 29, 30). This underscores the need for increased efforts in preventing postpartum depression. Previous research and reviews have identified various psychosocial, pregnancy-related, and biological factors underscoring the multifaceted etiology of postpartum depression (32-37).

This thesis found that the late pregnancy levels of GDF15 and postpartum HPA axis dynamics, i.e., CAR and absolute evening cortisol levels, were associated differently with maternal mental health in the early postpartum.

In Paper I, we found that lower CAR was associated with higher well-being. These findings imply that the blunted CAR and overall circadian rhythm are components of a physiological phenomenon in which HPA-axis "dysregulation" or maybe rather adaptation might be a protective factor for maternal mental health. In contrast, we did not find an association between GDF15 during late pregnancy and postpartum mental health (Paper II). Nor did we find an association between GDF15 during late pregnancy and CAR early postpartum, as demonstrated by Cimino et al. in a study in rats showing that chronic infusion of GDF15 was associated with higher levels of corticosterone (115). The differences in the associations between the specific biomarker and mental well-being could result from different functions of the biomarker. Cortisol regulates stress and the association with mental well-bring might be due to a healthy adaptation to motherhood. It may include conserving energy for lactation, protecting against stress-induced lactation inhibition, strengthening the immune function, and possibly supporting emotional well-being and bonding between mothers and infants (84, 163, 164).

GDF15 is triggered in response to stimuli inducing cell stress (88, 89). Elevated GDF15 levels outside pregnancy have previously been associated with mental illness (95-97). In pregnant

populations, high levels of GDF15 have primarily been associated with hyperemesis gravidarum, and recently, maternal sensitivity to GDF15 has been suggested to play a role (103-106). However, our study was the first to evaluate the association between GDF15 and mental well-being; thus, replication studies are needed, as well as studies focusing more on vulnerable women.

Further, as suggested by Fejzo et al., the differences could also result from some women being particularly sensitive to changes in GDF15 (106). Likewise, desensitization of the glucocorticoid system at a molecular level has also been suggested to be associated with antenatal depressive symptoms (165). The sensitivity to hormonal fluctuations during the perinatal transition at a molecular level has also involved estrogens, as shown in the work by Mehta et al. and Guintivano et al., who found that measures of methylation of estrogen-responsive genes might be a promising biomarker for predicting postpartum depression in the future (64-66).

Regarding estrogens, we observed a statistically significant correlation between GDF15 and E2 and E3. Some studies have found that levels of E2 affect maternal mental health, but in other studies, no association has been found (55-61). Yet, the disparities might also be a result of some women being susceptible to hormonal fluctuations during the perinatal transition (64-66). Two RCTs have demonstrated the effect of estradiol treatment on manifest depressive episodes postpartum; one RCT showed a significant impact of estradiol treatment, and the other showed reduced depressive symptoms when compared to a placebo (166, 167). Additionally, the bidirectional findings presented in this thesis regarding specific biomarkers and postpartum mental health suggest that direct links between individual biomarkers and maternal mental well-being may not be straightforward. Instead, they likely participate in a multifaceted interplay involving factors beyond the biological, as suggested by other researchers (35, 168-170). This interplay may also involve interactions between genetics and environmental factors, or biological risk status may act as a potential moderator (35, 170).

Moreover, there could be distinct risk profiles between healthy and at-risk women. No studies have evaluated the preventive potential of estradiol on postpartum depression. In the MAMA trial, we have proposed a preventive strategy targeted at women at high risk of postpartum depression. In the MAMA trial (Paper III), we built on the results of earlier research pointing to estrogen sensitivity and that maybe some women are at particular risk of experiencing depressive symptoms during the perinatal period, possibly implying specific hormone-sensitive profiles (64, 66, 117).

In Paper IV, we found that knowledge about genetic risk could introduce hope regarding possible prevention while simultaneously creating expectations about becoming a self-fulfilling prophecy.

If genomic risk markers for estrogen sensitivity and postpartum depression are introduced in the future, it should be followed by an intervention or with psychoeducational support, which has been shown to reduce depression-related prognostic pessimism and to mitigate the stigma experienced by women dealing with postpartum depression (171-173).

CONCLUSION

The results across the papers included in this thesis emphasize the unique biological landscape and related effects during the perinatal transition.

We found that healthy postpartum women exhibit a blunted CAR and significantly lower absolute evening cortisol levels compared to healthy non-perinatal women. Additionally, lower CAR was significantly associated with higher mental well-being and lower mental distress.

Our findings of GDF15 correlation across tissues suggest that GDF15 is globally produced in the placenta. The correlation with estrogen levels underscores GDF15's essential role in pregnancy. However, we did not demonstrate a direct association between GDF15 and mental health well-being or cortisol measures, i.e., CAR and absolute cortisol at awakening.

Finally, the results from the qualitative study revealed that women with a history of postpartum depression perceived testing for genomic risk markers associated with hormonal sensitivity and postpartum depression with ambiguity. While some viewed knowledge about these markers as promising for future prevention, others expressed concerns about the possibility of triggering depressive symptoms.

Collectively, the findings from the papers incorporated in this thesis suggest that the transition from elevated levels of cortisol and GDF15 during pregnancy to substantially reduced levels postpartum manifests diverse impacts on mental health during the early postpartum phase. It implies that dysregulation of the HPA axis may constitute an adaptive response, whereas GDF15 appears unrelated to mental health outcomes. This underscores the complexity of the biological and psychological factors influencing postpartum mental health, suggesting differences at the subgroup level and the existence of more intricate risk profiles.

PERSPECTIVES

Understanding the interplay between biological, psychological, and social factors and perinatal mental health facilitates the identification of at-risk groups, enabling early intervention and support for women at increased risk of perinatal mental disorders. Additionally, knowledge of interactions allows for the development of tailored treatment or preventive strategies that consider individual biological and hormonal variations, thereby enhancing the effectiveness of interventions for perinatal mental health. Future studies should replicate and extend our findings in relevant study designs. This will help obtain a more comprehensive understanding of the relationships between CAR and maternal mental well-being also in women at high risk for postpartum depression, e.g., women with prior depressive episodes or other robust risk factors like premenstrual syndrome, violent experiences, and unintended pregnancies (36).

In the protocol development for Paper III, we built upon previous knowledge by integrating it into a clinical trial investigating the preventive effects of estrogen supplementation on postpartum depression in high-risk women. Nevertheless, more knowledge is needed to understand the interplay since postpartum depression is probably caused by more than biological factors. Future research might consider biological risk status not solely as a causal factor but also as a potential moderator and combine biological factors with psychosocial, pregnancy-related, birth-related, and early postpartum-related factors in risk-profiling women (35, 170). Moreover, an improved understanding of subgroups within perinatal depression may allow for the identification of more tailored targets (174), thus offering unique opportunities for prevention.

In preventing postpartum depression, midwives play a pivotal role in identifying and closely monitoring women who are at high risk (175). In Denmark, pregnant women with current or past psychiatric disorders are advised by the Danish Health Authorities to undergo monitoring by a specialized team of midwives and obstetricians with expertise and a particular interest in addressing psychiatric challenges (176). The women and their partners are provided with extended time during midwifery consultations and are offered additional antenatal care opportunities (176). Midwifery care is defined as *"…optimising normal biological, psychological, social, and cultural processes of reproduction and early life; timely prevention and management of complications (…)"* (177). Consequently, midwives possess advanced skills in recognizing deviations from normal pregnancies, including identifying potential risks, and excel in establishing trusting relationships with expectant mothers and their partners. Educating midwives about the biological

influences on perinatal mental health would enable them to provide comprehensive counselling and support, potentially reducing stigma and fostering mental well-being among pregnant women and new mothers. This could involve integrating mental preparation modules into pregnancy courses, addressing the psychological aspects of parenthood and perinatal mental illness risk factors, as well as offering counseling on protective strategies such as stress reduction and improving sleep quality (178). These initiatives could be enhanced by involving partners to underscore their significance in maternal mental health and to highlight the importance of their own mental well-being as well (179, 180).

Further, improved screening methods informed by an understanding of the contribution of biological factors in integrated risk models could enhance the accuracy of perinatal mental disorder identification and subsequent intervention. Early detection of risk through screening allows for prompt intervention, potentially mitigating symptom severity and improving long-term outcomes (181). Inquiring about risk factors, such as past medical history, along with assessing current mental health status, enhances the initiation of referrals for further support or treatments focused on mental health concerns (182). This approach has also been linked to increased help-seeking behaviors throughout the perinatal period (183).

Pregnant women with a history of mental illness often fear experiencing postpartum depression, thus possibly making them motivated to accept support from healthcare professionals (184). However, it's crucial to acknowledge that some women may feel stigmatized and judged when identified as vulnerable during pregnancy (185). Therefore, providing psychoeducation about the biological influences on perinatal mental health may help mitigate these concerns.

Effective prevention of postpartum depression requires a collaborative effort involving a transdisciplinary healthcare team comprising psychiatrists, obstetricians, midwives, psychologists, and, crucially, the women and their partners. The effectiveness of collaborative care interventions has been assessed solely in non-perinatal depressed populations and treatment of perinatal depression, showing positive results (186, 187). However, the potential as a preventive intervention against perinatal depression remains unexplored. By working closely together, such transdisciplinary teams could provide comprehensive support, which may be based on combined biological and psychosocial risk profiles, tailored to individual needs, ultimately contributing to improved maternal mental health outcomes to benefit families and their infants' future health.

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APPENDICES

APPENDIX I, PAPER I

Journal Pre-proof

The Cortisol Awakening Response is blunted in healthy women early postpartum

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The Cortisol Awakening Response is blunted in healthy women early postpartum

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Abstract

Introduction: The dynamic capacity of the hypothalamic-pituitary-adrenal (HPA) axis supports healthy adaptions to stress and play a key role in maintaining mental health. Perinatal adaptations in the HPA-axis dynamics in terms of the Cortisol Awakening Response (CAR), may be involved in dysregulation of perinatal mental health. We aimed to determine if CAR and absolute evening cortisol early postpartum differed from non-perinatal women and evaluate the association between the CAR and maternal mental well-being.

Methods: The CAR was computed as the area under the curve with respect to increase from baseline from serial home-sampling of saliva across 0-60 minutes from awakening. We evaluated differences in CAR and absolute evening cortisol between postpartum women (N=50, mean postpartum days: 38, SD: ±11) and non-perinatal women (N=91) in a multiple linear regression model. We also evaluated the association between CAR and maternal mental well-being in a multiple linear regression model.

Results: We found that healthy postpartum women had a blunted CAR ($p<0.001$) corresponding to 84% reduction and 80% lower absolute evening cortisol ($p<0.001$) relative to non-perinatal healthy women. In the postpartum group, there was a trend-level association between lower CAR and higher scores on the WHO Well-Being Index (WHO-5) ($p=0.048$) and lower Edinburgh Postnatal Depression Scale (EPDS) scores ($p=0.04$).

Conclusion: Our data emphasize the unique hormonal landscape during the postpartum period in terms of blunted CAR and lower absolute evening cortisol in healthy women early postpartum compared to non-perinatal. Our findings show a potential association between a reduced CAR and

improved mental well-being during early motherhood, which suggests that reduced CAR might reflect healthy adjustment to early motherhood.

Keywords

Postpartum depression, postpartum well-being, cortisol, Cortisol Awakening Response, stress hormone dynamics, Hypothalamic-Pituitary-Adrenal (HPA) axis

Introduction

The transition to motherhood is one of the most profound psychological and physiological transitions in a woman's life (1). While most women navigate this transition healthily, around 10-15% experience postpartum depression, with the highest risk occurring typically within the first two months after childbirth (2, 3). Promoting mental health postpartum is crucial to minimize human suffering, the high economic burden associated with postpartum depression and optimize infant outcomes (4-7). Little is known about the physiological mechanisms supporting maternal mental health; however, emerging evidence suggests that endocrine systems can be involved in both maintaining and dysregulating perinatal mental health (1, 8, 9).

The Hypothalamic-Pituitary-Adrenal (HPA) axis, an endocrine system, that governs cortisol patterns and responses to stress, undergoes significant changes during pregnancy and postpartum (10-13).

During pregnancy, elevated estradiol levels and the placenta-produced Corticotropin-Releasing Hormone (CRH) cause a two- to three-fold increase in cortisol levels (10, 11, 14). In the early postpartum period, the HPA axis faces an acute withdrawal of placenta produced CRH and combined with desensitized adrenocorticotropic hormone (ACTH) receptors, leading to a decline in cortisol levels (14). The absolute cortisol levels gradually return to its pre-pregnant state within two to three months postpartum (15, 16). The physiological role of the low cortisol postpartum has not yet been established. However, a delayed or prolonged transition to the HPA axis' pre-pregnancy state, including both absolute levels and the restoring of cortisol dynamics, could potentially disrupt the physiological and psychological capacity to cope with stress effectively. This may in turn influence mental health during the vulnerable early postpartum phase, where the risk of developing mental distress and manifest depressive episodes is increased (3).

A 2016 systematic review linked elevated cortisol levels in the first week postpartum with depressive symptoms (17). However, it also revealed inconsistent findings for cortisol levels later postpartum and the association with depression, with some studies showing no association and others suggesting lower cortisol levels in women with depressive symptoms (17). The association between the dynamic aspects of the HPA-axis (CAR) and postpartum depression was inconclusive due to limited data (17). To our knowledge, two studies on CAR and postpartum depression have been published since the review. De Rezende et al. found a lower CAR in both euthymic and depressed women six months postpartum compared to non-perinatal women, and Scheyer et al. found no association between CAR and postpartum depression at three months postpartum (18, 19). Additionally, Iliadis et al. found elevated postpartum evening cortisol levels in women with depressive symptoms and Corwin et al. reported that higher daily total cortisol area under the curve (AUC) was associated with depressive symptoms 14 days postpartum suggesting dysregulated HPA axis activity (20, 21).

Before evaluating the clinical implications of cortisol on postpartum mental health, it is crucial to establish reference knowledge about both the absolute cortisol levels and the cortisol dynamics, in terms of CAR, in healthy postpartum women compared to non-perinatal women and how it relates to mental well-being. To our knowledge, only two studies have addressed CAR in postpartum women compared to non-perinatal women and yielded inconsistent results. Rezende et al. found that the CAR was significantly lower in healthy women six months postpartum (N=51) compared to non-perinatal women, whereas Taylor et al. found no difference in CAR between healthy women 6-8 weeks postpartum (N=79) and non-perinatal women (18, 22). However, the CAR in both studies was calculated from two cortisol samples at awakening and 30 min after awakening (18, 22) as such not capturing the full dynamics, i.e., the downregulation phase of the CAR, which index the inhibitory capacity after stimulated cortisol secretion. The recommendation when measuring the CAR is to base

it on four to five cortisol samples to enhance precision and capture relevant temporal patterns of post awakening cortisol dynamics (23, 24).

Understanding the interactions between cortisol dynamics and mental well-being postpartum can help illuminate critical mechanisms underlying the risk and robustness for mental health in the postpartum period.

In this study, we aimed 1) to determine if the HPA-axis dynamics in terms of the Cortisol Awakening Response (CAR) in women early postpartum differ from non-perinatal women; 2) to evaluate if absolute evening cortisol levels in women early postpartum differ non-perinatal women; and 3) within the postpartum women group, evaluate the association between HPA-axis dynamics in terms of the Cortisol Awakening Response (CAR) and maternal mental well-being.

Methods

Participants

We used data from The Center for Integrated Molecular Brain Imaging (Cimbi) database, which provides a unique dataset of HPA-axis characterization in healthy volunteers (25), and data from postpartum women from a recently completed study (ClinicalTrials.gov Identifier: NCT03795688). In the study, 100 healthy pregnant women were included (26). To ensure that the postpartum women did not have an undiscovered serious psychiatric condition, we employed the Mini-International Neuropsychiatric Interview (M.I.N.I.) (27). The natural cycling controls came from the CIMBI database, where healthy volunteers are eligible for inclusion only if they have no history of psychiatric disorders, have no severe somatic illness, are not using psychotropic medication, have no significant lifetime history of drug abuse, are not pregnant or breastfeeding, and are fluent in Danish (25). Working with postpartum women who underwent cesarean section allowed us to obtain cerebrospinal fluid for other measures of interest in the study (26). Indications for having a cesarean section were

fetal breech position, previous cesarean section, placenta previa, previous rupture of anal sphincter, and uterine fibroid or previous myomectomy, i.e., not motivated by maternal request or mental health problems. Approximately 30% of the postpartum women were enrolled in the study during the 2020 Severe Acute Respiratory Syndrome Coronavirus-2 (COVID-19) pandemic.

We included data from natural cycling women and postpartum women between 18 to 45 years of age with concurrent data on cortisol dynamics (CAR), absolute evening cortisol levels and psychometrics recruited between 2011 and 2020. (28). We excluded women who had a body mass index (BMI) ≥ 35 kg/m^2 if there was no status on use of contraceptives or use of corticosteroids. Some of the women had missing first time point saliva samples making them ineligible for the CAR determination. However, if they had a cortisol sample from the evening, they were still eligible for the absolute evening cortisol analyses.

For the CAR analyses, we excluded three women who had more than 10 minutes from wake-up to first saliva sample and nine women with a CAR time interval below 50 minutes or more than 70 minutes. This resulted in a study population of 50 postpartum women and 91 non-perinatal women.

For the absolute evening cortisol analyses, we only excluded women if they had missing data. This resulted in a study population of 50 postpartum women and 99 non-perinatal women. Flowcharts of the study populations from the CIMBI database are depicted in Figure 1.

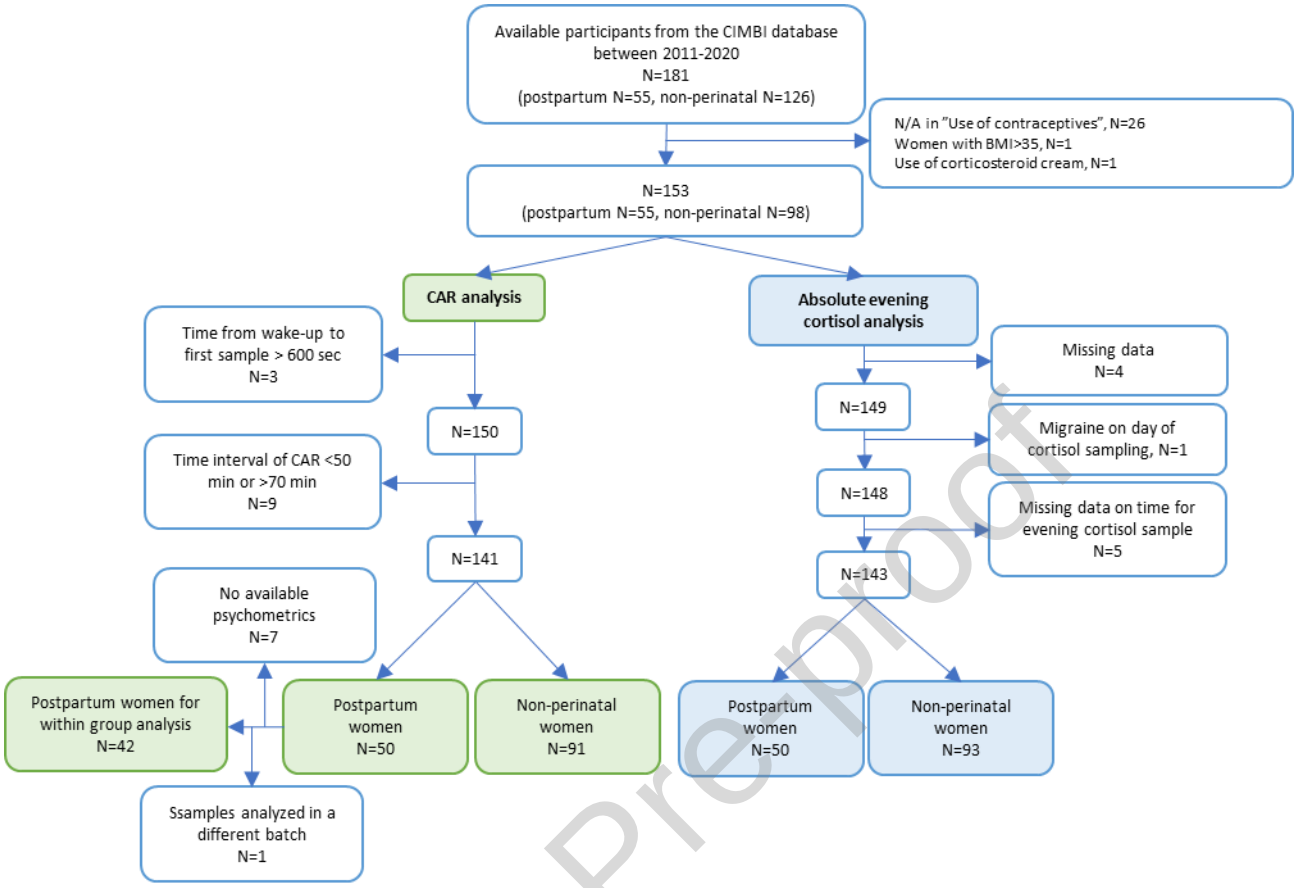

FIGURE 1 | Overview on study population selection from the CIMBI database. Participants recruited from the Center for Integrated Molecular Brain Imaging (CIMBI) database. Flowchart of the study population for CAR analysis and for the Absolute evening cortisol analysis.

Cortisol measurements

Serial saliva samples were sampled at home and collected at 3-5 weeks postpartum for the postpartum women. Participants were instructed to sample saliva immediately after awakening in the morning and again after 15, 30, 45, and 60 minutes and at 10 PM before going to sleep using Salivette® Cortisol tubes (Sarstedt, Nümbrecht, Germany). They received written instructions and a self-reported form to evaluate sample procedure compliance. The participants were trained in the sampling technique prior to the sampling day. The first hour after waking up until the fifth sample was taken,

participants were instructed to abstain from meals, drinks (other than water), brushing teeth, and smoking.

The saliva samples were stored in the refrigerator at maximum 5˚C until brought to the laboratory, either by mail or in-person. To enhance compliance, several text reminders were sent to the postpartum women. Samples were subsequently stored at -80 ˚C until analyses. We used cortisol data from six separate batch analyses, analyzed using two distinct assay types (CLIA and ECLIA).

Psychometrics

We quantified levels of maternal mental well-being 3-6 weeks postpartum using self-reported questionnaires. Our primary measure of interest was the World Health Organization Well-Being Index (WHO-5) as a proxy for levels of mental well-being within this group of overall healthy women. To cover potential associations with subclinical symptoms of mental distress, we also conducted explorative analyses using the measures from the Edinburgh Postnatal Depression Scale (EPDS), the Profile of Mood States (POMS) and the Perceived Stress Scale (Cohen's PSS) (29-32).

The WHO-5 determine the subjective psychological well-being covering the past two weeks. The WHO-5 contains positively phrased items, and the respondent is asked to rate how well each of the 5 statements applies to one when considering the last 14 days (29, 33). Lower score indicates worse well-being.

The EPDS is designed to screen for possible depression in new mothers. It is the most common depression screening tool in postnatal care and assess depressive symptoms during the past seven days, and scores ranges from 0-30. Higher score indicates more depressive symptoms (31).

The POMS assess short-term mood states (i.e. *yesterday and today)* which are understood to be transient and frequently fluctuating (30). It utilizes a 0-4 -point Likert scale to assess ranges of moods. There are six moods subscales. We used the Total Mood Disturbance (TMD) to assess the overall mental state. Higher score indicates worse mood state.

The Cohen's PSS assess if women have perceived their lives as being characterized by unpredictability, lack of control, and excessive demands during the past month. Score ranges from 0-40, and higher score indicates more perceived stress (32).

Data analysis

The demographic variables were described using medians, interquartile range, means and standard deviations.

To determine HPA-axis dynamics in terms of the CAR we computed the area under the curve with respect to increase from baseline (AUCi) from the five-serial home-sampling of saliva across 0-60 minutes from awakening. In case of missing one of the samples apart from #1, the area under the curve with respect to increase (AUCi) was computed using the first and only three additional cortisol samples. Additionally, the cortisol awakening response (CAR) sampling time was approximately 60 minutes for most participants in both groups (mean: 61 min, SD 2.6, range: 55-70 min). To address this, we extrapolated the AUCi values by adjusting the sampling time to 60 minutes. The AUCi corrected for time was used in our analyses.

Data on CAR and absolute evening cortisol from women early postpartum were compared with data from non-perinatal women using a multiple linear regression model adjusted for age and cortisol levels at awakening (sample #1).

We performed a sensitivity test using the cortisol "Area (nmol/L*min) under the cortisol curve above baseline" to restrict the analyses to evaluate positive contributions to the CAR. Further, to address potential effects of batch variation and assay type of the cortisol measurements, we applied the Generalized Least Squares (GLS) test. This allowed us to accommodate random variations within each batch and assay type in the analysis thus accounting for technical analysis differences. We performed sensitivity analysis by including variables that potentially could affect the CAR, i.e., sleep quality (evaluated by the Pittsburgh Sleep Quality Index (PSQI) Global score), POMS TMD, and Cohen PSS score, in our model.

In the postpartum group, we evaluated the association between cortisol dynamics (CAR) and maternal well-being (WHO-5, EPDS, POMS, PSS) using multiple linear regression models. We included estradiol (E2) levels into the regression model due to their association with postpartum well-being in previous studies (8, 34-36) and included parity to account for its potential influence, given differences observed in cortisol levels between primiparous and multiparous women (37). In the within postpartum group analyses, we did not do any statistical analyses on categorical WHO-5 scores (i.e., ≤50 or >50) since only six women had scores below the threshold of 50 indicating depression (29). To adjust for multiple comparisons in our exploratory analyses on mental distress, we applied Bonferroni-Holm correction method. This correction ensured that the probability of making at least one Type I error across all tests remained at or below the chosen alpha level (0.05). For the CAR and WHO-5 analysis, we performed robustness analyses, adjusting for time from birth to cortisol sampling and cortisol levels at awakening.

For the absolute evening cortisol analyses, one outlier with a value of 24.45 nmol/L was excluded due to a migraine attack the same day. Another outlier with a value of 21.00 nmol/L was kept in the analysis since we were unaware of any potential bias to her data; however, we provide results from a sensitivity analysis by leaving this value out of the model.

In 40 out of 154 samples (36 of the postpartum and 4 of the non-perinatal women), the absolute evening cortisol values were below the lower detection limit of 0.55 nmol/L, accordingly we imputed these values to the value of 0.54 nmol/L.

All data were analyzed using the statistical software RStudio 4.2.2. Results were considered statistically significant at the 5% level.

Results

Sociodemographic, cortisol and psychometric data are presented in Table 1. The postpartum women and the non-perinatal women differed on several parameters: the postpartum women were older than the non-perinatal women, they woke up later in the morning, went to bed earlier in the evening, and reported worse sleep quality. The non-perinatal women were normal weight (mean BMI: 23.2 (SD 3.1)), and 90% were non-smokers.

The cortisol samples were collected from postpartum women at an average of 38 days after giving birth (SD 11 days, range 15-69 days), and the questionnaires were answered at an average of 34 days after giving birth (SD 10 days, range 16-63 days). In the first cortisol sample taken upon awakening, the postpartum women exhibited significantly lower absolute cortisol levels at awakening than the non-perinatal women, as expected, with a mean of 6.0 nmol/L (SD 2.7) and 11.8 nmol/L (SD 5.6), respectively.

TABLE 1 | Demographic and psychometric data, cortisol and hormonal parameters

Parameters	**Postpartum women (N=50)**	**Non-perinatal women (N=91)**	**p-value**

Age (Mean, (SD))	34.2 (3.8)	25.1 (3.8)	<0.001
Parity			
- Nulliparous (%)	22 (44)	5 (5)	
- Multiparous (%)	28 (56)	83 (91)	
Days after birth for cortisol sample (Median days (SD))	38.0 (11)	-	
Time of first cortisol sample (mean hour mins (SD))	07:29 (77)	07:11 (67)	0.065
Time of evening cortisol sample (mean hour min (SD))	22:37 (01:01)	23:04 (01:06)	0.013
Cortisol levels at awakening (mean nmol/L (SD))	6.0 (2.7)	11.8 (5.6)	<0.001
Evening cortisol levels (mean nmol/L (SD))*	0.9 (0.9)	4.5 (3.0)	<0.001
AUCi (mean (SD))	38.4 (166)	240 (309)	<0.001
AUCi time corr (mean (SD))	38.2 (166)	238 (303)	<0.001
Length of CAR parameters (mean min (SD min))	60.9 (2.9)	60.5 (2.5)	0.38
E2 (mean pmol/L (SD))	62.8 (50.6)	-	
	Postpartum women (N=42)	**Non-perinatal women (N=91)**	
Cohen PSS score (0-40) (mean (SD))	6.8 (5.7)	8.5 (6.1)	0.13
EPDS score (0-30) (mean (SD))	3.7 (3.0)	-	
POMS - TMD score (-32-200) (mean (SD))	1.3 (13.3)	3.0 (16.5)	0.53
WHO-5 score (0-100) (mean (SD))	67.1 (16.5)	-	
PSQI Global score (0-21) (mean (SD))	7.0 (2.8)	3.9 (1.8)	<0.001

Mean/median and standard deviation are shown for clinical parameters and psychometrics in each group. AUCi, area under the curve with respect to increase from baseline at awakening. PSQI Global, Pittsburgh Sleep Quality Index Global score; POMS, Profile Of Mood States "Total Mood Disturbance score"; POMS Vigor, POMS, Profile Of Mood States "Vigor-Activity factor" , WHO-5, WHO-5 Well-being index

*Values below the lower detection limit of 0.55 nmol/L were imputed with the value of 0.54 nmol/L

Cortisol awakening response (CAR) early postpartum compared to healthy non-perinatal women

The mean CAR (AUCi) in the postpartum women was 38.2 nmol/L*minutes and 238 nmol/L*minutes in the non-perinatal women, corresponding to 84% lower levels in the postpartum women. In the model adjusting for age and cortisol levels at awakening, the postpartum women had

a significantly lower CAR than the non-perinatal women (β = -303, 95% CI [-429; -178], p<0.001) (Table 2). For every unit increase in cortisol levels at awakening, there was a decrease of approximately 22 units in AUCi (p<0.001), when keeping other variables constant. The AUCi for the two groups is depicted in Figure 2a. The unadjusted mean cortisol values across 0-60 minutes from awakening in the two groups are depicted in Figure 2b.

The CAR was not significantly associated with sampling time postpartum, i.e., number of days postpartum (p=0.08).

The sensitivity test using the cortisol "Area (nmol/L*min) under the cortisol curve above baseline" to restrict the analyses to evaluate the positive contributions to the CAR did not alter the conclusion of a significant difference between the two groups (β=-178, 95% CI [-284; -71], p<0.001). Further, we also performed sensitivity analyses to exclude spurious cortisol batch effects and assay type, which cannot be efficiently adjusted, potentially driving our findings. This was not the case, and the result remained robust and consistent, with a statistically significant difference between the two groups (β = -408, 95% CI [-622; -194], p<0.001). Also, when ignoring data from batches with < 3 samples in total, effect sizes, and significance values were similar to the main analysis, including all batches (β =-305, 95% CI [-431; -178], p<0.001), N=50 postpartum women and N=89 non-perinatal women). Finally, the postpartum women reported worse sleep quality than the non-perinatal women; however, including the PSQI global score and other mental health factors in our model did not affect the result (β=-434, 95% CI [-663; -201], p<0.001) (Supplementary Table 1).

TABLE 2 | Cortisol awakening response (AUCi) and absolute evening cortisol in women early postpartum compared to healthy non-perinatal women

	Cortisol awakening response (AUCi)*

Covariates for adjustment	Effect (nmol/L*minutes)	95% CI	p-value
Adjusted for age and cortisol at awakening	-303	[-429; -178]	<0.001
Crude analysis	*-200*	*[-292; -108]*	*<0.001*

Absolute evening cortisol

Covariates for adjustment	Effect (nmol/L)	95% CI	p-value
Adjusted for age	-3.46	[-4.64; -2.28]	<0.001
Crude analysis	*-3.92*	*[-4.76; -3.08]*	*<0.001*

*Time corrected AUCi: AUCi divided by the actual sampling time in minutes multiplied by 60 minutes
Cortisol awakening response: N=50 postpartum women and N=91 non-perinatal women
Absolute evening cortisol: N=50 postpartum women and N=93 non-perinatal women

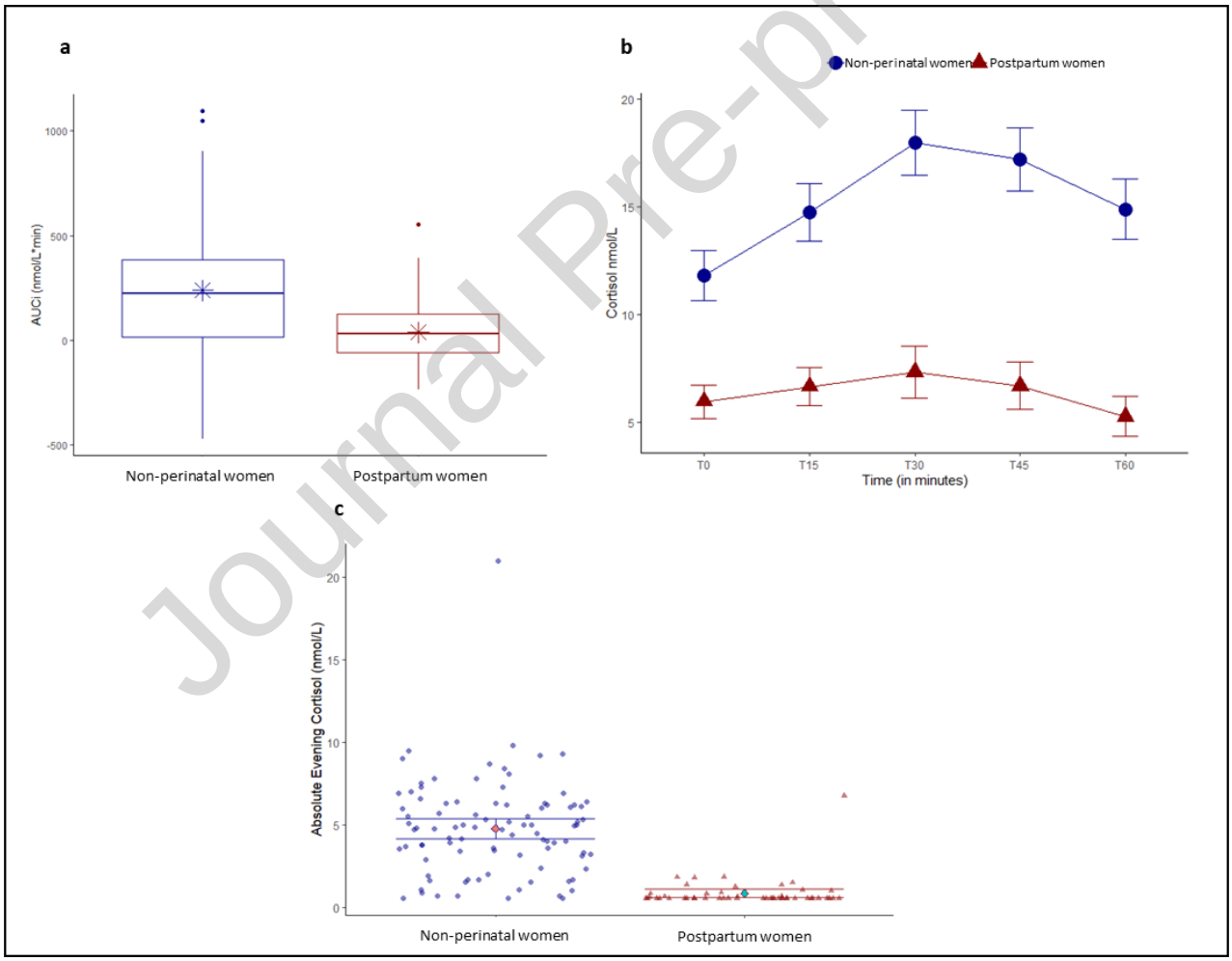

FIGURE 2 | a) Boxplot of the Cortisol Awakening Response (AUCi) in postpartum women (n=50) and non-perinatal women (n=91), b) Saliva cortisol concentrations during the first hour after awakening in postpartum women (n=50) and non-perinatal women (n=91). Error bars represent a 95% confidence interval for the corresponding mean, and c) Absolute

evening cortisol in postpartum women (n=50) and non-perinatal women (n=93). Absolute evening cortisol values is shown for each individual. Error bars represent a 95% confidence interval for the corresponding mean.

Absolute evening cortisol early postpartum compared to healthy non-perinatal women

Absolute evening cortisol level was collected at mean time = 22:37 Central European Time (CET) (SD = 01:01) for postpartum women and 23:04 CET (SD = 01:06) for non-perinatal women.

The absolute evening cortisol levels for the postpartum women were 80% lower than those of the non-perinatal women with a mean of 0.9 nmol/L compared to a mean of 4.5 nmol/L, respectively. Similarly, in our model adjusting for age, the postpartum women had significantly lower absolute evening cortisol compared to the non-perinatal women (β = -3.46, 95% CI [-4.64; -2.28], $p < 0.001$), as shown in Figure 2c.

The sensitivity analysis in which we left the outlier in the non-perinatal group out did not change the results or the estimate for the non-perinatal group (β = -3.05, 95% CI: [-4.05; -2.07], $p < 0.001$). Since the data did not meet the assumption of normal distribution, we used the Mann-Whitney U test to supplement the linear regression. The difference between the two groups remained statistically significant ($p < 0.001$).

Cortisol awakening response (CAR) and maternal mental well-being

Only six women scored below 50 on the WHO-5 scale and one woman scored above 11 on the EPDS scale, indicating that the women overall had high psychological well-being.

We found statistically significant association between CAR (AUCi) and total score on WHO-5 (β = -0.04, 95% CI [-0.07; -0.00], $p = 0.048$) after controlling for the effect of parity and E2 (Table 3). The results indicate that lower CAR postpartum might be associated with higher well-being in healthy

women and vice versa. This aligns with the finding that levels of subclinical depressive symptoms, i.e., EPDS score, and CAR appeared to be positively correlated (β = 0.007, 95% CI [0.002; 0.013], p(corrected) = 0.04).

On the other hand, the CAR was not significantly associated with POMS score (β = 0.02, 95% CI [-0.007; 0.05] p(corrected) = 0.21) or Cohen's PSS scores (β = 0.01, 95% CI [-0.00; 0.02], p(corrected) = 0.21).

The robustness analyses for WHO-5 and CAR, adjusting for time from birth to cortisol sampling did not affect the results (β= -0.04, 95% CI [-0.07; -0.00], p=0.048).

The associations between CAR and WHO-5 total score, EPDS score, POMS score, and Cohen PSS score is visualized in Figure 3.

TABLE 3 | Cortisol awakening response (AUCi)* and maternal mental well-being

Covariates for adjustment	Effect (nmol/L*minutes)	95% CI	p-value (uncorrected)	Corrected p-value**
Main analysis				
WHO-5				
Adjusted for E2 and parity	-0.04	[-0.07; -0.00]	0.048	
Crude analysis	*-0.04*	*[-0.07; 0.002]*	*0.037*	
Exploratory anlyses				
EPDS				
Adjusted for E2 and parity	0.007	[0.002; 0.013]	0.01	0.04
Crude analysis	*0.008*	*[0.002; 0.014]*	*0.01*	
POMS TMD				
Adjusted for E2 and parity	0.02	[-0.007; 0.05]	0.13	0.21
Crude analysis	*0.02*	*[-0.005; 0.05]*	*0.11*	
Cohen PSS				
Adjusted for E2 and parity	0.01	[-0.00; 0.02]	0.10	0.21
Crude analysis	*0.01*	*[-0.00; 0.02]*	*0.05*	

WHO-5, World Health Organization Well-Being Index; POMS TMD, Profile of Mood States "Total Mood Disturbance score"; Cohen PSS, The Perceived Stress Scale; EPDS, Edinburgh Postnatal Depression Scale

*Time corrected AUCi: AUCi divided by the actual sampling time in minutes multiplied by 60 minutes

**Bonferroni-Holm correction applied through p-value adjustment

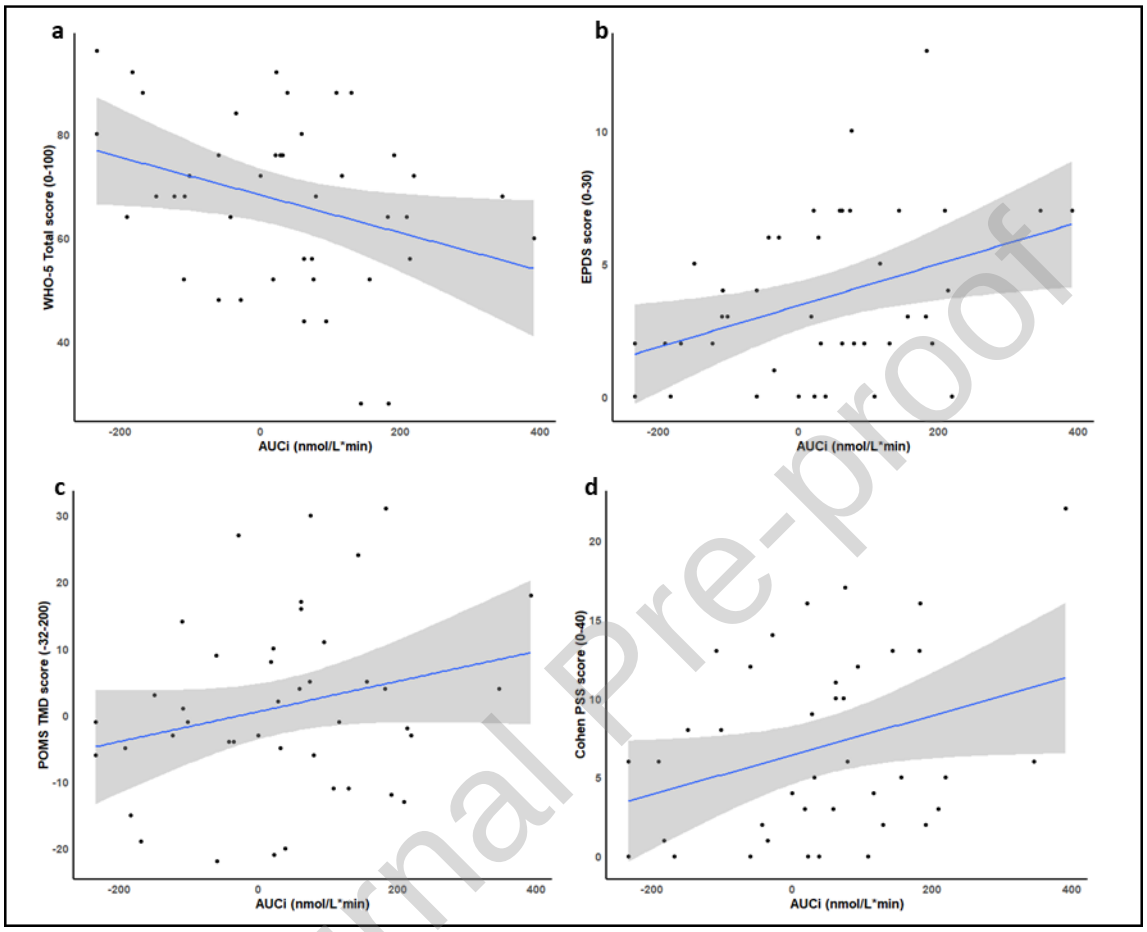

Figure 3 | Scatter plot of the correlation between the cortisol awakening response (AUCi) and a) WHO-5, World Health Organization Well-Being Index total score, b) EPDS, Edinburgh Postnatal Depression Scale Sum Score, c) POMS, Profile of Mood States Total Mood Disturbance score, d) Cohen PSS, Perceived Stress Scale score

Discussion

We found that healthy women early postpartum had marked reductions in HPA-axis dynamics, i.e., an estimated 84% lower CAR relative to healthy non-perinatal women. Also, notably, we identified 80% lower absolute evening cortisol in the postpartum women relative to non-perinatal women. In

the postpartum group, there was a statistically significant association between lower CAR and higher well-being (WHO-5) and lower mental distress (EPDS). However, we did not find an association between CAR and transient mood states (POMS) or perceived stress (Cohen's PSS).

Our finding of blunted CAR in healthy women early postpartum relative no non-perinatal women further add to the literature that the pregnancy induced suppression of cortisol persists at least until 5-6 weeks postpartum, indicating that it most likely represents a normal physiological phenomenon (15, 16). The blunted CAR and the lower absolute evening cortisol in women early postpartum may result from the high levels of CRH in pregnancy, desensitizing the anterior pituitary to CRH (14). Moreover, it has been suggested that pregnancy induced CRH receptor desensitization in the hypothalamus and its higher regulatory inputs may be linked to decreased CRH secretion, potentially resulting in mild adrenal suppression after childbirth, possibly lasting between 5-12 weeks postpartum (14-16). Our findings of a lower CAR in healthy postpartum women confirm the results by de Rezende et al. (18), who showed a lower CAR in euthymic women at six months postpartum. Conversely, in a study by Taylor et al., at 7.5 weeks postpartum, the euthymic women displayed the same CAR as the non-perinatal women (22). However, the CAR in both studies was calculated from two cortisol samples, which potentially could have produced inaccurate results (23, 38).

Our results taken together with the existing evidence on the CAR postpartum in healthy women support that a blunted CAR is part of a physiological phenomenon of the perinatal transition (14-16, 18).

We found that healthy postpartum women had significantly lower absolute evening cortisol than non-perinatal women. This mean absolute evening cortisol level of 0.9 nmol/L corresponds closely to the results reported by Iliadis et al., who found an average evening cortisol level of 0.89 nmol/L in women without postpartum depressive symptoms (21). These findings together with the blunted CAR show

that the overall circadian rhythm is affected during the postpartum period, suggesting that it is part of a normal and healthy mechanism of the perinatal transition.

Within our healthy postpartum women group, we found an association between a lower CAR and both higher well-being and lower mental distress. Supporting our results, Corwin et al. found that the total salivary cortisol output (AUC) in a healthy population was significantly higher in depressed women compared to euthymic women suggesting that a "dysregulation" might protect maternal mental health (20). This contrasts with findings from studies of depressed women postpartum; where Taylor et al. and De Rezende et al. observed a reduced CAR in depressed postpartum women compared to non-depressed postpartum women at 7.5 weeks postpartum and six months postpartum, respectively (18, 22). However, two studies of healthy women by Cheng et al. and Scheyer did not find any associations between CAR and depressive symptoms at 4-6 weeks and 3 months postpartum, respectively (19, 39). The CAR in these studies was assessed at different time points postpartum, and, in three out of four studies, the CAR was calculated from two cortisol samples not capturing the downregulation of the response to baseline, which might explain the discrepancies between the results (18, 19, 22, 23, 38, 39). Also notably, these cohorts had maladaptive responses to their postpartum transition in terms of depressive symptoms, which may well be linked to other patterns of associations between HPA-axis dynamics and mental state than in women with healthy perinatal transitions. Moreover, cortisol is influenced by numerous factors, including stressors, sleep patterns, and hormonal changes (40-42). The temporal dynamics of cortisol secretion, influenced by the circadian rhythm and different sleep patterns postpartum, might contribute to the inconsistency across studies conducted at different times postpartum. This illuminates the complexity of the relationship between cortisol levels and mental health during the perinatal period (43). The heterogeneity in findings on CAR and postpartum mental health align with similar conflicts in systematic reviews regarding CAR and major depressive disorder outside the perinatal period (43, 44).

Taken together, our findings are consistent with the notion that a blunted CAR is part of a healthy pre- to postpartum transition and may serve multiple adaptive purposes in the early postpartum period, such as conserving energy for lactation, protecting against stress-related lactation inhibition, enhancing immune function, promoting calmness, and potentially facilitating maternal-infant bonding and emotional well-being (1, 20, 45). Future studies should replicate and extend our findings in relevant study designs to obtain a more comprehensive understanding of the relationships between CAR and maternal mental well-being also in women at high risk for postpartum depression, e.g., women with prior depressive episodes or other robust risk factors.

Our findings highlight the need for further research to elucidate the underlying adaptive and maladaptive responses that may drive the associations between CAR and postpartum well-being and potentially informing precision prevention of mental distress and postpartum depression.

Strength and Limitations

This study has several strengths. First, we followed the recommended practice of using five saliva samples to calculate the CAR, ensuring a more precise representation of post-awakening cortisol dynamics. This method captures both the increase and inhibitory feedback capacity, enhancing our understanding of cortisol temporal patterns (23, 38). Second, our evaluation of cortisol dynamics and mental well-being is a strength, given the multifaceted nature of postpartum mental health. We included a broader range of measures covering both positive and negative aspects of mental state, providing a more comprehensive view of how cortisol levels relate to postpartum mental state. Third, the fact that the mental well-being scales used in the study correlated as expected validates their convergent measurement of the same underlying mental state.

The study has limitations. First, self-reported saliva sample times for the CAR may be less accurate due to early morning disruptions from infant care, potentially introducing bias (46, 47). However, we encouraged women to be precise with sampling times and to choose another day if uncertain about their awakening time, improving data reliability. Including sleep quality in our model didn't impact the result. Second, we did not have access to data on breastfeeding, smoking, and pre-pregnancy BMI, which could potentially influence our outcomes (38). Nevertheless, it's worth noting that, even though we didn't have the precise numbers for breastfeeding, BMI, and smoking, the majority of women in our study were breastfeeding, had a normal BMI, and most Danish women do not smoke during pregnancy and only 6 of the non-perinatal women were smokers (48, 49). Third, imputing values below the lower detection limit for evening cortisol might slightly overestimate lower values. Still, since postpartum women generally had lower levels than non-postpartum women, our assessment conservatively represents the actual difference. Also, we may have missed meaningful relationships due to limited statistical power to detect smaller effect sizes.

Conclusion

Our study shows substantial differences in the cortisol dynamics, in terms of CAR, and absolute evening cortisol levels between healthy early postpartum women and healthy non-perinatal women, emphasizing that the overall circadian rhythm is blunted postpartum. While we observed an association between lower CAR and higher well-being and lower mental distress within the postpartum cohort, no such associations were found for more transient mood states or perceived stress. These findings highlight the complexity of cortisol's role in postpartum mental health and suggest that the blunted CAR may subserve healthy adaption to early motherhood. Further studies with larger sample sizes and other clinical spectra is needed to advance the understanding of cortisol

dynamics in reproductive mental health and how it may inform relevant, personalized strategies for promoting maternal mental health.

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Ethical approval statement

All studies included in this study were formally registered and granted approval by the Capital Region's Committee on Health Research Ethics in Denmark (VEK H-2-2010-108, VEK H-6-2014-057, VEK H-4-2012-105, VEK (KF)01-2006-20, VEK H-15004506, VEK H-15017713, VEK H-18029563, VEK H-2-2014-070, and VEK H-4-2011-103) (25). Furthermore, these studies were carried out in strict adherence to the principles outlined in the Declaration of Helsinki. Prior to their involvement, all participants voluntarily provided written consent.

Conflict of interest

VGF discloses that she has received honorarium in form of consulting fees from SAGE Therapeutics, Lundbeck Pharma A/S, Janssen Cilag A/S and Gedeon-Richter A/S. All other authors disclose no potential conflict of interest.

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CRediT authorship contribution statement

Stinne Høgh: Conceptualization, Methodology, Investigation, Formal analyses, Writing first draft. **Emilie Ø. Lange:** Conceptualization, Methodology, Formal analyses, Writing and editing. **Kristian Larsen:** Formal analysis, Writing and editing. **Emma S. Høgsted:** Investigation, Writing and Editing. **Hanne K. Hegaard:** Resources, Writing and Editing. **Camilla Borgsted:** Project administration, Conceptualization, Methodology, Investigation, Writing and Editing. **Vibe G. Frokjaer:** Conceptualization, Methodology, Investigation, Supervision over investigation and analyses, Writing and Editing, Funding acquisition. All authors reviewed and approved the final manuscript as submitted.

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Declaration of Interest Statement

VGF discloses that she has received honoraria in the form of consulting fees from SAGE Therapeutics, Lundbeck Pharma A/S, Janssen Cilag A/S, and Gedeon-Richter A/S. All other authors have no conflicts of interest.

Highlights

- Healthy postpartum women had a blunted CAR corresponding to 84% reduction compared to non-perinatal women
- Healthy postpartum women had 80% lower absolute evening cortisol compared to non-perinatal women
- We note a potential association between lower CAR and higher well-being postpartum
- We suggest that reduced CAR reflects a healthy adjustment to early motherhood.

APPENDIX II, PAPER II

GDF15 levels are not associated with Cortisol Awakening Response or mental health across the perinatal transition

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Conflict of interest

VGF discloses that she has received honorarium in form of consulting fees from SAGE Therapeutics, Lundbeck Pharma A/S, Janssen Cilag A/S and Gedeon-Richter A/S. ABK and CC are co-founders of Ousia Pharma ApS. All other authors disclose no potential conflict of interest.

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Word count, Abstract: 400

Word count, Main text: 2993

Tweetable statement: Novel research finds no link between GDF15 levels, Cortisol Awakening Response, or mental health during the perinatal transition. Shedding light on hormonal dynamics in pregnancy.

Short Title: GDF15 and mental health across the perinatal transition

AJOG at a Glance:

1) Placenta produced hormones and cytokines and their changes across the perinatal period may be critical for healthy adaptations to motherhood and symptoms of mental distress.
2) The cytokine GDF15 appear to play a role for pregnancy related nausea but the role in perinatal mental health is unknown.
3) We found no association between GDF15 and mental health outcomes postpartum and hypothesize that (variations in) nausea responses to pregnancy induced GDF15 are unrelated to mental health effects of GDF15.

Abstract

Background: During the perinatal transition, hormonal changes, including the decline of placenta produced sex steroids, cortisol, and inflammatory factors, play a vital role in mental health adaptation to motherhood. Growth Differentiation Factor 15 (GDF15) increases substantially during pregnancy, primarily produced by the placenta. Elevated levels of GDF15 have been associated with mental health problems in non-perinatal populations, higher corticosterone levels, and increased estrogen receptor activity. However, the role of GDF15 in mental health during the perinatal transition remains unknown. This longitudinal study is the first to evaluate levels of GDF15 in cerebrospinal fluid (cGDF15) and the association with mental health postpartum, along with mapping plasma GDF15 (pGDF15) level changes from late pregnancy to early postpartum.

Objectives: The primary aims were to 1) evaluate the association between pregnancy cGDF15 levels and cortisol early postpartum, 2) evaluate the association between pregnancy cGDF15 levels and mental health in pregnancy and postpartum, and 3) evaluate the association between pGDF15 and estrogens and high-sensitivity C-reactive protein (CRP).

Study Design: We included data from 95 women scheduled for a cesarean section and obtained CSF and plasma levels of GDF15. We quantified *GDF15* mRNA levels in placenta biopsies. Estrogens, high-sensitivity CRP, and mental health measures were further collected 0 to 1 day before the cesarean section. At five weeks postpartum, mental health measures and saliva samples for cortisol analyses were collected. Correlation analyses for GDF15 in CSF, plasma, and placenta mRNA were performed, along with association analyses for pregnancy cGDF15, Cortisol Awakening Response, and mental health outcomes.

Results: We demonstrated a high correlation between cGDF15 and pGDF15 ($r=0.52$; $p<0.001$) and found that both cGDF15 and pGDF15 correlated with placenta *GDF15* mRNA * placenta weight ($r=0.62$, $p<0.001$ and $r=0.44$, $p=0.008$, respectively). During late pregnancy, both estradiol (E2) and estriol (E3) were significantly associated with pGDF15 levels (E2: $p=0.002$; E3: $p(\text{corrected})<0.001$). Finally, we found that markers for postpartum mental health status, such as self-reported mental well-being and the Cortisol Awakening Response or absolute cortisol at awakening, were not associated with cGDF15 levels.

Conclusion: This novel study points to the unique hormonal landscape across the perinatal transition and the specific role of GDF15 in pregnancy, which appears uncoupled with perinatal mental health outcomes. The observed correlations between GDF15 across tissues suggest its global production in

the placenta and may guide future study designs. Our findings, raise the hypothesis that GDF15 associated hyperemesis gravidarum is independent on mental health effects of GDF15.

Keywords

Growth Differentiation Factor 15, pregnancy, placenta, hormones, postpartum depression, postpartum well-being, cortisol, Cortisol Awakening Response, stress hormone dynamics

Introduction

Emerging evidence suggests that the dynamic hormonal changes occurring in the perinatal transition may contribute to regulating mechanisms crucial for mental health when transitioning to motherhood (1-4).

Growth Differentiation Factor 15 (GDF15), a cytokine that belongs to the transforming growth factor β (TGF-β) superfamily, is highly expressed in the human placenta (5, 6). GDF15 is an autocrine regulator of macrophage activation and is triggered in response to stimuli inducing cellular stress (7, 8). GDF15 exerts its functions through the glial-derived neurotrophic factor receptor alpha-like (GFRAL) receptor in the hindbrain (9).

During pregnancy, levels of GDF15 increase significantly as pregnancy progresses, reaching up to a 200-fold increase in serum and a more than a 3-fold increase in cerebrospinal fluid (CSF) compared to non-pregnant levels (5, 10, 11). Notably, the increase in GDF15 levels is far more prominent in humans and non-human primates compared to mice and rats, indicating a potentially vital role for GDF15 in primate pregnancy (10, 11).

Although research on GDF15 in pregnancy remains in the early stages, high levels of GDF15 have been considered a potential biomarker for pregnancy complications such as preeclampsia, gestational diabetes, miscarriage and in particular hyperemesis gravidarum (12-18). By combining findings in human participants and mice, Fejzo et al. recently suggested that the severity of nausea and vomiting results from the interaction between fetal-derived GDF15 and the mother's sensitivity to GDF15 (17). Hyperemesis gravidarum has been significantly associated with the risk of depressive symptoms (19). This suggests that the physiological effects of elevated GDF15 may extend beyond the somatic impact and play a role in the mental well-being of pregnant women or, vice versa, that suboptimal mental health and (stress) coping capacities shape how somatic distress (e.g., nausea) is perceived.

Recently, GDF15 has been associated with activating the endocrine stress response through the Hypothalamic-Pituitary-Adrenal (HPA) axis in rodents (20), suggesting that GDF15 functions as part of the organism's coping mechanism to significant external threats or stress (20). During pregnancy, the HPA axis undergoes notable changes with increased cortisol levels and reduced dynamics (21, 22). In the early postpartum period, cortisol levels decrease and gradually return to pre-pregnancy dynamics (21, 23). Impaired HPA axis dynamics have been associated with postpartum depression and mental distress, although data remains limited (24-28).

In the field of mental health, emerging evidence points to elevated levels of GDF15 in patients affected by psychosis and depression (29-31). Additionally, estrogen receptor activity and GDF15

expression appear to be related (32). In the peripartum period, estradiol has been associated with depressive symptoms both in risk models of depression and in postpartum women (33-36).

In this study, we leverage a unique dataset from women across the perinatal transition with the same subject measures on GDF15 in CSF (cGDF15), plasma (pGDF15), mRNA expression in placental tissue, psychometrics, and cortisol dynamics. We aim to 1) evaluate the association between pregnancy cGDF15 levels and cortisol early postpartum, 2) evaluate the association between pregnancy cGDF15 levels and mental health in pregnancy, and postpartum and 4) evaluate the association between pGDF15 and estrogens and high-sensitivity C-reactive protein (hs-CRP) in late pregnancy.

Methods

Participants

We used data from a recently completed study (ClinicalTrials.gov Identifier: NCT03795688), including healthy pregnant women from Copenhagen University Hospitals (Rigshospitalet and Herlev Hospital) in Denmark from March 2019 to November 2020 (37). We included women aged 18-40 years with GDF15 measures. To obtain CSF for pregnancy GDF15 quantification, we included women scheduled for a planned cesarean section (C-section), due to fetal breech position, previous C-section, previous myomectomy, obstructing fibroid, previous rupture of the anal sphincter, and placenta previa. We excluded women with a history of severe somatic or psychiatric illness, pre-pregnancy Body Mass Index below 18 or above 35, severe postpartum hemorrhage, severe neonatal morbidity, use of antidepressants, substance abuse, or non-fluency in Danish. For a detailed overview of the number of participants included in the analysis for each specific aim, see Tables 2 and 3.

CSF for GDF15 protein analysis

As part of the he anesthetic procedures during spinal anesthesia for the C-section. anesthesiologists extracted 0.5–1 ml of CSF. The CSF was promptly transferred to dry ice and stored at -80°C until GDF15 ELISA measurements. GDF15 was measured in CSF using the Quantikine ELISA Human GDF-15 Immunoassay (ELISA, R&D systems, catalog no. DGD150). The ELISA assays were used according to the manufacturer's protocol.

Placenta biopsies for GDF15 mRNA analysis

Biopsies were taken from healthy cotyledons within 30 minutes from birth on the maternal side using an 8 mm sterile punch biopsy. The biopsy was dipped in a cold phosphate-buffered saline solution 4-5 times, and added 1 mL RNAlater. Samples were stored at 4°C for 24-48 hours, then drained of RNAlater and transferred to -80°C until RNA extraction.

RNA extraction & cDNA synthesis

Placental tissue was homogenized in Trizol reagent (QIAzol Lysis Reagent, Qiagen) using a stainless-steel bead (Qiagen) and a TissueLyser LT (Qiagen) for 3 min at 20 Hz. For a detailed description please see Klein et al (10).

qPCR for mRNA quantification

SYBR green qPCR was performed using PrecisionPLUS qPCR Mastermix containing SYBR green (Primer Design, #PrecisionPLUS). Primers used for *GDF15* were F: GCTACGACCTGCTAACC; R: GATCCCAGCCGCACTTCTG. *RPL13A* was used as a reference gene and was amplified using the following primers: F: AAGCCAAGATCCACTACCGG; R: TTGAGGACCTCTGTGTATTTGTC. The qPCR was performed in 384-well plates on a Light Cycler 480 Real-Time PCR machine using 2 min preincubation at 95°C followed by 45 cycles of 60 sec at 60°C. Melting curves were performed stepwise by increasing the temperature from 60°C to 95°C. Quantification of mRNA expression was performed according to the delta-delta Ct method.

Blood samples

The plasma samples for GDF15, estrogen, and hs-CRP analyses were collected on the day of C-section and approximately five weeks postpartum. The samples were stored at -80°C until analyses by a collaborating lab. Concentrations of estrone (E1), Estradiol (E2), and estriol (E3) were measured as detailed in the methodology described by Frederiksen et al. (38). E3 was exclusively analyzed during pregnancy, as levels are typically below the limit of detection in non-pregnant states (38, 39). Plasma GDF15 was measured as described for CSF (see above).

Cortisol measurements

Participants conducted serial saliva samples in their homes at 5 weeks postpartum. Specific instructions and procedures were according to guidelines described by Stalder et al. and described in detail in earlier manuscripts (40, 41).

To assess the Cortisol Awakening Response (CAR), we computed the area under the curve with respect to the increase from baseline (AUCi) based on five sequential saliva samples collected within 0-60 minutes from awakening.

Psychometry

Our primary mental well-being outcome was the World Health Organization Well-Being Index (WHO-5) score. Exploratory outcomes were Total Mood Disturbance (TMD) scores from the Profile of Mood States (POMS), and the Edinburgh Postnatal Depression Scale (EPDS) score (42-44).

The WHO-5 measures psychological well-being and consists of five positively worded mood and general life satisfaction statements. The total raw score ranges from 0 (worst possible well-being) to 100 (best possible well-being) (42).

The POMS is a psychological questionnaire designed to assess a person's transient, fluctuating emotional states. The TMD score assesses the overall levels of mental distress. The score ranges from -32 to 200, with higher scores indicating more mental distress (43).

The EPDS is a widely used self-report questionnaire designed to identify and assess symptoms of postpartum depression in mothers. The total score can range from 0 to 30, with a higher score suggesting a greater severity of postnatal depression symptoms (44).

Data analysis

Demographic variables were described using medians, ranges, means, and standard deviations, as appropriate.

The correlation between cGDF15 and pGDF15, and placenta *GDF15* mRNA, was assessed using pairwise Pearson correlation coefficients, including 95% confidence intervals (CI). Additionally, we conducted pairwise Pearson correlation coefficient analyses between cGDF15 and pGDF15 and placenta *GDF15* mRNA multiplied by placental weight.

The association between cGDF15 and mental health outcomes was evaluated in multiple linear regression models adjusted for age, E2, and hs-CRP. The WHO-5 outcome was considered our

primary outcome in the mental health analysis.

The association between cGDF15 and cortisol outcomes was evaluated in multiple linear regression models adjusted for age, E2, and hs-CRP. The CAR outcome was considered our primary outcome in the cortisol analysis. We excluded one woman whose cortisol samples were analyzed in a different batch than the others.

The association between pGDF15 and estrogens (E1, E2, E3) and hs-CRP was evaluated in a linear regression model.

For all outcomes, we initially assessed if all assumptions for conducting linear regression were met before conducting the analysis. To adjust for multiple comparisons in our exploratory analyses, we applied the Bonferroni-Holm correction method.

We performed sensitivity analyses on pGDF15 and cortisol/mental health outcomes and on cGDF15 and E1, E2, E3, and hs-CRP outcomes to evaluate if a potential effect of GDF15 was dependent on where the GDF15 was derived (CSF or plasma).

Results

The average age of the 95 women included in the study was 33.9 years, with 38% being nulliparous. Additionally, 46% were carrying female fetuses, cesarean sections were conducted at an average gestational age of 39+0, ranging from 261 to 289 days (Table 1).

The mental distress and well-being scores of the participants fell within the normal spectrum (42, 45).

Placental GDF15 mRNA and pregnancy GDF15 levels in CSF and serum

GDF15 levels exhibited a significant decrease from pregnancy to postpartum, with pregnancy levels approximately 172 times higher than postpartum levels (Table 2 and Figure 1). Notably, cGDF15 levels and pGDF15 levels correlated significantly ($r=0.52$; $p<0.001$) (N=89, Table 2 and Figure 2). Interestingly, placental *GDF15* mRNA and cGDF15 correlated significantly ($r=0.43$, $p=0.01$), indicating an association between gene expression and cerebrospinal fluid levels. Conversely, only at trend level were placental *GDF15* mRNA and pGDF15 correlated. However, we found a significant correlation between pGDF15 and placenta *GDF15* mRNA*placental weight (N=34, Table 2 and Figure 1).

Pregnancy cGDF15 levels and Cortisol Awakening response (CAR)/cortisol at awakening

We did not find an association between pregnancy cGDF15 levels and CAR or cortisol levels at awakening (β = 34.9, 95% CI [-144.6; 214.5], p=0.70, and β = -1.8, 95% CI [-4.5; 0.9], p(corrected)=0.38, respectively) (N=47, Table 3 and Figure 3).

Pregnancy cGDF15 levels and mental well-being in pregnancy and postpartum

Our study revealed no significant association between cGDF15 levels and mental well-being or mental distress in late pregnancy, as assessed by the WHO-5 (β=-5.9, 95% CI [-15.7; 4.0], p=0.24) or the POMS TMD (β=6.5, 95% CI [-6.2; 19.2], p=0.31) (N=70, Table 3 and Figure 4).

Similarly, no significant association was observed between cGDF15 levels during late pregnancy and mental well-being in the early postpartum period, in both unadjusted and adjusted models (N=57, Table 3 and Figure 4).

Pregnancy pGDF15 levels, hs-CRP and estrogens (E1, E2, E3)

Levels of E1 during pregnancy were approximately 277 times higher than postpartum levels, while the levels of E2 during pregnancy were approximately 1468 times higher than postpartum levels (Table 1). Hs-CRP also decreased from pregnancy to postpartum, however not as dramatic as the estrogens. PGDF15 levels were significantly associated with both E2 and E3 levels during late pregnancy (E2: β=195.7, 95% CI [71.1; 320.3], p=0.002, E3: β=177.6, 95% CI [96.6; 258.7], p (corrected)<0.001) (N=95, Table 3 and Figure 5). Our sensitivity analysis showed that for CSF-derived GDF15, only E3 was significantly associated with GDF15 (Table A.1).

Comment

Principal Findings

This longitudinal study is the first to compare levels of human GDF15 in CSF, plasma, and mRNA in placenta, along with pGDF15 changes from pregnancy to early postpartum. We showed that cGDF15 and pGDF15 were highly correlated and that cGDF15 and pGDF15 correlated with placenta *GDF15* mRNA*placental weight. Moreover, we found an association between pGDF15 levels and E2 and E3 in pregnancy. Finally, we demonstrated that mental well-being and cortisol measures, i.e., CAR and absolute cortisol at awakening, were not associated with cGDF15 levels.

Results in the Context of What is Known

We found a mean late pregnancy cGDF15 of 0.442 ng/mL, which aligns with findings by Andersson-Hall et al. reporting a mean late pregnancy cGDF15 of 0.385 ng/mL (11).

We demonstrated a significant decline in pGDF15 levels from pregnancy to the postpartum period, aligning with previous observations (11, 18). Andersson-Hall et al. reported findings from the third trimester and six months postpartum in one study and five years postpartum in another (11, 18). Our research contributes to the body of evidence by illuminating GDF15 decline in the early postpartum, however no data on the immediate postpartum are yet available.

We found that cGDF15 and pGDF15 were highly correlated and that cGDF15 correlated the strongest with placenta *GDF15* mRNA and even stronger correlations were seen when accounting for placenta weight, which no studies have evaluated previously. This is consistent with the notion that placental *GDF15* mRNA is produced globally throughout the placenta and aligns with Moore et al. and Turco et al., who showed that GDF15 is primarily produced by trophoblastic cells placed throughout the placenta (5, 46). Similar to our findings, Andersson-Hall et al. reported a strong correlation between cGDF15 and serum GDF15 (11). Interestingly, in their study, it was only in the pregnant state that a correlation between cGDF15 and serum GDF15 was found (11). Taken together this suggests that cGDF15 serves as a more precise indicator of the placental-derived GDF15 and that GDF15 may play a vital role during human pregnancy which may differ from other physiological roles in the non-pregnant context, e.g., immunomodulatory functions. Nonetheless, the correlation between cGDF15 and pGDF15 indicates that the more easily obtainable pGDF15 can function as an indicator for pregnancy GDF15 levels.

GDF15 has, in several studies, been associated with pregnancy nausea and vomiting (16-18). Recently, using mouse models and human cohorts data, Fejzo et al. demonstrated that the severity of nausea and vomiting in pregnancy was influenced by the interaction between fetal-derived GDF15 and the mother's sensitivity to GDF15, and largely influenced by previous exposure to the hormone (17). The potential impact of GDF15 on nausea and vomiting in pregnancy may extend to mental health issues during pregnancy or postpartum, as it could affect the dynamics of the HPA-axis and well-being parameters. Cimino et al. showed in rats that chronic infusion of GDF15 was associated with higher levels of corticosterone (20). However, we did not find any association between GDF15 and HPA-axis dynamics or mental health scores.

Although GDF levels in some studies have been demonstrated to be associated with infant sex, such that mothers of female infants have increased levels of GDF15, we did not replicate these findings in pGDF15 nor in cGDf15 (Figure A.1) (11, 18).

Clinical and Research Implications

We found a statistically significant association between pGDF15 and E2 and E3, for cGDF15 this was most pronounced for E3 as expected, given that GDF15 levels can only be measured in CSF during pregnancy due to very low levels in the non-perinatal state. Additionally, since both GDF15 and estrogens are major placental endocrine products, and putatively play a vital role in pregnancy, it is likely that they at least partly index the placenta's functional mass.

This study did not find an association between cGDF15 and outcomes of relevance for mental health in terms of HPA-axis dynamics, absolute cortisol at awakening, or mental wellbeing or distress scores. Therefore, the impaired mental health associated with nausea and vomiting and hyperemesis gravidarum appears to have different origins than the effects of GDF15.

Previous work shows that women with hyperemesis gravidarum have an increased risk of mental health issues (19). The causal pathway remains uncertain, with ambiguity regarding whether mental health influences the occurrence of hyperemesis gravidarum or vice versa. Notably, most studies incorporated into a meta-analysis by Mitchell et al. excluded women with a history of psychiatric illness (19). Our findings of no association between GDF15 and mental health outcomes imply that GDF15 is not an underlying factor of lower mental health in pregnancies complicated by hyperemesis gravidarum. Thus, future studies should focus on the association between nausea and vomiting and/or hyperemesis gravidarum, and GDF15 in the context of perinatal mental health including in high-risk groups of pregnant women. Also, in the light of our findings, we consider it unlikely that desensitization of GDF15 receptors, as suggested by Fejzo et al., explains the entire association between GDF15 and hyperemesis gravidarum (17). An alternative plausible neurobiological link between nausea in pregnancy and perinatal mental health is the serotonergic brain signaling system which appears to play a role in perinatal mental health (35) as well as appetite regulation and nausea (47).

Strengths and limitations

The strengths of this study include the longitudinal design, which allows us to assess pGDF15 both during pregnancy and postpartum in the same individuals. Further, this is the first study to evaluate

the correlation between GDF15 derived from both CSF, plasma, and placenta mRNA from same subjects and at the same time point during pregnancy. Including several mental health outcomes in the study enable a more thorough analysis and interpretation of the association.

The study was limited by no access to data on pre-pregnancy body weight measures, which could potentially influence our outcomes as suggested by others (16, 18). Another limitation in this study, is that we did not investigate whether individuals were carriers of the histidine (H) the aspartate (D) variant at position 202 in the pro-peptide (position 6 in the mature peptide) (17), which may have underestimated GDF15 measurements for some individuals.

Conclusions

This novel longitudinal study compares GDF15 levels in CSF, plasma, and placenta across pregnancy. The highly correlated CSF and plasma GDF15, in addition to the association with placenta *GDF15* mRNA and placental weight, indicates that GDF15 is produced globally in placenta. The study identifies a dramatic pGDF15 decline from pregnancy to postpartum, i.e., 35 days postpartum. While a correlation with estrogen levels underscores GDF15's role in pregnancy, no direct association with mental health outcomes was found. Recognizing the need for a more thorough understanding of GDF15's role in pregnancy, future research should focus on the association between nausea and vomiting, GDF15, and mental health, possibly involving the serotonergic system.

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Ethical approval statement

This study is approved by the Capital Region's Committee on Health Research Ethics in Denmark (VEK H-18029563). The research adhered strictly to the Declaration of Helsinki (48). All participants provided informed, written consent before volunteering for the study.

CRediT authorship contribution statement

Stinne Høgh: Conceptualization, Methodology, Investigation, Formal analyses, Writing first draft. **Camilla Borgsted:** Project administration, Conceptualization, Methodology, Investigation, Writing

and Editing. **Hanne K. Hegaard:** Validation, Resources, Writing and Editing. **Kristina M. Renault:** Validation, Writing and Editing. **Silvia EP Bruzzone:** Validation, Writing and Editing. **Christoffer Clemmensen:** Validation, Writing and editing, Funding acquisition. **Anders B. Klein:** Conceptualization, Methodology, Investigation, Writing and Editing. **Vibe G. Frokjaer:** Conceptualization, Methodology, Investigation, Supervision over investigation and analyses, Writing and Editing, Funding acquisition. All authors reviewed and approved the final manuscript as submitted.

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Table and Figure Legends

Table 1. Demographic, GDF15, cortisol and psychometric parameters

Table 2. Correlation between GDF15 levels in CSF/plasma, placenta *GDF15* mRNA and weight

Table 3. Associations for GDF15 in CSF and plasma during late pregnancy

Figure 1. Change in plasma GDF15 from late pregnancy to early postpartum

Figure 2. Correlation between GDF15 in plasma and cerebrospinal fluid (CSF) and Placenta *GDF15* mRNA

Figure 3. Correlation between pregnancy CSF GDF15 and Cortisol Awakening Response (AUCi) postpartum and Cortisol levels at awakening postpartum

Figure 4. Correlation between pregnancy CSF GDF15 and well-being scores in pregnancy and postpartum

Figure 5. Scatterplot of the correlation between plasma GDF15 and estrone (E1), estradiol (E2) and estriol (E3) during pregnancy.

Table 1. Demographic, GDF15, cortisol and psychometric parameters

Parameters	Late pregnancy (N=95)	Early postpartum (N=75)	N (pregnancy)
General			
Age, years (Mean, (SD))	33.9 (3.9)	-	95
Parity, Nulliparous/Multiparous (%)	37 / 57 (39/60)	-	94
Sex of child, Male/Female	52/43	-	95
Gestational age at C-section, days (mean (SD))	273 (5)	-	55
Placental weight, grams (mean (SD))	654 (137)	-	95
Biological measures			
GDF15 CSF levels, ng/mL (mean (SD))	0.442 (0.298)	-	89
GDF15 plasma levels, ng/mL (mean (SD))	85.9 (42)	0.50 (0.18)	95
High sensitivity CRP, mg/L (median (range))	2.56 (0.51-19.17)	2.0 (0.37-16.75)	94
Estrone (E1), pmol/L (median (range))	23959 (4330-113185)	86.4 (15.27-816.85)	95
Estradiol (E2), pmol/L (median (range))	75296 (36391-169174)	51.3 (6.86-1307.66)	95
Estriol (E3), pmol/L (median (range))	46812 (19741-114877)	-	95
Cortisol levels at awakening, nmol/L (mean (SD))	-	6.1 (2.7)	47
CAR (mean AUCi (SD))	-	31.4 (158.8)	47
Psychometry			
Days postpartum for well-being (mean (SD))	-	35 (9.1)	
WHO-5 (0-100) (mean (SD))	66.3 (14.0)	67.1 (14.4)	57
EPDS (0-30) (mean (SD))	-	3.6 (2.8)	53
POMS (-32-200) (mean (SD))	10.6 (17.9)	1.63 (13.0)	53

Mean/median and standard deviation are shown for clinical parameters and psychometrics in each group. CSF, cerebrospinal fluid; CAR, Cortisol Awakening Response; AUCi, area under the curve with respect to increase from baseline at awakening. WHO-5, WHO-5 Well-being index; EPDS, Edinburgh Postnatal Depression Scale; POMS, Profile of mood states "Total Mood Disturbance score"

TABLE 2 | Correlation between GDF15 levels in CSF/plasma, placenta GDF15 mRNA and weight

CSF GSF15 correlations	***r***	**p**	**N**
CSF GDF15 and Plasma GDF15	0.52	**<0.001**	89
CSF GDF15 and placental weight	0.24	**0.02**	89
CSF GDF15 and placenta *GDF15* mRNA	0.43	**0.01**	34
CSF GDF15 and placenta *GDF15* mRNA*placental weight	0.62	**<0.001**	34
Plasma GDF15 correlations			
Plasma GDF15 and placental weight	0.13	0.22	95
Plasma GDF15 and placenta *GDF15* mRNA	0.30	0.09	34
Plasma GDF15 and placenta *GDF15* mRNA*placental weight	0.44	**0.008**	34

CSF, Cerebrospinal Fluid; Plasma and CSF GDF15 levels in ng/mL, placenta *GDF15* mRNA levels relative to reference Rpl13a gene

TABLE 3 | Associations for GDF15 in CSF and plasma during late pregnancy

	Crude Model*				Model A**			
	β	**95% CI**	**p**	**p (corrected)**	**β**	**95% CI**	**p**	**p (corrected)**
CSF GDF15 (ng/mL)								
Pregnancy								
WHO-5	-5.9	[-15.7; 4.0]	0.24		-5.1	[-15.4; 5.3]	0.33	*Primary*
POMS TMD	6.5	[-6.2; 19.2]	0.31		6.7	[-6.9; 20.3]	0.33	0.66
Postpartum								
WHO-5	-3.0	[-15.6; 9.7]	0.64		-4.1	[-17.2; 9.1]	0.54	*Primary*
POMS TMD	0.3	[-11.2; 11.9]	0.96		0.4	[-11.9; 12.7]	0.95	1.0
EPDS	0.3	[-2.2; 2.8]	0.82		0.2	[-2.5; 2.9]	0.89	1.0
CAR (AUCi)	44.3	[-123.9; 212.6]	0.60		34.9	[-144.6; 214.5]	0.70	*Primary*
Cortisol at awakening	-1.8	[-4.6; 1.0]	0.20		-1.8	[-4.5, 0.9]	0.19	0.38
Plasma GDF15 (ng/mL)								
Pregnancy								
s-Estradiol (E2)	195.7	[71.1; 320.3]	**0.002**	*Primary*				
s-hs-CRP	-0.01	[-0.03; 0.007]	0.24	0.48				
s-Estrone (E1)	-49.7	[-139.5; 40.2]	0.28	0.48				
s-Estriol (E3)	177.6	[96.6; 258.7]	<0.001	**<0.001**				

CSF, cerebrospinal fluid; CAR, Cortisol Awakening Response; AUCi, area under the curve with respect to increase from baseline at awakening. WHO-5, WHO-5 Well-being index; EPDS, Edinburgh Postnatal Depression Scale; POMS, Profile Of Mood States "Total Mood Disturbance score".

*Linear regression analysis with blood serum, mental health, and cortisol variables as dependent variables and GDF15 measurements as independent variables. β depicts the standardized beta coefficient for GDF15.

**Model A is adjusted for maternal age, hs-CRP and E2. Bonferroni-Holm correction applied through p-value adjustment.

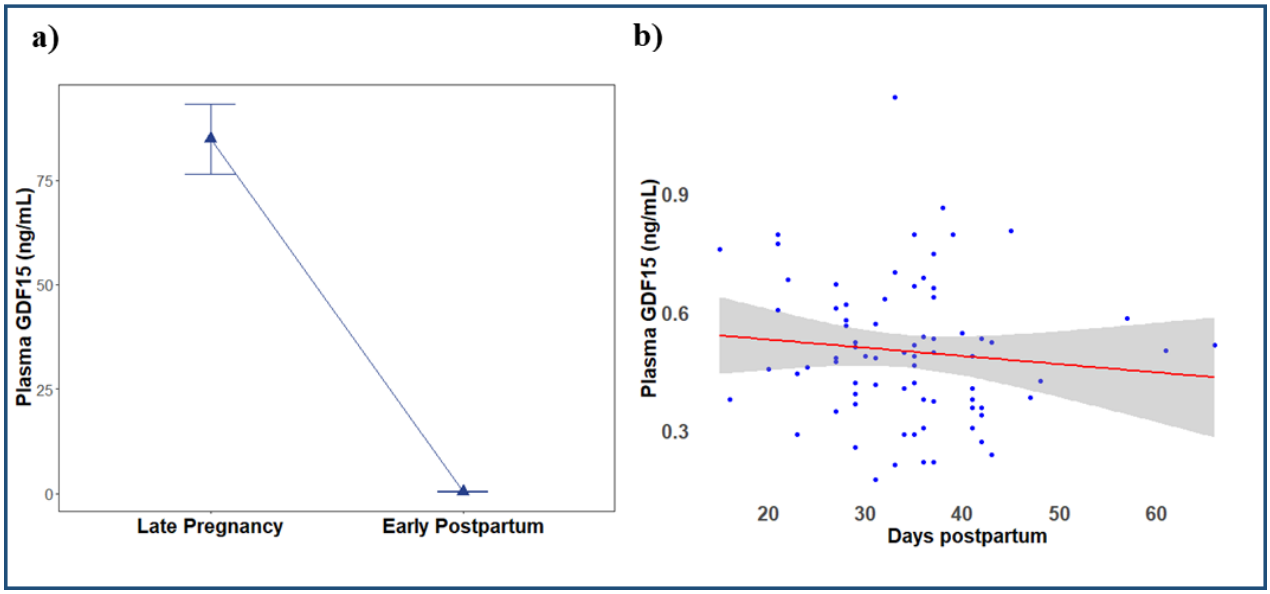

FIGURE 1 | a) Change in plasma GDF15 from late pregnancy to early postpartum. Errorbars represent a 95% confidence interval for the corresponding mean, b) Scatterplot of the relationship between days postpartum and Plasma GDF15 levels.

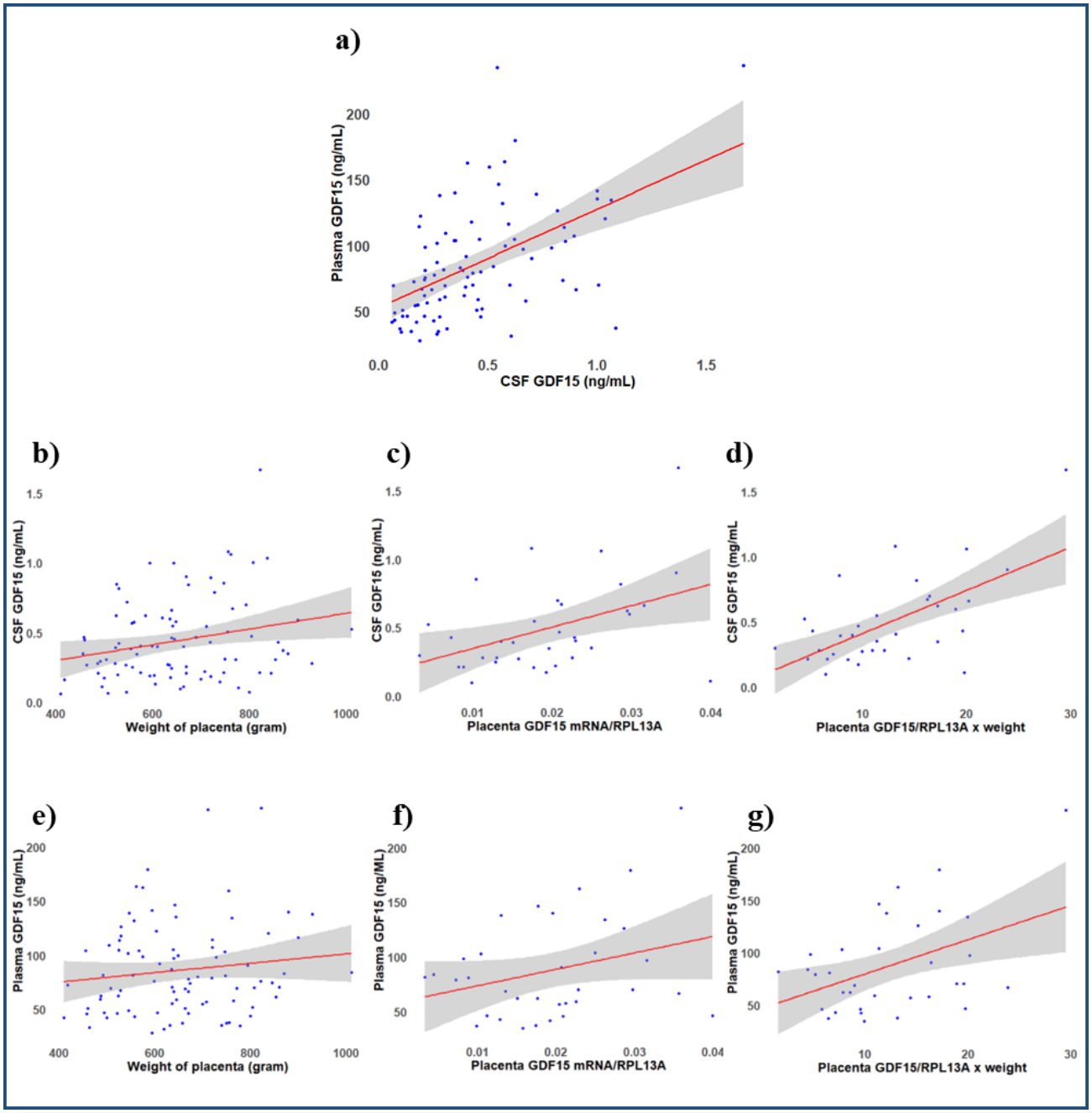

FIGURE 2 | Scatterplot of the correlation between a) GDF15 in plasma and cerebrospinal fluid (CSF), b) Placental weight and CSF GDF15, c) Placenta *GDF15* mRNA and CSF GDF15, d) Placenta *GDF15* mRNA * placenta weight and CSF GDF15, e) Placental weight and plasma GDF15, f) Placenta *GDF15* mRNA and Plasma GDF15, g) Placenta *GDF15* mRNA * placenta weight and Plasma GDF15

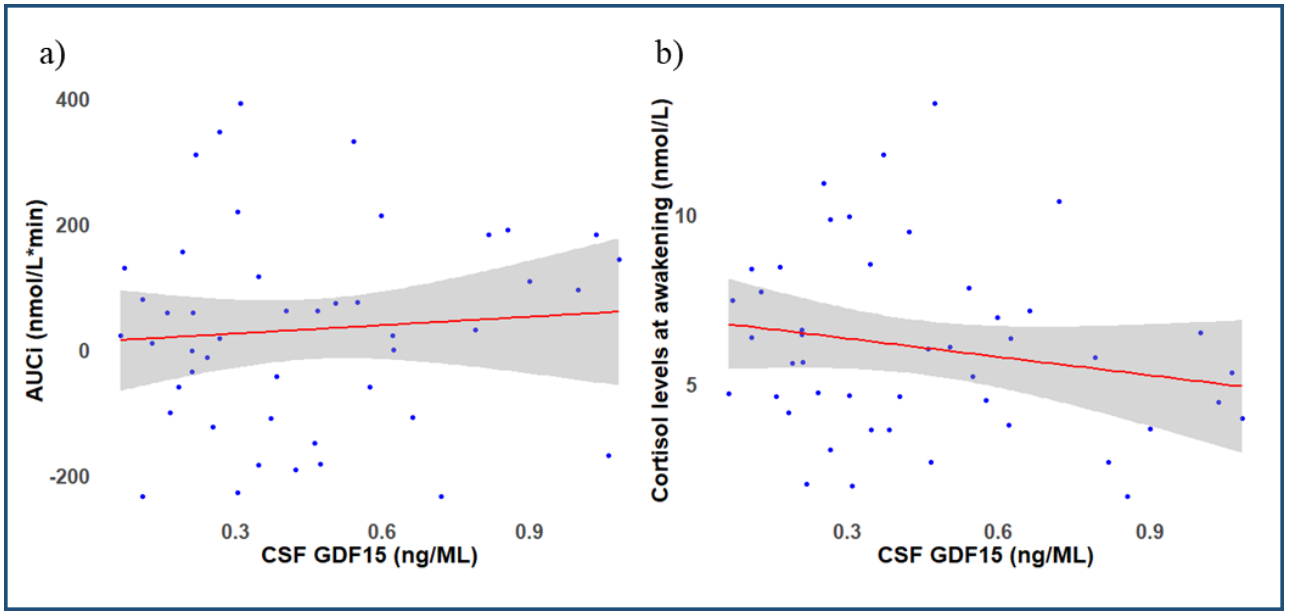

FIGURE 3 | Scatterplot of the correlation between pregnancy CSF GDF15 and a) Cortisol Awakening Response (AUCi) postpartum and b) Cortisol levels at awakening postpartum.

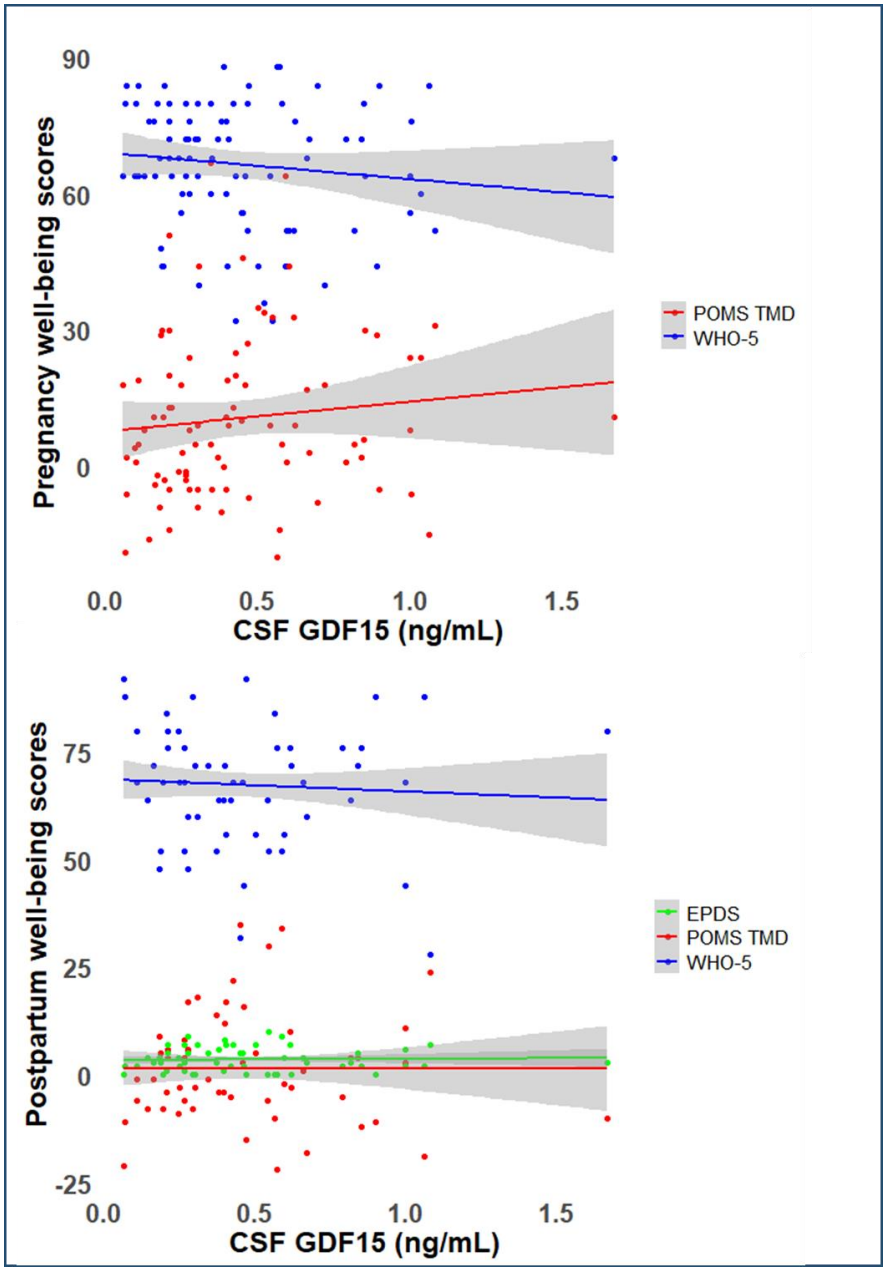

FIGURE 4 | Scatterplot of the correlation between pregnancy CSF GDF15 and well-being scores in pregnancy and postpartum.

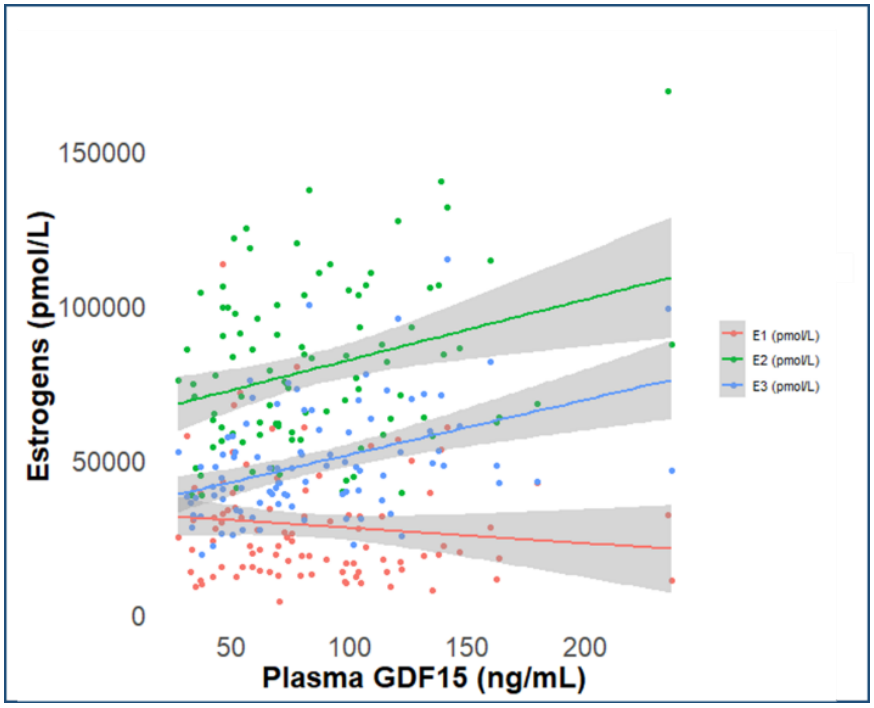

FIGURE 5 | Scatterplot of the correlation between plasma GDF15 and estrone (E1), estradiol (E2) and estriol (E3) during pregnancy.

Appendices

Table A.1. Association between cGDF15 levels and estrogens.

Figure A.1. Maternal GDF15 levels in late pregnancy depending on fetal sex.

Table A.1 | Association between cGDF15 levels and estrogens

	Effect (β)	**95% CI**	**p**
S-Estrone (E1)	-6724	[-19420; 5971]	0.30
S-Estradiol (E2)	13660	[-5113; 32433]	0.15
S-Estriol (E3)	17007	[4320; 29693]	**0.009**

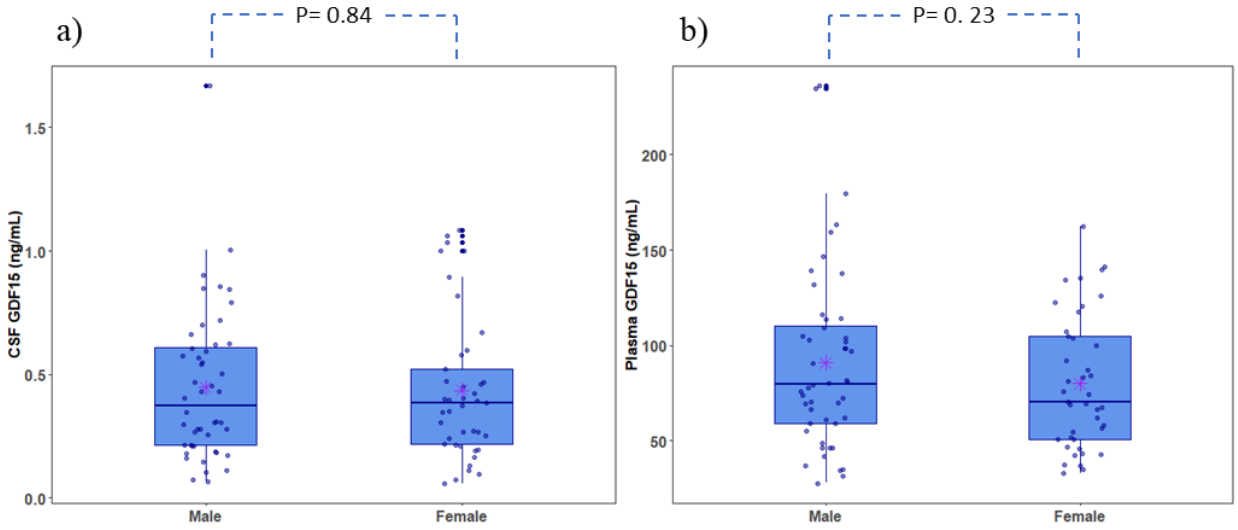

Figure A.1 | Maternal GDF15 levels in late pregnancy depending on fetal sex. Boxplot of GDF15 in a) cerebrospinal fluid (CSF) and b) plasma. P-values of linear regression analyses.

APPENDIX III, PAPER III

BMJ Open

Short-term oestrogen as a strategy to prevent postpartum depression in high-risk women: protocol for the double-blind, randomised, placebo-controlled MAMA clinical trial

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ABSTRACT

Introduction Postpartum depression affects 10%–15% of women and has a recurrence rate of 40% in subsequent pregnancies. Women who develop postpartum depression are suspected to be more sensitive to the rapid and large fluctuations in sex steroid hormones, particularly estradiol, during pregnancy and postpartum. This trial aims to evaluate the preventive effect of 3 weeks transdermal estradiol treatment immediately postpartum on depressive episodes in women at high risk for developing postpartum depression.

Methods and analysis The Maternal Mental Health Trial is a double-blind, randomised and placebo-controlled clinical trial. The trial involves three departments of obstetrics organised under Copenhagen University Hospital in Denmark. Women who are singleton pregnant with a history of perinatal depression are eligible to participate. Participants will be randomised to receive either transdermal estradiol patches (200 µg/day) or placebo patches for 3 weeks immediately postpartum. The primary outcome is clinical depression, according to the Diagnostic and Statistical Manual of Mental Disorders-V criteria of Major Depressive Disorder with onset at any time between 0 and 6 months postpartum. Secondary outcomes include, but are not limited to, symptoms of depression postpartum, exclusive breastfeeding, cortisol dynamics, maternal distress sensitivity and cognitive function. The primary statistical analysis will be performed based on the intention-to-treat principle. With the inclusion of 220 participants and a 20% expected dropout rate, we anticipate 80% power to detect a 50% reduction in postpartum depressive episodes while controlling the type 1 error at 5%.

Ethics and dissemination The study protocol is approved by the Regional Committees on Health Research Ethics in the Capital Region of Denmark, the Danish Medicines Agency and the Centre for Data Protection Compliance in the Capital Region of Denmark. We will present results at scientific meetings and in peer-reviewed journals and in other formats to engage policymakers and the public.

Strengths and limitations of this study

- ► First trial to evaluate short-term transdermal estradiol treatment to prevent postpartum depression in women at high risk.
- ► Double-blind, randomised placebo-controlled multicentre design.
- ► It may pose a challenge for breastfeeding women to participate in a trial involving a drug.
- ► Evaluation of offspring neurodevelopmental and physical health consequences of intervention is limited due to a relatively short follow-up period of 6 months.

Trial registration number NCT04685148.

INTRODUCTION

Major Depressive Disorder (MDD) is currently the leading cause of disability worldwide[1] and affects more women than men.[1] Women are at increased risk for depression when the endogenous sex steroid hormone milieu changes such as in puberty, during late pregnancy to postpartum and during the menopausal transition.[2 3] This includes postpartum depression (PPD) that affects 10%–15% of mothers and has a recurrence rate of 40%.[4–6] PPD is a disabling disorder that affects the entire family, including infant development and future health.[7–9]

The underlying risk and resilience mechanisms in MDD, including PPD, are far from clear.[10–12] Consequently, current treatment and preventive strategies are suboptimal. Women who develop PPD might be particularly sensitive to the transition from high

levels of sex steroid hormones, in particular, estradiol, in pregnancy to low levels in the hormone withdrawal phase postpartum.[2 13] Thus, PPD is most likely to have a distinct pathophysiology, which may provide a unique opportunity for protecting mental health by targeted short-term prevention in the immediate postpartum period. Intriguingly, recent human data have provided evidence for sex hormone manipulation to provoke subclinical depressive symptoms in about 12% of healthy volunteers. The phenomenon was linked to changes in estradiol, which were induced by the pharmacological manipulation with a gonadotrophin-releasing hormone agonist[14 15] that first stimulates and subsequently suppresses ovarian hormone production, primarily estradiol, to menopausal levels. Estradiol affects critical domains and key brain regions known to be dysfunctional in women with MDD.[13 16] Estradiol sensitivity appears to predispose to PPD, which can be demonstrated at the level of gene transcription in clinical cohorts[3 17 18] and is also directly supported by recent research results in a sex hormone manipulation model.[17 19] Such peripheral markers of estradiol sensitivity may, therefore, prove useful in identifying individuals at excess risk for PPD and may help direct preventive efforts. Also, the hypothalamic–pituitary–adrenal (HPA) axis appears to be involved in the dysregulation of perinatal mental health both in human studies,[20] possibly in interaction with psychological stressors and interaction with endocrine profiles,[21] and in rats, where blunting of HPA-axis dynamics was related to depressive-like behaviour in a model of PPD.[22] Furthermore, compromised serotonin signalling may be involved in the mechanisms by which sex hormone transitions add to the risk of developing depressive symptoms, at least in a certain subgroup of susceptible individuals.[14 18 19] This has been shown in a human sex hormone manipulation study[15] and may also affect HPA axis dynamic capacities.[23 24] Evidence for serotonergic contributions to risk for depressive symptoms across peripartum also comes from rodent work, which, in particular, highlights estradiol-dependent changes in key features of the serotonin signalling system.[25–27] Some disturbances of serotonin signalling may, from high estradiol stimulated states (ie, late pregnancy), carry over to the early postpartum phase. Also, earlier findings of ours[14 28] and others[29] support that the brain architecture of hormonal transition includes key targetable features beyond serotonin that ostensibly contribute to an increased risk for depressive episodes and are most likely linked to the estradiol *withdrawal* phase.

Transdermal estradiol emerges as a promising preventive treatment for PPD.[30] Previously, a randomised controlled trial (RCT) showed effect of transdermal estradiol treatment on manifest PPD.[31] Meanwhile, a recent pilot RCT with transdermal estradiol as a treatment for PPD failed to achieve its primary outcome but, notably, did reduce depressive symptoms postpartum compared with placebo.[32] Another study aimed to evaluate the efficacy of transdermal estradiol compared with placebo as treatment of PPD, with sertraline as an antidepressant comparator. However, this study was stopped as it revealed non-significant estradiol concentration differences between the treatment groups.[33] Furthermore, transdermal estradiol appears to be effective in preventing clinically significant depressive symptoms among perimenopausal women, which is another group of women who undergo a hormonal transition.[34]

Rather than treating manifest depressive episodes postpartum, we propose a different approach: to target and potentially prevent early postpartum risk mechanisms and to direct this preventive strategy towards women at high risk. This immediate and early postpartum timing corresponds to the peak risk period and covers the peak of hormonal decline postpartum.[4 35]

This trial aims (1) to evaluate the preventive effect of transdermal estradiol treatment for 3 weeks immediately postpartum on depressive episodes in a subgroup of women who are at high risk due to a history of perinatal depression and (2) to determine if a set of genomic biomarkers can identify women within this high-risk group who benefit from the intervention (or vice versa become depressed in the placebo group) and, thus inform future personalised prevention or treatment.

METHODS

The Maternal Mental Health (MAMA) Trial is designed as a double-blind, 1:1 randomised, placebo-controlled multicentre trial. The MAMA trial was designed in accordance with the Consolidated Standards of Reporting Trials recommendation for RCTs[36] (figure 1) and with the Standard Protocol Items: Recommendations for Interventional Trials guidelines for reporting trial protocols (additional file 1).[37]

Study setting

The trial is conducted in a multicentre setting involving maternity wards at four university hospitals in the Capital Region of Denmark. The four maternity wards have more than 20 000 deliveries in total per year, of which a minimum of 250 are deliveries of women with previous perinatal depression (estimated by personal communication with midwives from the specialised team for women with psychiatric history and social challenges).[38]

The three maternity units have teams of experienced midwives, nurses and obstetricians working with pregnant women with current or previous psychiatric disorders and collaborate with psychiatrists in severe cases. The teams at two maternity units include psychologists and all teams include social workers, who are specialised in taking care of women with psychiatric history and social challenges. At all three maternity units, women with a history of perinatal depression are offered to stay with their infant at the postnatal ward after the delivery, typically for 2–5 days.

Eligibility criteria

Women who are singleton pregnant in gestational week ≥34+0 with a history of perinatal depression (onset

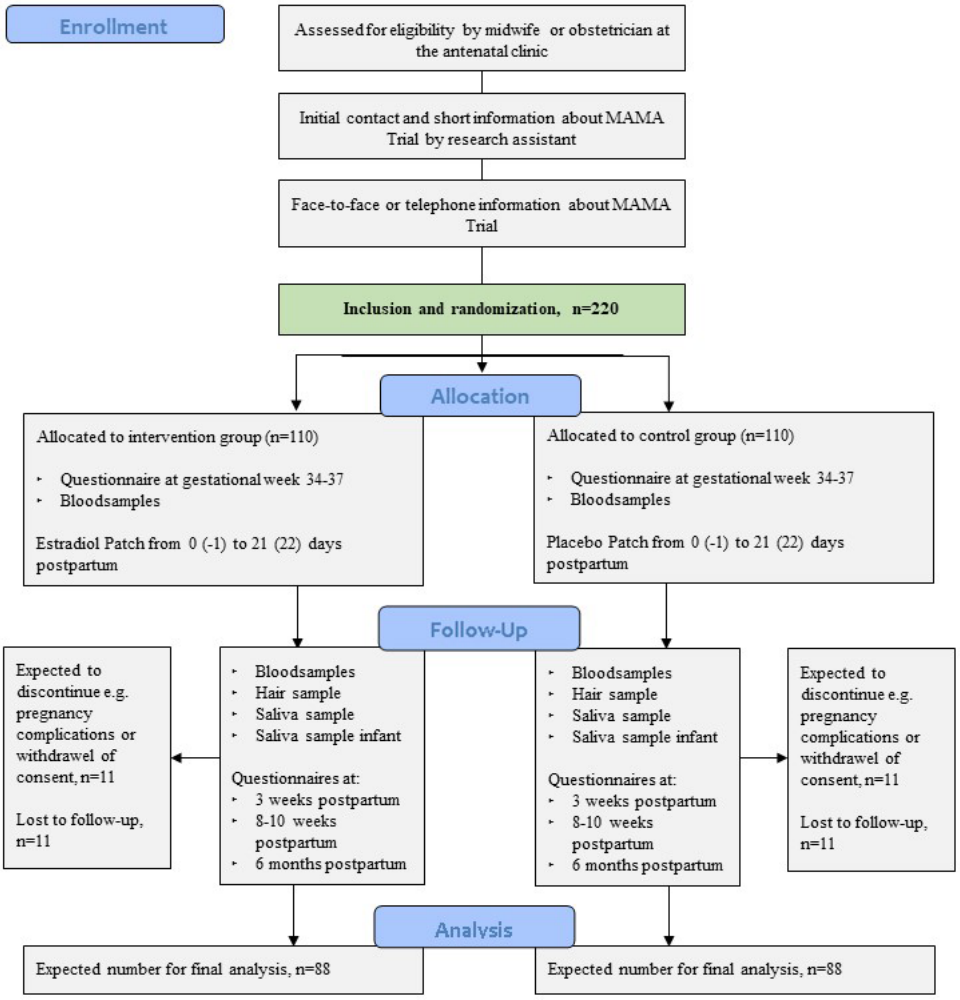

Figure 1 CONSORT flow diagram of MAMA trial. CONSORT, Consolidated Standards of Reporting Trials; MAMA, Maternal Mental Health.

during pregnancy or before 6 months postpartum) and aged 18–45 years are eligible to participate. The women must be unmedicated and otherwise untreated for MDD at inclusion.

Exclusion criteria include moderate to severe depression with onset in the current pregnancy; severe psychiatric disorders; previous suicide attempts without having a depressive episode; neurological disorders; severe somatic illness; risk factors for any thromboembolic disorders; deep vein thrombosis or pulmonary embolism in the current pregnancy; pregnancy-induced hypertension or preeclampsia; contraindication for estrogenic treatment; use of psychotropic drugs; non-fluency in Danish; prepregnancy body mass index >35 kg/m^2; ongoing alcohol or illicit drug abuse; severe postpartum haemorrhage (>1500 mL); severe illness or perinatal death of the infant.

A full list of inclusion and exclusion criteria is provided in table 1.

Interventions

Participants will be randomised to either transdermal estradiol patches (200 µg/day, Vivelle Dot) or placebo patches for 3 weeks. The patches will be administered by a research assistant either on the day of delivery or day after the delivery. After detailed instructions, the trial participants self-administer the patches two times weekly for 3 weeks. Participants confirm the subsequent administrations by filling in a form on the date for changing the patch to monitor adherence. If participants fail to administer their patches for 4 days or more of the 3-week period, they will be considered non-adherent and treated as such in the analyses.

We will perform an interim analysis on the first 40 participants to assess estradiol levels during transdermal estradiol treatment compared with placebo (ie, blood samples at 3 weeks postpartum). Should the interim analysis indicate that the treatment is insufficient for changing estradiol levels, we will consider if there are elements of the intervention that need to be modified, such as the dose or type of administration.

Side effects

Using transdermal estradiol for a limited time in the immediate postpartum period is not expected to pose any unacceptable risk or adverse side effects to the

Table 1 Inclusion and exclusion criteria of the MAMA trial

Inclusion	Exclusion
► Singleton pregnant in gestational week ≥34+0 ► History of perinatal depression ► Age between 18 and 45 years	► Moderate to severe depression with onset during the current pregnancy. ► Severe psychiatric disorders (eg, disorders with psychotic symptoms, schizophrenia, bipolar disorders, inpatient eating disorders and inpatient obsessive-compulsive disorders). ► Previous suicide attempts without having a depressive episode. ► Use of psychotropic pharmacology, except for short-term sleep support treatment. ► Severe somatic illness. ► History of or ongoing cancer. ► History of venous thromboembolism, myocardial infarction, cerebrovascular thromboembolism or thrombophilia, or other risk factors clinically assessed after thrombophilia screening. ► Deep vein thrombosis or pulmonary embolism in current pregnancy. ► Pregnancy-induced hypertension or preeclampsia. ► Other contraindications for oestrogen treatment (eg, acute liver failure, severe varicose veins). ► Non-fluent in Danish or pronounced vision or hearing loss. ► Prepregnancy BMI>35 kg/m^2. ► Ongoing alcohol or drug abuse. ► Severe postpartum haemorrhage (>1500 mL). ► Severe illness or perinatal death of the infant.

BMI, body mass index; MAMA, Maternal Mental Health.

participants. However, there are some known side effects to consider such as risk of deep vein thrombosis, which is already increased due to pregnancy and childbirth.[39] Women who are at a known increased risk of venous thromboembolism will not be included in the trial.

The most frequently reported side effects of transdermal estradiol treatment are headache, redness or irritation of the skin (where the patch was worn), bloating, nausea and abdominal pain.

There are only a few trials investigating potential side effects regarding breastfeeding. A study that investigated 100 µg transdermal estradiol per 24 hours found no traces in breast milk.[40] Another study evaluated between 50 and 200 µg transdermal estradiol and found no significant association between the mother's and the infant's serum estradiol level.[41] In the same study, no associations were found between the infant's growth, measured by weight, length and head circumference and estradiol treatment.[41] In the MAMA trial, participants will be monitored and assessed through a breastfeeding questionnaire developed for the purpose and by using both weight change as percentage of birth weight and frequency of exclusive breastfeeding (without supplemental nutrition) after 3 weeks as outcome parameters.

Participants will be prompted to contact the investigators if they experience any adverse side effects. Moreover, participants will be asked about side effects 1 week postpartum and 3 weeks postpartum (at the end of the trial drug administration). The investigator at each site will assess severity and possible association to trial medication. Serious adverse events will be reported to the sponsor investigator, monitor, and relevant authorities immediately, and unblinding is permissible.

Outcomes

The primary outcome of interest is clinical depression, according to the Diagnostic and Statistical Manual of Mental Disorders criteria of MDD with onset at any time between week 0 and 6 months postpartum diagnosed by a medical doctor specialised in psychiatry.

The participants will be screened for depressive symptoms at the follow-up assessments 3 and 8–10 weeks postpartum by the Edinburgh Postnatal Depression Scale (EPDS) and the Major Depression Inventory (MDI). Moreover, the participants will be screened for depressive symptoms at 8–10 weeks postpartum by a semistructured interview using the 17 items Hamilton Depression Rating Scale (HAMD-17). In case the participants present depressive symptoms (EPDS score of ≥9 or MDI score ≥20 or HAMD-17 ≥11) or if there is a clinical suspicion without scores on EPDS, MDI or HAMD-17 over cut-off, the participants will be booked for a clinical psychiatric interview with a doctor specialised in psychiatry. The participants are also encouraged to contact the research team if they experience depressive symptoms outside the follow-up assessments. Moreover, if the participants are admitted to the hospital, the researchers get a notification through the regional e-health platform. At the follow-up assessment 6 months postpartum, the participants are also asked about any depressive episodes during the follow-up period.

Secondary outcomes include, but are not limited to, symptoms of depression postpartum, exclusive breastfeeding, cortisol dynamics, maternal distress sensitivity, cognitive function and developmental functioning of the infants. The secondary outcomes are described in table 2.

Recruitment

The women are assessed for eligibility by a midwife or obstetrician when attending antenatal care at the outpatient clinic (figure 2). Eligible participants who verbally consent to receive more information about the trial are subsequently contacted by telephone for more detailed information about participating in the trial. On request, a face-to-face meeting is also possible. Written information is provided after the telephone (or face-to-face) information. A second call/face-to-face meeting is arranged to give the potential participant and her partner at time to consider participation and to have the opportunity to ask questions. Written informed consent will be obtained before inclusion in the MAMA trial.

Allocation and blinding

Participant allocation to active or placebo group will be conducted by the capital region pharmacy. The participants will be block randomised with a fixed block size (unknown to the investigator). Trial participants, clinical care providers, research assistants, investigators, outcome assessors and data analysists will all be blinded to allocation.

Participant timeline

Participation in the MAMA trial includes four visits lasting between 20 min and 3 hours. In addition, the participants are asked to answer an online questionnaire at 6 months postpartum (table 3).

All participants have follow-up assessments at 1 week postpartum (by telephone to assess any side effects), 3 weeks postpartum (at the hospital), 8–10 weeks postpartum (at the hospital) and 6 months postpartum (by telephone).

Baseline assessment at gestational week ≥34+0

The participants receive a basic physical screening, including somatic status and medical history, blood pressure measurement and routine blood samples. Furthermore, at baseline, we perform cognitive testing, and the participants are asked to fill in the baseline questionnaires covering questions on sociodemographic, lifestyle and obstetric history (table 3). The participants will randomly undergo toxicology urine tests for detection of drug abuse within the last month.

Psychometrics

Depressive symptom severity will be assessed with a face-to-face, semistructured interviews (Hamilton Depression Rating Scale six items, HAMD-6) by a trained healthcare professional.[42]

Self-reported questionnaires are filled in at gestational week ≥34+0 (for screening purposes and baseline measurements) and 3 weeks, 8–10 weeks and 6 months postpartum (table 3).

Psychometric measures of psychological traits include trait questionnaires indexing personality (NEO Personality Inventory and State Trait Anxiety Inventory (STAI)-trait); early life stress information (Child Abuse and Trauma Scale); quality of parental bonding (Parental Bonding Instrument) and substance abuse and psychiatric family history (Online Stimulant and Family History Assessment).

Furthermore, psychometric measures of psychological states include questionnaires indexing mental well-being (WHO-5)[43]; depressive symptoms (EPDS and MDI)[44]; anxiety (STAI-state)[45]; stress (Cohen Perceived Stress Scale, Cohen Parental Stress Scale, PSS); antenatal attachment (Maternal Antenatal Attachment Scale)[46]; sleep quality (Pittsburgh Sleep Quality Index)[47]; pleasure (Snaith-Hamilton Pleasure Scale), obsessive compulsive behaviour (Obsessive-Compulsive Inventory); PSS[48]; parental reflection functioning (Parental Reflective Functioning Questionnaire)[49] and parental sense of competence (Parenting Sense of Competence) Scale.[50] Finally, the infants' social-emotional development is assessed by the mother and the assessor in cooperation by using the semistructured Ages & Stages Questionnaires, second edition.[51]

Neuropsychological tests

Neuropsychological testing covers a range of emotion-independent (cold) and emotion-dependent (hot) cognitive domains. Cognitive testing is placed two times in the study programme, at baseline and follow-up assessment week 8–10 postpartum.

Cold cognitive task domains include simple reaction time, declarative verbal memory (Verbal Affective Memory Test-24),[52] working memory (WAIS-IV Letter-Number Sequence)[53] and cognitive flexibility (Intra-Extra Dimensional) and hot cognitive task domains include facial emotion recognition (ERT-e) and emotion detection threshold (IM).[54]

Further infant emotion recognition (infant emotion detection) is assessed using the iMotions software, V.8.0, and integrated hardware.[55 56] Two infant video clips, a 'distress' and a 'laughter' video, will be shown to the participants. The 'distress video' displays a 4-month old infant crying heavily, unattended by a caregiver. The 'laughter video' displays a mother and her quadruplets all laughing continuously. During the infant videos, participants' facial expressions and galvanic skin responses will be recorded using specialised software. Participants will be instructed to watch the videos passively, and the duration of the task is less than 2 min.

Also, set of 50 infant vocalisations of each 2 s duration in five intensities: most happy, moderately happy, neutral, moderately distressed and most distressed will be played and the women will rate how happy or distressed they think the infant is on a continuous numerical Likert scale ranging from −4 (most distressed) to +4 (most

Table 2 Secondary outcomes of the MAMA trial

Secondary outcomes	
Maternal well-being	Level of depressive symptoms postpartum measured as mean continuous score on the Edinburgh Postnatal Depression Scale (EPDS) at 8–10 weeks postpartum.
	Level of depressive symptoms postpartum measured as mean continuous score at the Hamilton Depression Rating Scale, 6 items (HAMD-6) at 8–10 weeks postpartum.
	Maternal mental well-being measured as mean continuous score at WHO-5 Well-Being Index (WHO-5).
	Level of anxiety postpartum measured as continuous score at the State Trait Anxiety Inventory (STAI) at 8–10 weeks postpartum.
	Maternal sleep quality rated by mean continuous score on the Pittsburgh Sleep Quality Index (PSQI) 8–10 weeks postpartum.
Maternal capacity	Level of maternal antenatal attachment to the unborn child rated by mean continuous score on the Maternal Antenatal Attachment Scale at third trimester of pregnancy.
	Level of parental stress measured as mean continuous score at the Parental Stress Scale (PSS) at 8–10 weeks postpartum.
	Level of parental reflection measured as mean continuous score at the Parental Reflective Functioning Questionnaire (PRFQ) and Parenting Sense of Competence scale (PSOC), 8–10 weeks postpartum.
	Proportion of women who exclusively breastfeed their infants at 8–10 weeks postpartum (questionnaire).
Maternal cognitive performance	Performance on non-emotional (cold) cognitive domains including: reaction time assessed with the Simple Reaction Time (SRT) task; declarative memory performance assessed with Verbal Affective Memory Test (VAMT-24); working memory performance assessed with WAIS-IV Letter-Number Sequence (LNS); and cognitive flexibility assessed with the Intra-Extra Dimensional Set Shifting task (IED).
	Performance on emotional (hot) cognitive domains including emotion recognition assessed with the Emotion Recognition Task-eyes (ERT) and emotion detection threshold assessed with the Emotional Intensity Morphing Task (IM).
	Performance on the iMotions Infant Emotion Test (maternal distress sensitivity and infant emotion detection (IET)) including eye-tracking and facial emotion analysis, and galvanic skin response to emotional infant vocalisations and videos.
Maternal biological markers	Genome-wide genetic polymorphisms, gene expression and DNA methylation levels from peripheral blood at baseline (third trimester of pregnancy) and 3 weeks postpartum.
	Cortisol dynamics measured as Cortisol Awakening Response (CAR) in saliva indexed as area under the curve with respect to baseline from 0 to 60 min from awakening at 3–5 weeks postpartum.
	Evening cortisol concentrations in saliva 3–5 weeks postpartum.
	Hair cortisol level dynamics, that is, concentration of cortisol in hair from mother, estimating cortisol exposure up to 6 months prior to delivery.
	Estradiol level in third trimester of pregnancy, that is, gestational week >34. Estradiol level in peripheral blood.
	Postpartum estradiol level in peripheral blood measured at 3 weeks postpartum.
	Changes in estradiol level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.
	Postpartum progesterone level in peripheral blood measured at 3 weeks postpartum.
	Changes in progesterone level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.
	Allopregnanolone level in third trimester of pregnancy, that is, gestational week >34. Allopregnanolone level in peripheral blood.
	Postpartum allopregnanolone level in peripheral blood measured at 3 weeks postpartum.
	Changes in allopregnanolone level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.
Infant outcomes	Epigenetic markers for HPA axis control (stress hormone axis), FKBP5 methylation index, from the infant.
	Developmental functioning of the infants rated by mean continuous score at Bayley Scales of Infant and Toddler Development—third edition (Bayley-III).
	Social-emotional development in the infants rated by mean continuous score at the Ages and Stages Questionnaire—Social-Emotional, second edition (ASQ:SE-2).

HPA, hypothalamic–pituitary–adrenal ; MAMA, Maternal Mental Health.

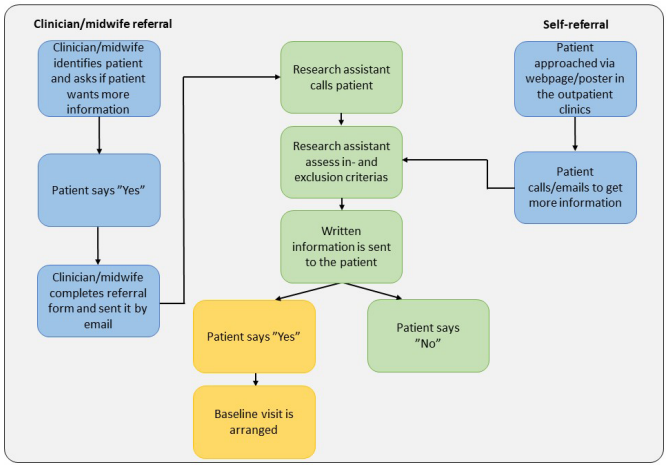

Figure 2 Recruitment pathways for the MAMA trial. MAMA, Maternal Mental Health.

happy).[57–59] During the display of infant vocalisations and faces, participants' facial expressions will be recorded and rated using iMotions software and integrated hardware.

In addition, the infants' fine and gross motor function and prelinguistic behaviours will be assessed by Bayley Scales of Infant and Toddler Development—third edition (Bayley-III) at 8–10 weeks of age. The interaction lasts approximately 20 min.[60]

Information from medical records

Information on pregnancy and delivery outcomes will be obtained from patient records. The information comprises: labour onset (spontaneous or induced); use of epidural anaesthesia during delivery (yes or no); duration of labour (measured in hours); mode of delivery (spontaneous delivery, vacuum extraction or caesarean section (emergency or planned)); blood loss at delivery (measured in ml); gestational age at delivery (measured in weeks and days); birth weight (measured in grams); Apgar score at 5 min; admission to neonatal intensive care unit; hospitalisation and complications postpartum.

Saliva

Saliva is collected to determine the total cortisol output across 1 day as well as dynamics of the HPA-axis, as indexed by Cortisol Awakening Response.[61 62] Serial saliva samples will be sampled at home and collected 3–5 weeks postpartum. Participants will be instructed to take samples immediately after awakening in the morning and again after 15, 30, 45 and 60 min and at 22:00 before going to sleep. Saliva will be collected with Salivette Cortisol (Sarstedt, Nümbrecht, Germany). To ensure accurate saliva sampling, participants will be instructed about the procedure by trained study personnel and receive written take-home instructions for use. Compliance is assessed by a self-reported form.

Hair cortisol

Hair samples (20 mg) will be collected 0–1 day postpartum from the mother to determine cortisol levels during pregnancy.[63] The concentration is determined in the 3 cm hair closest to the scalp and in the 3–6 cm hair from the scalp. Based on an average hair growth of 1 cm/month, this represents cortisol in the last 3 months and 3–6 months, respectively, before the sample is collected.

Blood samples

At baseline, all participants are screened for somatic disease markers to exclude somatic conditions with possible influence on depressive symptoms. Corresponding blood samples for determining relevant blood biomarkers including sex steroid hormones, DNA, PAXgene tubes for mRNA (for candidate biomarker[18 64]) analyses are taken at inclusion in late pregnancy and at follow-up 3 weeks postpartum.

Serum estradiol, estrone and estriol concentrations will be quantified by a specific and validated by isotope dilution online TurboFlow-Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology.[65]

DNA samples from the infant saliva

Buccal swabs from the infant are collected 0–1 day postpartum for epigenetic data and to determine genetic polymorphisms of importance for stress regulation.[66 67]

Data management

Questionnaire and neuropsychological paper-and-pencil data are managed and stored using REDCap, a secure web application for managing online questionnaires.[68] Computer-based neuropsychological data are managed and stored in the Center for Integrated Molecular Brain Imaging (Cimbi) database while biological data are stored in the Cimbi biobank at Neurobiological Research Unit at Copenhagen University Hospital, Rigshospitalet.[69]

Sample size

The recurrence (baseline) risk for PPD is 40% within 6 months postpartum.[4] We calculated that a sample of 2*88 complete cases would provide the trial with 80% power (at a two-sided alpha level of 0.05) to detect a reduction in PPD of 50%. Thus, with a study number of 2*110, the design is considered solid and can tolerate 22% dropouts. Since women with a history of perinatal depression are at high risk of depressive episodes, we expect that at least 50 participants will develop manifest depressive episodes untreated and more will display subclinical depressive symptoms. This will allow for comparison of depressive episode frequencies between active and placebo groups and correlation analyses with relevant outcome parameters.

Statistical analysis

The primary statistical analysis will be performed based on the intention-to-treat principle. We will compare data on the primary outcome in terms of proportions of depressive episodes in the group with transdermal estradiol treatment versus placebo with a melded binomial test.[70] We will also report a p value from a Pearson's χ^2 test.

Table 3 Time schedule of enrolment, intervention and outcome measures of the MAMA trial

	Study period						
	Gestational week <34	Gestational week ≥34+0	0–1 day postpartum	1 week postpartum	3 weeks postpartum	8–10 weeks postpartum	6 month postpartum
Timepoint		Baseline			T1	T2	T3
Face-to-face meeting		X	X		X	X	
Enrolment							
Eligibility screening	X						
Informed consent		X					
Allocation			X				
Intervention							
Transdermal estradiol/placebo patch			◆	———	◆		
Assessment of side effects				X	X	X	
Outcomes and measures							
Medical history		X					
Obstetric history		X					
Socio-demography/ lifestyle		X					
Blood samples		X			X		
Blood pressure		X	X		X		
Saliva sample mother (cortisol)					X		
Saliva sample infant (DNA)			X				
Hair sample mother			X				
HAMD-6		X				X	
STAI-AD		X					
CATS		X					
OS-FHAM short		X					
PBI (mother)		X					
PBI (father)		X					
NEO-P-IR		X					
EPDS		X			X	X	X
WHO-5		X			X	X	X
SHAPS		X			X	X	X
MDI		X			X	X	X
Cohen PSS		X			X	X	X
RRS		X			X	X	X
STAI		X			X	X	X
PSQI		X			X	X	X
OCI		X			X	X	X
MAAS		X					
PSS					X	X	X

Continued

Table 3 Continued

	Study period						
	Gestational week <34	Gestational week ≥34+0	0–1 day postpartum	1 week postpartum	3 weeks postpartum	8–10 weeks postpartum	6 month postpartum
PCOS					X	X	X
PRFQ							X
Breast feeding					X	X	X
ASQ:SE-2						X	
Bayley-III						X	
Neuropsychological tests		X				X	

ASQ:SE-2, Ages and Stages Questionnaire; Bayley-III, Bayley Scales of Infant and Toddler Development—third edition; CATS, Child Abuse and Trauma Scale; EPDS, Edinburgh Postnatal Depression Scale; MAAS, maternal Antenatal Attachment Scale; MDI, Major Depression Inventory; NEO-PI-R, Revised NEO Personality Inventory; OCI, Obsessive-Compulsive Inventory; PBI, Parental Bonding Instrument; PCOS, Parents' Sense of Competence Scale; PRFQ, Parental Reflective Functioning Questionnaire; PSQI, Pittsburgh Sleep Quality Index; PSS, Parental Stress Scale; Cohen PSS, Perceived Stress Scale; RRS, Rumination Response Scale; SHAPS, Snaith-Hamilton Pleasure Scale; OS-FHAM short, substance abuse and family history; STAI, State-Trait Anxiety Inventory—State; STAI-AD, State Trait Anxiety Inventory-Trait; WHO-5, WHO-5 Well-being index.

Demographic and other baseline variables will be displayed using descriptive statistics (eg, means, SD, median, IQR, proportions). We will test the group balance achieved by randomisation by appropriate statistical tests (eg, Student's t tests, Pearson's χ^2 tests).

Secondary outcomes with a continuous distribution will be compared between groups using the mean (Student's t test). Pearson's χ^2 test will be used for binary categorical data.

In case of participant dropout and when possible, we will use a mixed effect model to model the distribution of the outcome over time. It will enable us to include patients for which the outcome is missing (eg, depression score at 8–10 weeks) but have an intermediate measurement (eg, depression score at 3 weeks). We will test the mean or proportion difference between the two groups using a Wald test based on mixed-effects model estimates. Compared with complete-case analysis, this approach will be valid under weaker assumptions about the dropout mechanism and provide more precise estimates (ie, higher statistical power). Missing data due to participant drop-out will be handled using imputation when the cause of censoring makes the primary outcome predictable (eg, questionnaire information on psychological distress may inform expected primary outcome). Participants who experience depression at one assessment will be classified as depressed even when they may have missing values at other assessments. To deal with potential non-random dropout, we will use inverse probability of censoring weighting, where the censoring weights are estimated by a Cox regression with, for example, treatment group or depression severity as covariates.

As an attempt to estimate the causal treatment effect in the case that non-adherence is not occurring at random, we will also use an instrumental variable approach using randomisation as an instrument.[71 72]

Development of novel genomic biomarkers for PPD

This will build on gene expression and/or DNA methylation profiles of genes involved in hormonal signalling pathways.

Oestrogen sensitivity as a predictor of estradiol patch versus placebo group differences in postpartum depressive episodes in high-risk women

Based on earlier observed gene expression profiles of women in third trimester who later developed a PPD[3 17] and women who in a sex-hormone manipulation study responded with depressive symptoms,[19] we will test if such patterns translate in the current study. Specifically, we will derive a polygenic gene expression profile score (PGES) based on the effect sizes of the previous studies and test if this 'cumulative PGES' is higher in estradiol patch-treated women who did *not* develop a postpartum depressive episode (defined as interview-based diagnosis) compared with women who did. Likewise, we will evaluate if women in the placebo group who developed a postpartum depressive episode has a higher 'cumulative PGES' relative to placebo-treated women who did not develop such an episode. Similarly, for the DNA methylation from the same set of transcripts, we will derive a combined polygenic DNA methylation profile score (PDMS) and perform the same set of analyses. All the above analyses will be repeated using EPDS scores at 8–10 weeks postpartum as the outcome measure of depressive symptoms in order to capture any patterns with subclinical depression.

An exploratory analysis will be performed on genome wide gene expression and DNA methylation profiles. The results for these analyses will be corrected for multiple testing using the Bonferroni method for the number of tests performed for gene expression and the study wide epigenome wide significant p value threshold

of p<9×10–8.[73] First, individual gene-level analyses will be performed via linear mixed-effects models with random intercepts fitted to example gene expression/DNA methylation differences between the time points across the groups. Next, to determine whether the genes identified to be different across the groups are enriched for oestrogen-signalling genes, targeted examination of oestrogen signalling pathway genes will be performed via a Monte-Carlo approach. Finally, gene expression/DNA methylation dynamics across time will be examined via more advanced and statistically powerful methods such as K-means clustering and time-course Gene Set analysis[74] to identify sets if genes showing similar patterns via longitudinal gene set trajectories. Such gene set methods can be applied to the set of differentially expression/methylated genes from above or implemented on the predefined set of oestrogen pathway genes to identify specific group dynamics of genes over the two time points.

Oestrogen sensitivity as a predictor of recurrent PPD

Second, based on the same earlier observed gene expression/methylation profiles of women in third trimester who later developed a PPD, we will test whether such patterns translate in the current study. Thus, we will test if the 'cumulative PGES and PDMS' defined above are associated with the onset of a depressive episode (interview-based diagnosis). This analysis will disregard any potential effects of oestrogen versus placebo treatment.

Again, to capture potential patterns of associations with subclinical PPD, we will also perform the analysis using EPDS scores at 8–10 weeks postpartum.

Patient and public involvement

During the preparation and design phase of the MAMA trial, we interviewed pregnant women with a history of perinatal depression who would have been considered eligible participants. The interviews lasted approximately 30 min. They were asked about their willingness to participate in such trial, about possible concerns regarding the trial medication and the amount of time required to participate in the trial. In addition, they were asked to assess the recruitment strategy.

They provided valuable insight, which led to considerations of ethical issues as well as feasibility, resulting in changes in the number of questionnaires to fill out postpartum and in the recruitment strategy.

During the trial, participants are invited to comment on any concerns on the setup or discomfort regarding the intervention. The comments will be taken into considerations for possible adjustments. We will comply with the General Data Protection Regulation by sending e-mails with sensitive personal information to the participants through an online digital mailbox (e-Boks). Using personal registration numbers, e-Boks is subjected to very restrictive legislation and is controlled and approved by the Danish Data Protection Agency.

ETHICS AND DISSEMINATION

Ethical considerations

The short-term transdermal estradiol treatment is not expected to pose unacceptable or intolerable side effects, disrupt breastfeeding or pass to the infant in any dosages that may pose a risk to the infant. Should adverse side effects for mother or infant occur or be suspected, the treatment will be discontinued immediately. When removing the patch, serum concentrations of estradiol return to baseline levels within 24 hours. Participants who develop mental distress or depressive symptoms that approach clinical thresholds will be referred to relevant care by a trained clinician. All potential participants receive oral and written information about the trial and all enrolled participants will provide written informed consent prior to inclusion. The partner receives written information about the trial regarding the infant and is offered oral information. Both parents provide written informed consent for the participation of the infant. Participants and parents can at any time withdraw their consent.

All potentially sensitive personal data will be anonymised. The trial will adhere to the Declaration of Helsinki.[75]

Approvals and registrations

The trial protocol is approved by the Regional Committees on Health Research Ethics in the Capital Region of Denmark (H-20036213), the Danish Medicines Agency (EudraCT: 2020-001592-33) and the Knowledge Centre on Data protection Compliance in the Capital Region of Denmark (P-2020–712). The manufacturers of estradiol patches and placebo patches have been notified about the trial, as standard procedure from Danish Medicines Agency. They have not contributed to the protocol, nor have they made financial contributions to the trial.

Dissemination

We will present results at scientific meetings and in peer-reviewed journals. Results will be presented to policymakers and engage the public, for example, via news media.

The results of this trial may be integrated in future recommendations on the clinical management of women at high-risk for PPD.

Monitoring

The trial is monitored ongoing by the Danish units for Good Clinical Practice in accordance with International Conference on Harmonisation for Good Clinical Practice.

Trial status

The MAMA trial was initiated on 4 January 2021. The first participant was included on 3 February 2021, the inclusion period is expected to run for 2–3 years, and the trial is expected to be completed by December 2026.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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APPENDIX IV, PAPER IV

Women's perceptions of biological causes and potentials of genomic risk markers in postpartum depression

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Short title: Perceptions of causes and potentiality of genomic risk markers in postpartum depression

Abstract

Introduction

Postpartum depression affects 10-15% of women and has a recurrence of up to 40% in subsequent pregnancies. Novel evidence suggests that genomic markers for enhanced sensitivity to estradiol signaling may help identify women at high risk of postpartum depression. Therefore, we explored their perceptions of testing for genomic risk markers that potentially identify women at risk for developing postpartum depression.

Methods

We conducted semi-structured interviews with 13 Danish women who had a history of postpartum depression using a phenomenological approach. A transdisciplinary group of researchers analyzed the interviews thematically. Through the concept of *potentiality*, we unfolded the women's perceptions regarding testing for genomic risk markers to profile their risk of postpartum depression.

Results

We identified three key themes. *1) Biology as a contributing factor to postpartum depression*. Only a few women thought postpartum depression could be related to a sensitivity to hormonal changes across pregnancy and postpartum. *2) The role of external events in making sense of postpartum depression.* Most women perceived their postpartum depression as primarily triggered by external factors rather than biological factors. *3) The ambiguous potentiality of testing for genomic risk markers of postpartum depression.* Testing for genomic risk markers was envisioned by some women as having the potential to prevent postpartum depression and reduce stigma. Yet, at the same time, knowing their risk marker status was perceived as holding the potential to induce depressive symptoms.

Conclusion

Our data show that to women with a history of postpartum depression, the potentiality of testing for genomic risk markers capturing hormonal sensitivity was envisioned with ambiguity. To some women, knowledge about genomic risk markers introduced hope regarding possible prevention and, at the same time, it introduced concerns about inducing depressive symptoms. We suggest considering such perceptions if implementing new genomic risk marker technologies in risk profiling to direct preventive strategies.

Introduction

Postpartum depression affects 10-15% of women, and the risk of recurrence is up to 40% in subsequent pregnancies (1, 2). Women experience postpartum depression as a time of hopelessness, loss of control, guilt, and reduced ability to experience pleasure (3, 4). They often describe feeling stigmatized and embarrassed about their inability to cope with the situation and fear judgment from others (5). Further, women suffering from postpartum depression attribute their depressive symptoms to personal vulnerability rather than illness (6). Postpartum depression is a disabling disorder affecting the entire family and poses a risk for neurodevelopmental disorders and suboptimal health in the offspring (7, 8). Preventive initiatives are suboptimal at best, and only counseling has, in high-risk groups, been associated with a lower likelihood of postpartum depression (9). So far, few studies on preventive strategies approach biological contributions to risk for postpartum depression and identifying women at high risk could help guide preventive initiatives (10, 11).

Even though the etiology of perinatal depression is far from clear, gene-by-environment factors are putatively involved (12). Identifying genomic risk markers enhanced sensitivity to estrogen signaling may help identify women at risk. In addition, this information may guide preventive strategies to protect postpartum mental health (11-16). Efforts to provide insight into the pathophysiology of relevant subgroups of diseases through genomic risk markers and possibly predict the risk of developing the disease is referred to as precision medicine. Genetic testing and precision medicine have been introduced in psychiatry, targeting disorders like schizophrenia and major depressive disorder (17, 18), and are in an exploratory stage within disorders like postpartum depression (12-16). Precision medicine is often articulated as hopeful, filled with power and potential (19). In particular, the concept of potentiality has been employed to explore people's understanding of the transformations that biomedical and genomic technologies may initiate (20, 21). According to Taussig and colleagues, potentiality depicts something imaginable that may or may not (yet or never) exist. As a concept, potentiality captures human capacities to imagine how something will develop in the future and encompass both desirable and/or undesirable outcomes. Accordingly, potentiality has a temporal complexity by being latently present and yet open to future modification and transformation (22). Following Taussig and colleagues' anthropological outline of the concept of potentiality, we employ the notion of potentiality to understand how women perceive hormone sensitivity testing and knowledge about genetic risk (22).

Previous studies have focused on facilitators and barriers to genetic testing and precision medicine in psychiatry. They include approval of testing but, at the same time, also a fear of unfavorable consequences (23, 24). However, to understand women's perceptions of hormone sensitivity testing and precision medicine in postpartum depression, it is essential not only to understand the facilitators and barriers but also to gain insight into how the women perceive the potential of knowing and transforming the risk of postpartum depression and how genomic knowledge plays a role in this transformation.

Among women with a history of postpartum depression, we explored their perceptions of testing for genomic risk markers that potentially identify women at risk for developing postpartum depression.

Materials and Methods

We conducted a qualitative interview study with women with a history of postpartum depression. The interview study was a part of a broader research study called Maternal Mental Health (MAMA) Trial aiming to evaluate the preventive effect of three weeks of transdermal estradiol treatment immediately postpartum on depressive episodes in women at high risk of postpartum depression (10). As a part of the clinical MAMA Trial, the participants were also asked to donate blood for analyses of unique candidate markers to evaluate if genomic markers of estrogen sensitivity qualify as a predictor of postpartum depression risk (10).

We conducted and reported the study in accordance with the Consolidated criteria for reporting qualitative research (COREQ) (25).

Participants

Women enrolled in the MAMA Trial from January to April 2022 were asked if they were interested in participating in the interview study.

We used purposive sampling to ensure that women varied in their socio-economic background and that both women with and without recurrence of postpartum depression were represented (26). The inclusion criteria for participating in the interview study were a self-reported history of perinatal depression (during pregnancy or within six months postpartum period) qualified by retrospective interviews by trained clinicians and confirmed by a medical doctor specialized in psychiatry.

In the subsequent postpartum period, we evaluated if the women developed recurrence as diagnosed through a clinical interview with a medical doctor specialized in psychiatry according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criteria of Major Depressive Disorder (within six months postpartum). When the women had their last in-person trial visit at ten weeks postpartum, they were asked if they were interested in participating in the interview study. The first author (SH) provided them with an information letter and stressed that participation was entirely voluntary and would not affect their continued participation in the MAMA trial. Three women did not reply to attempts of contact with information about the study.

The first author (SH) had no personal relationship with any participants; however, since they were also a part of the clinical MAMA Trial, she had met them before when collecting data earlier in the trial. In that sense, the first author was familiar with some of the participants' stories prior to the interview. This might have created a space of trust and confidentiality during the interviews. The first author stressed the importance of telling their stories, pretending it was the first time the interviewer heard them.

Setting

The study comprised women from the Capital Region of Denmark. The capital region of Denmark covers more than 20,000 deliveries per year; of these, approximately 250 are deliveries of women with a history of postpartum depression.

Data collection

We performed individual, semi-structured interviews between three and five months after their second birth, lasting around one hour on average (ranging from 45 minutes to one hour and 45 minutes), from January to March 2022. All interviews were performed in the participants' homes, some with their 3-5 months old infant present during the interview. The first author (SH) conducted all interviews. The last author (LEN) participated in one interview, supervising the first author.

The transdisciplinary research group consisted of two midwives, two anthropologists, one obstetrician, and one psychiatrist developed an interview guide prior to the interviews. We based the interview themes (Table 1) on existing literature on women’s experience of postpartum depression, perceptions of genetic testing in psychiatry and field notes from recruitment interviews for the clinical

MAMA Trial (more than 100 recruitment interviews) (3, 6, 23, 24). Following the initial two interviews, we adjusted the interview guide. Specifically, we included more information about genomic risk marker testing to encourage women to reflect more on the biological aspects of risk for postpartum depression. To access the women's experience of having postpartum depression, we used a narrative interview technique, opening the interview with the phrase: "Please, tell me your story about the last time you had a baby". When the women came to a natural end in their story, we used the predefined questions from our interview guide. If the women themselves mentioned biology as a part of the explanation for their depression, we further explored their perceptions around the theme. If not, we initiated by asking them: "What do you think about a potential biological/genomic factor contributing to your postpartum depression?"

We were curious about how the women defined biology in relation to postpartum depression and their perceptions of hormone sensitivity testing for risk of postpartum depression. Therefore, we asked them about their perceptions of the biology contributing to their depression and how they would perceive it if we, *hypothetically*, could point at them being predisposed to postpartum depression through testing for genomic risk markers.

The interviews were audiotaped and transcribed ad verbatim. The first author transcribed six interviews, and student assistants transcribed the rest.

Data analysis

We drew our analysis on a phenomenological tradition, exploring human experience and sense-making (27). The interviews were analyzed using NVivo software (28). We used a thematic analysis with six phases described by Braun and Clarke (29). In line with these phases, 1) we read the interviews and initial meaning was noted, 2) we worked systematically through the data and extracted relevant parts of the interviews that seemed interesting according to our research question into codes, 3) we searched for themes across the codes, 4) we reviewed the themes in relation to the coding and generated a thematic map of the analysis, 5) a collective thematic framework was defined involving all authors, and finally, 6) findings were written up.

We ensured rich data reflections by involving researchers from different fields (midwifery, obstetrics, psychiatry, and anthropology) in the analysis. We found data redundancy after 12 interviews

indicating data saturation and decided to stop at 13 interviews. All participants were given fictional names.

Ethics Approval

This interview study is part of the clinical Maternal Mental Health (MAMA) Trial and is approved by the Regional Committees on Health Research Ethics in the Capital Region of Denmark (H-20036213), the Danish Medicines Agency (EudraCT: 2020-001592-33) and the Knowledge Centre on Data protection Compliance in the Capital Region of Denmark (P-2020-712). The women provided written informed consent before being included in the study.

Results

We conducted 13 interviews with women with a history of postpartum depression, typically 2-3 years ago with one exception of 11 years ago, and who had experienced pregnancy and birth after the depression. All the women had an infant of 3-5 months of age at the time of the interview.

Most of the interviewed women were of Danish origin, between 30-35 years of age, had higher educational degrees, and three had a recurrence of postpartum depression (Table 2).

Key themes

Through the analysis, we identified three key themes 1) Biology as a contributing factor to postpartum depression, 2) The role of external events in making sense of postpartum depression, and 3) The ambiguous potentiality of testing for genomic risk markers of postpartum depression.

Biology as a contributing factor to postpartum depression

Most women did not spontaneously mention biological contributions to their postpartum depression. When asked about their perceptions of biology possibly contributing to their depression, it became evident that it played a minor role in their understanding of postpartum depression.

Heredity as a contributing factor to postpartum depression

When reflecting upon the association between biology and postpartum depression, the women primarily associated biology with genes and heredity. However, most women did not perceive their genes as the single cause for their depression; it was closely intertwined with life conditions and specific past experiences.

> *"I thought it was me. That I have a flaw that makes me highly anxious. And since I've been depressed before, I think that I'm at risk. Then of course I thought about how difficult the birth was and that I was totally deprived of sleep, not to mention the immense shock of giving birth for the first time. Being responsible for a tiny baby and, well, for a long time I thought the reason was that, with Emil, I was terribly stressed because I breast fed and he had to eat. But it wasn't like that this time; it wasn't something like that making me feel frazzled in the beginning this time, so I can't quite (…) So, but yeah, I definitely believe that there's something about me, genetically, that makes me prone to it."*
>
> (Ida, postpartum depression with her first child and anxiety with her second child)

To Ida, the potential of testing for genomic risk markers was inevitably linked to reflections on the causality of disease. She perceived her depression as resulting from stressful living during the weeks leading up to the birth, the physical transition from pregnancy, through birth to postpartum, combined with her history of depressive episodes.

Hormones as a contributing factor to postpartum depression

Despite participating in MAMA Trial involving estradiol for preventing postpartum depression, only a few women thought that postpartum depression could be related to a sensitivity to hormonal fluctuations occurring during pregnancy and postpartum.

> *"I believe that it has something to do with the hormone levels in my body that make me more susceptible. Not only that, but my mother has anxiety, and my father is an alcoholic, which means they are battling with something psychologically. So, that's why I was convinced that it purely had to do with my genes, without really understanding how it works (…) Is there something or other in my upbringing, that is, in my abilities,*

I mean, in the resources I have available, that makes me unable to handle more than this or otherwise I'll just fall apart? Or am I just built differently, which means that I must cope in another way, sort of, to be able to manage? Or what do I have to learn, that others have learned, that I really can't. I find that interesting."

(Mette, no recurrence of postpartum depression)

Like Mette, the women speculated that there had to be some explanation related to their hormones. Karen had experienced a traumatic birth and attributed the experience as the cause of her first postpartum depression. After her second birth, she experienced a recurrence of postpartum depression. However, after experiencing a good pregnancy and birth, she revised her perceptions of the cause of her depression.

"The typical thing about it was that it occurred three days after I gave birth. It came out of nowhere. I had a really good pregnancy and, before that, was fine mentally and physically, and happy with my life, my work, my home, and my children and all. There was nothing during my pregnancy. Not a glimpse of anything, not before my pregnancy either. She [the psychologist] was like; it appears to be exceptionally hormonal when it happens in one night."

(Karen, recurrence of postpartum depression)

In contrast to many other women, Karen did not have a story of traumatic external events, and she was convinced that her hormones affected her risk of postpartum depression.

The role of external events in making sense of postpartum depression

Listening to the women's stories, it became evident that they all were searching for an explanation of why they suffered from depression at a time that was supposed to be the happiest of their lives.

Imagined ideals of a good mother

Most of the women strived to be the "perfect mom." The quote of Sandra illustrates the high expectations to motherhood.

> *"I guess you just want to do your best, don't you? You'd like to be able to do it well enough, in a way, to be able to do it 100% perfectly. This is also something that I've talked to the psychologist about. It's not because I normally do everything perfectly either. But when it comes to my children, it's just like it has to be perfect."*
>
> (Sandra, recurrence of postpartum depression)

After birth, Kirsten struggled with breastfeeding, overshadowing all other aspects of becoming a mother. She described feelings of anxiety, stress, and self-blame. She had all her identity as a mother tied up in successful breastfeeding, and when failing at breastfeeding, it became difficult for her to envision herself as a good mother.

> *"I remember just sitting there. I was so unhappy; I couldn't stop crying and felt like a bad mom. Just the fact that it wasn't as simple as letting the baby nurse at my breast and off we go. I thought that that was what was happening. My expectations didn't match reality at all. That made me think about whether there was something wrong with me. It's me who can't, I can't figure it out; am I even suited to being a mother if I can't do something as basic as breastfeeding my own child?"*
>
> (Kirsten, no recurrence of postpartum depression)

The quote of Kirsten and Sandra is representative of many of the interviewed women. The contrast between ideals of motherhood and real life left the women with overwhelming feelings of failing in motherhood, negatively impacting their self-understanding, and feeding their guilt.

Lise gave birth by emergency caesarean section which was an overwhelming experience.

> *"Of course, it's been a combination of many things, but I had a really complicated birth with Esther the first time (...). So, my initiation into motherhood was, uhm, totally ... I got off to a completely bad start, I'd say. I was incredibly drained mentally and physically. Not just the birth but the cesarian section. I didn't get to hold her immediately when she came out. They had to help her, which meant Jens got her. I got her when I woke up in recovery. if you've ever experienced having so much medicine in your body, that needs to be expelled. You're oddly wide awake. Then they give you the child as though it was any old child. Yeah, now you have to breastfeed and everything. I was like; please get this baby away from me. I couldn't cope at all."*

(Lise, no recurrence of postpartum depression)

With the words, "Then they give you the child as though it was any old child," Lise expressed that experiencing critical events during birth challenged her attachment to her newborn. To Lise, postpartum depression was affected by experiencing a traumatic birth. Lise framed it as she "got off to a completely bad start."

Lack of support from the healthcare system and relatives

An overarching theme for the women was the lack of care and support as the cause of their depression. The women described it as relatives neglecting them emotionally or the healthcare professionals failing them in their care, thus leaving them with the impression that staff members were unconcerned about their mental and physical well-being.

Simone characterized her personality as worrisome. During pregnancy, Simone experienced a healthcare system that took over monitoring the well-being of her fetus by ultrasound because he was too small and induced her labor. She believed that her postpartum depression resulted from being removed from sensing how her fetus was doing and how her pregnancy was progressing, combined with her being worrisome. This experience led her to lose confidence and feel disempowered in her motherhood.

> *"I find it difficult to describe it other than I just remember being excessively worried and frightened all the time. Well, nervous (…). They looked at him and said everything looks good, just like when he was in the womb. We can see that your baby is doing well in the womb, so you don't have to sense it yourself. The same was true during the birth. I shouldn't even notice when the birth began. I was kicked into gear and had to just hit the ground running at a thousand miles a minute. I was somehow removed from what was happening. I think that's also probably why it took so long for me to connect with him afterward."*
>
> (Simone, no recurrence of postpartum depression)

Others, like Emma, described failings in care from the healthcare system, in which she felt left alone, contributing to her postpartum depression. Due to postpartum hemorrhage, Emma went to the operating room immediately postpartum, leaving her husband to care for their newborn daughter by

himself. He was so nervous that he vomited, which the medical staff mistook for sickness, and he was sent home. Being new parents and separated, combined with the impression that the professional caregivers not caring about them, was stressful for Emma and her partner.

> *"Yeah, he [her partner] had such a rough start, and also a rough start for me to be alone and feeling like I couldn't take care of myself. Uhm … well, when you lie there and can't move and she's lying next to you. Then they stuff a piece of paper in your hand, telling you that you're expected to manage on your own now and that food is available on the ground floor at such and such a time. Even if you made plans with someone, they were not respected because the person you made them with had left. So, it starts off with the feeling of total neglect and abandonment."*
>
> (Emma, no recurrence of postpartum depression)

The ambiguous potentiality of testing for genomic risk markers of postpartum depression

When introduced in the interview, some women envisioned testing for genomic risk markers as holding the potential to prevent depression and reduce stigma. Yet, at the same time, knowing their risk score was perceived as having the power to enhance awareness of depressive symptoms thus holding the potential to become a self-fulfilling prophecy.

Biology as a hidden force in postpartum depression

The majority of the women spontaneously addressed the stigma surrounding postpartum depression by stressing that they had never thought it was "someone like me" who would get postpartum depression. Instead, they considered themselves to be personally-, socially-, and physically resourceful and, by that, not at risk for depression.

A few women anticipated that the effects of testing for risk markers would include reduced guilt, stigma, and shame. The following quotes of Maja and Karen illustrate that perceiving biology as a cause of postpartum depression would relieve them from responsibility and entail accepting the postpartum depression as a life circumstance. To Karen, the test for risk markers represented a

knowledge that she expected would inevitably manifest itself in the future. Importantly, this means that Karen understood the risk of postpartum depression conveyed through the genomic risk marker test as outside her power to control or change.

> *"Sometimes it's easier to be vulnerable for biological reasons than due to a broken mind, right? It might be less shameful that way, I guess."*
>
> (Maja, recurrence of postpartum depression)

> *"I think it would also reassure me if I could explain it to myself, well, that I'm not crazy, that it has something to do with my body. It's chemistry; it's biology. Something is happening inside me, and we don't know what it is (…). It would give tremendous peace of mind to know, well, that that's what your DNA is like; it has nothing to do with me or, well, it's me, it's my DNA, but I couldn't have done anything differently. It's just a condition in life that I have. It's not really my fault or something I should have done or not done like that; it's the way it is."*
>
> (Karen, recurrence of postpartum depression)

Regitze experienced that the ability to localize postpartum depression in biology or hormones, presented by the clinical MAMA Trial, established her future as open and provided her with the possibility to understand, know and act upon postpartum depression.

> *"I know my body extremely well, and I'm incredibly aware of what my period can do to me. (…) That's also why it's quite logical if it's something biological or hormonal. That there's a natural explanation for why this happens to some people and why it doesn't happen to others. That makes a lot of sense. I hope, and this sounds utterly insane, but I HOPE that this is why people get postpartum depression because then something can be done about it."*
>
> (Regitze, no recurrence of postpartum depression)

A couple of the women pointed out that genomic risk marker testing would give them biological proof of their risk of depression, which they imagined as more valued and respected by healthcare professionals.

"The first time I went to the doctor, she was like, well, let's just wait and see what happens. If you give people this kind of information: I have this gene, whatever it is, we can try to have a serious conversation instead of you just brushing me off with the fact that you're also a first-time mom and you're probably just overreacting and feeling like everything is too much right now. I think maybe the healthcare system would take you more seriously."

(Astrid, no recurrence of postpartum depression)

"That would be brilliant because then it would get caught early. Maybe you would be put in touch with a psychologist without delay (…). I just think that it would be taken seriously in a completely different way as well. Both in terms of your health but also for the family (…). I think it would be handled differently when it's precisely something that you specifically can see on a blood test, a bit like diabetes or something."

(Kirsten, no recurrence of postpartum depression)

In previous encounters with healthcare professionals, the women had experienced the healthcare professionals not listening to them and wanting to normalize and tone down their depressive symptoms and distress. Hence, they were left with enhanced feelings of guilt and shame. To Kirsten, genomic knowledge carried the potential to transform her interaction with healthcare professionals and her family.

The potential to modify the risk of postpartum depression

In contrast to Karen, Lise envisioned that knowing her biological risk would somehow render her future closed; she imagined that knowing her risk could lead to an exaggerated awareness and reinforcement of or even trigger depressive symptoms. In that way, testing for genomic risk markers related to postpartum depression was perceived as carrying a risk of becoming a self-fulfilling prophecy. The potentiality of the genomic risk marker test was perceived as possibly leading to manifest disease. Two women pointed to the different potential of screening for physical diseases and psychiatric disorders. They perceived testing for genomic risk markers of psychiatric disorders as

having transformative power in which anticipation of psychiatric symptoms could potentially trigger or enhance symptoms.

> *"I think that it [genetic screening] can be both positive and negative. Because it's not always, I believe, positive to know what can potentially happen. Because, on the one hand, you could say that it's terribly smart in terms of prevention, but I also can't help but think that, well, it can also be a contributing factor in terms of being prone to something happening."*
>
> (Lise, no recurrence of depression)

Secondly, they all pointed at the transformative potential of the genomic risk marker test in the sense that knowing about a risk of postpartum depression revealed a preventable future. If it was not in their power to modify the risk themselves, it could be modified by others, thus leaving their future open.

> *"If you had that blood test taken and you got the result that you were at increased risk, then a lot of other things should be made available to make you better at … (…) well, it should be helpful and not just information, I think. So, yes, what you're told must be accompanied by more. Otherwise, I wouldn't…."*
>
> (Ida, postpartum depression with her first child and anxiety with her second child)

One woman speculated if knowing the risk could be misused by social system authorities.

> *"I would probably take it, I think. But I also think the anxiety I have towards all the social services stuff and about forced child removal and all, that I would like to know ahead of time; what happens if someone is told they have something, and it triggers an avalanche, like completely automatically? What does it mean if I'm told I have something? What does it mean, then? This would likely affect whether I would take it or not."*
>
> (Kirsten, no recurrence of postpartum depression)

When Kirsten had postpartum depression, she feared that the social services would remove her baby if they found out she had depressive symptoms. Even though Kirsten's fear of the social services is not representative of the interviewed women, it points to the perceived potency of a genomic risk marker test and a fear that the most valuable, namely their child, may be at stake.

Discussion

In this qualitative study, we found that most women perceived their postpartum depression as predominantly influenced by external factors rather than biological ones. Only a few women believed postpartum depression could be related to a sensitivity to hormonal fluctuations. Generally, the women envisioned testing for genomic risk markers with ambiguity, i.e., holding the potential to prevent postpartum depression and reduce stigma, and, at the same time, holding the potential to become a self-fulfilling prophecy.

According to Goffman, stigma is an attribute that communicates devalued stereotypes and excludes a person from social acceptability (30). A sub-group of stigma is self-stigma, which is when a person internalizes the attitudes of the public stigma with a negative impact on self-esteem and self-image (31). Our analysis provided a nuanced illustration of stigma related to postpartum depression. To some of the interviewed women, having “biological proof” of their depressive symptoms held the potential to change their self-image and not feeling stigmatized. Notably, women who perceived genomic risk marker testing as mitigating feelings of guilt and shame associated with postpartum depression were exclusively those who had experienced a recurrence of depression. One might speculate if their heightened sensitivity to the stigma surrounding postpartum depression, which was still fresh in their minds, influenced this perception. However, we are cautious in concluding, as only two women articulated this perception, and the third woman with a recurrence did not discuss this aspect. The potential of reducing guilt through biogenetic explanations was also found in a meta-analysis by Kvaale et al. (32). In contrast, other studies have shown that genetic information about psychiatric conditions could increase stigma and discrimination in asymptomatic people (24, 33). Furthermore, women who perceived genomic risk marker testing as offering 'biological proof' had not experienced a recurrence of postpartum depression. This underscores the significant impact of the experience with depressive symptoms, persisting long after remission.

Most of the interviewed women perceived their postpartum depression as primarily influenced by external factors rather than biological factors. As the women made sense of the postpartum depression, they envisioned the risk as plastic and transformable, as something they and, especially their relatives and caregivers, could actively provoke and prevent. Thus, they perceived it as something that could be modified by experiencing traumatic events. Relating postpartum depression to external causes might leave the women with the hope of evading recurrence in a future pregnancy or postpartum. Predominantly, the women looked back and reflected on the dynamics of their high

expectations of motherhood and some experiences of lack of support in healthcare encounters. The importance of prioritizing care and attention postpartum and the psychological consequences of failing in care are similarly highlighted in other studies (34, 35).

The perceived potentiality of hormone sensitivity testing was ambiguous. In theory, testing for genomic risk markers of postpartum depression can induce hope and render the future open; however, as our analysis shows, it sometimes does the opposite. Knowledge about a genetic risk of postpartum depression introduced hope to some women regarding possible prevention. Creating hope for future prevention through genetic testing has similarly been demonstrated among people suffering from depression and unaffected people (24). In contrast to the hopeful nature of genetic testing articulated among researchers and some patients as enabling multiple alternative futures (22, 36-38), some women also pointed to the possibility of knowing their risk of postpartum depression as something that narrowed down their space for maneuvering and shaping their future. By attributing the depression to genetic causes, the women might have been forced to accept a possible recurrence in future pregnancies and were left with less hope. Anchoring postpartum depression in biology or at a molecular level seems to represent something deterministic to some of the women. In line with our findings, other studies have shown that knowledge about genetic causes for depression increased peoples' prognostic pessimism (32, 39). However, previous research has demonstrated that malleability-focused psychoeducation could reduce prognostic pessimism in individuals with depression (40). Two experimental studies that showed learning that a person was genetically susceptible to depression caused them to feel their depressive symptoms as more severe support our findings that knowledge about a risk represented the potential to induce depressive symptoms (41, 42). Further, the medical anthropologist Monica Konrad argues in her work that learning about a genetic risk makes people be seen as ill before they are ill (21).

Introducing genomic risk markers testing calls to be followed by an intervention that is not (yet) available or efficient psychoeducation support. Thus, ways to address ambiguities and concerns for women eligible for genomic risk marker testing must be developed before designing and implementing testing for postpartum depression based on genomic markers or more complex risk models.

Strengths and Limitations

This study is unique in qualitatively exploring the perceptions of testing for genomic risk markers related to postpartum depression among women with lived experiences of postpartum depression. In addition, the interviewed women had all participated in a clinical trial in which they wore short-term hormone patches to evaluate the preventive effect on recurrence of postpartum depression. This could have increased their awareness of postpartum depression and possibly increased the likelihood of them reflecting on the biological factors involved in their postpartum depression. Nevertheless, even with their involvement in the MAMA Trial, biology continued to play a minor role in their perception of postpartum depression. On the contrary, women who decided to participate in the MAMA Trial could have more positive expectations of genomic risk marker testing than those outside the trial. However, it is noteworthy that discussions surrounding genomic risk marker testing, a secondary objective of the MAMA Trial, were only briefly touched upon during interactions with participants Another strength was the research group's transdisciplinary nature, enhancing the study's reflexivity and validity (26).

One limitation of the study is that we asked the women about a hypothetical genomic risk marker test making it more abstract to reflect upon. We might have had different results if the genomic risk marker test had already been implemented in clinical practice and a relevant preventive strategy had been available. In addition, the women in our study were primarily of Danish origin. Future research would benefit from including women of other ethnicities to ensure diverse voices are heard and any unique issues related to these groups are explored.

Conclusion

Our study suggests that to women with a history of postpartum depression, the potentiality of testing for genomic risk markers related to postpartum depression was perceived with ambiguity.

Biological causes were represented in the women’s stories; however, external causes were always intertwined. Knowledge about hormonal sensitivity at the genomic level for developing postpartum depression could, to some, introduce hope regarding possible prevention. At the same time, it was perceived as having the power to enhance awareness of depressive symptoms and create imaginative negative expectations about the future. Before implementing new genetic technologies in risk profiling to direct preventive strategies, this essential knowledge needs to be considered.

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Author Contributions

SH, LEN and VGF created the idea for the project. SH, HKH, MNS, LEN and VGF designed the study. SH were responsible for conducting the interviews and main analysis with support from HKH and LEN. SH drafted the first version of the manuscript, with contributions on interpretation of data and critical revision of important intellectual content from HKH, KMR, MNS, LEN and VGF. All authors reviewed and approved the final manuscript for publication.

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Competing Interest Statements and Disclosures

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Table Legends

Table 1. Interview theme guide

Table 2. Selected characteristics of participating women (n=13)

Table 1. Interview theme guide

Introduction	The aim of the study, the possibility of withdrawal, audio recording, anonymity, and the way interview data will be disclosed.
Opening question	Please tell us your story about the previous birth, where you think it begins…
Guiding interview themes	• Expectations to motherhood • Experience with postpartum depression • Social interactions and relationships – *Healthcare professionals* – *Partner* – *Other relatives* • Experience with being pregnant again and thoughts of recurrence and prevention • Reflections about triggering factors in relation to postpartum depression • The value of genomic risk marker testing in mental illness and postpartum depression • Perceptions of testing for genomic risk markers related to enhanced sensitivity to estradiol signaling and postpartum depression. • Perceptions of prevention and treatment of postpartum depression. • Retrospective rationalizations. Looking back on the postpartum depression and creating explanations. • Thoughts about the future – future pregnancies and births • Questions and was there something we forgot to talk about • *Thank you*

Table 2. Selected characteristics of participating women (n=13)

Characteristics	Number of women
Maternal age (years)	
<30	1
30-35	10
>35	2
Living with partner	
Yes	13
No	0
Danish origin	
Yes	12
No	1
Highest qualification	
Compulsory education	2
1-2 years higher education	2
3-4 years higher education	4
Advanced degree	5
Time since first postpartum depression (years)	
1-3	7
4-5	4
>5	2
Recurrence of postpartum depression	
Yes	3
No	10

APPENDIX V, INTERVIEW GUIDE (PAPER IV)

Kvinders perspektiver på præcisionsmedicin og screening for hormonfølsomhed som forebyggelse af fødselsdepression

Formålet med projektet er at beskrive kvinders forestillinger og forventninger til præcisionsmedicin i relation til forebyggelse af fødselsdepression og screening i forhold til biologisk informerede strategier.

Vi vil inkludere 10-15 kvinder med tidligere fødselsdepression. Kvinderne inkluderes efter fødsel af deres andet/tredje barn (og efter deltagelse i MAMA).

Introduktion: Jeg hedder Stinne, tak fordi du har sagt ja til at deltage i interviewet. Du har deltaget i MAMA studiet, som handler om at forebygge fødselsdepression med behandling af østrogenplaster efter fødslen. Dette er IKKE en evaluering af projektet.

Formålet med projektet (NRU ved Vibe Frøkjær i samarbejde med KU Mette Nordahl Svendsen/Laura Navne) er at beskrive kvinders erfaringer og oplevelser med fødselsdepression og deres tanker om screening for hormonfølsomhed i en blodprøve i relation til at være i risiko for at udvikle fødselsdepression og dermed forebyggelse af fødselsdepression. Vi er interesserede i dine tanker og dem kan vi kun få ved at tale med dig.

Der vil være to dele i dette interview. Første del kommer til at handle om din oplevelse af forløbet ved første fødsel, som du husker den, og hvordan det så var at være gravid igen efter den oplevelse. Anden del kommer til at handle om det biologiske og med det mener vi en blodprøve, der kan undersøge hormonfølsomheden og dermed muligvis kunne sige noget om risiko for fødselsdepression.

Interviewet varer ca. 1 time, måske lidt mere. Jeg vil gerne optage på bånd så vi får alt med hvad du fortæller os. Du er anonym. Det er frivilligt, og du kan til enhver tid trække deltagelsen tilbage. Din deltagelse her og dette interview kommer ikke til at påvirke vores behandling af dig i den kliniske del af MAMA studiet.

Interview guide:

- **Fortæl om din oplevelse ved sidste fødsel (det som fylder hos dig)**
    a. Hvilke forventninger havde du til det at blive mor? Giv gerne eksempler, særlig episode.
    b. Hvis nogen spurgte din mand, hvordan du havde det med at blive mor, hvordan tror du så han ville beskrive hvordan du havde det sidste gang? Giv gerne eksempler, særlig episode.
    c. Talte du med nogen om hvordan du havde det?
        i. Familie
        ii. Venner
        iii. Kollegaer
    d. Var der nogen i sundhedsvæsnet, altså din egen læge, jordemoder, sundhedsplejerske, der efter fødslen spurgte dig om hvordan du havde det? (giv gerne eksempler på samtaler)
- **Du blev så gravid igen. Hvad tænkte du om det? Hvad havde du af forventninger og eventuelt bekymringer til ny graviditet?**
    a. Hvad havde du af tanker i forhold til at skulle være gravid og mor igen? Forventninger?
    b. Talte du med nogen om, hvordan du havde det? Eksempelvis mand, familie, venner eller kollegaer Fortæl hvad I snakkede om.
    c. Talte du med din jordemoder/sundhedsplejerske eller læge om det? Fortæl hvad I snakkede om.
    d. Gjorde du dig nogle tanker om, hvordan dette forløb kunne blive anderledes end det første?
    e. Da sundhedsplejersken kom på besøg efter denne fødsel, kan du fortælle om det? Hvad talte I om? Talte I så konkret om hvordan du havde det? Og hvad du evt. kunne gøre for at forebygge/håndtere at få det ligesom ved sidste fødsel?
- **Forestillinger om udløsende faktorer i forhold til fødselsdepression**
    a. Hvad tænker du om, at der muligvis kunne være en biologisk /genetisk faktor som var bidragende til din fødselsdepression?
    b. Hvad tænker du om, hvis en blodprøve der undersøgte hormonfølsomheden kunne vise at du var i risiko for fødselsdepression?

c. Hvad tror du dine forældre og venner vil sige om det?

- **Værdien af genetisk viden om psykisk sygdom**
    a. Er der andre i din familie, der har haft fødselsdepression eller efterfødselsreaktion?
    b. Helt overordnet, hvad tænker du om fordele ved at screene for hormonfølsomhed i relation til fødselsdepression?
    c. Helt overordnet, hvad tænker du om fordele ved at screene for hormonfølsomhed i relation til fødselsdepression?
    d. Ser du nogen dilemmaer i forhold til screening med din erfaring med fødselsdepression?
    e. Hvis vi kunne pege på at du havde en helt særlig markør i dine gener, som medførte at du var særligt følsom for hormonudsving og dermed disponeret til fødselsdepression, hvad tænker du så om det?
- **Hormoners bidrag til mental sundhed**
    a. Har du tidligere oplevet at dine hormoner påvirker dit humør og psykiske velbefindende? F.eks. i forbindelse med menstruation eller p-piller? Vil du prøve at beskrive hvordan hormoner påvirker dig hvordan/ hvordan du mærker hormoner?
    b. Har du selv oplevet at mærke hvordan dine hormoner påvirker dig psykisk i dine graviditeter/efter fødslen.
- **Forebyggelse og behandling af fødselsdepression**
    a. Hvis man i en blodprøve kunne afgøre om du var "hormonfølsom" hvad ville det så betyde for din motivation for forebyggelse af fødselsdepression?
    b. Hvad ville det betyde for din holdning til behandling hvis du igen skulle få en fødselsdepression?
    c. Hvad ville det betyde for dine pårørendes (eller partner/forældre/venners) holdning til behandling, hvis du igen skulle få en fødselsdepression?
    d. Der er nogen der har nævnt en bekymring for at andre kunne have adgang til resultatet af den test.
- **Hvad tænker du om, hvis du skulle blive gravid igen? I forhold til fødselsdepression?**