Cortisol metabolism, postnatal depression and weight changes in the first 12 months post-partum.

Short title: Postpartum cortisol metabolism

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Abstract

Background & Objectives Postnatal depression correlates with postpartum weight retention, and dysregulated cortisol metabolism is evident in depressed individuals. Cortisol metabolism, BMI and metabolic phenotype are robustly associated but the role of cortisol metabolism in postnatal mental health and weight loss has never been examined. **Design** A longitudinal observation. **Patients** 49 healthy women with uncomplicated pregnancy. **Measurements** BMI and urinary steroid metabolites at 1 week and 1, 3, 6 and 12 months postpartum**.** Validated urinary steroid metabolite ratios were measured to determine the activities of 11β-hydroxysteroid dehydrogenases (11β-HSD) that interconvert inactive cortisone and active cortisol and the 5α-reductases that clear cortisol to its inactive metabolites. Postnatal depression symptoms were measured at 1, 6 and 12 months. **Results** Low 5α-reductase activity was associated with greater weight loss across the first year, independent of demographics, breastfeeding and depression. Postpartum BMI change was unrelated to postnatal depression at any time. Symptoms of postnatal depression were related to higher cortisol metabolite production at 12 months, independent of demographics and breastfeeding. **Conclusions.** Greatest weight loss in the postpartum year was associated with lower conversion of cortisone to cortisol and lower conversion of cortisol to its metabolites, supporting previous work that demonstrates the facilitative role of lower 5α-reductase and 11β-HSD-1 in weight loss. Greater depression symptoms were associated with higher cortisol metabolite production rates. Whilst weight and mental health are both associated with

dysregulation of the HPA axis, there may be different pathways towards depressed and obese phenotypes in healthy postpartum samples.

Background

Maternal postpartum weight trajectories are important factors in immediate and long-term maternal physical and mental health. Two thirds of women retain weight after pregnancy[1]. The risk of depression and anxiety is significantly higher in women who retain two or more BMI units at 6 months postpartum[2]. Women who gain excess weight during pregnancy are 2.15 times as likely to be overweight and 4.49 times as likely to be obese two decades later[3]. Unsuccessful postpartum weight loss is a risk factor for long-term obesity[1,4] and postnatal adiposity tends to have centralised distribution thus increasing risk of disease[5]. There is considerable variability of postpartum weight change (e.g. mean (±SD) of +1.1kg (±7.6kg) at 14 months postpartum[6]) with lower weight retention predicted by married status, lower gestational weight gain and longer breastfeeding[6]. Maternal depressed mood has also been associated with poorer postpartum weight loss trajectories[2] and obesity is a risk factor for postnatal depression[7].

Dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis is common in obesity and mental health disorders, indicating a possible mechanistic link[8]. Women with higher prenatal depression scores have been shown to have higher salivary cortisol[9] and maternal distress in pregnancy is related to plasma cortisol levels[10] . However, some studies show weak associations[11-12]. This may be because relationships between pregnancy distress and circulating cortisol may not necessarily be linear or may vary with gestation[12]. For example, in one study of severely obese pregnant women, both very high and very low maternally reported anxiety was related to low serum cortisol levels[13]. Furthermore, higher serum cortisol

levels in late pregnancy predicts greater postpartum weight retention in severely obese women[13]. However, serum cortisol level does not mediate between mental health and postpartum weight retention[13]. In obese individuals, circulatory cortisol concentrations are often normal or low[14] due to enhanced excretion accompanied by increased metabolic clearance[15]. Therefore, investigation of the role of cortisol metabolism, rather than level, in postnatal mental health and weight loss/retention is warranted.

Little is known about the metabolic underpinnings of postpartum weight change or postpartum mental health. Steroid hormone metabolism has been linked to insulin resistance, obesity and the metabolic syndrome[16]. Longitudinal changes in glucocorticoid metabolism are associated with the development of adverse metabolic phenotypes, with higher total glucocorticoid production linked to increased BMI and abnormal glucose tolerance[17]. Examination of the activity of the enzymes 11ß-hydroxysteroid dehydrogenase type 1 (11ß-HSD-1, which converts inactive cortisone to cortisol), 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD-2, which converts cortisol to inactive cortisone) and 5α-reductase (which converts cortisol to its tetrahydrometabolites) has established their role in the development and maintenance of metabolic disease[17]. 11ß-HSD-1 is highly expressed in adipose tissue from obese individuals and inhibition of 11ß-HSD-1 results in weight loss and improvements in glycaemic control[18-21]. 11ß-HSD-2 is expressed in the kidney and has a role in controlling blood pressure via its blocking of cortisol's activation of the mineralocorticoid receptor. There is a positive relationship between 5α-reductase and markers of insulin resistance[22].

In addition to its role in metabolic disease, cortisol metabolism is implicated in a number of mental health problems, particularly depression, anxiety and psychosis[23-25]. 5α-reductase inhibitors have been associated with symptoms of depression[26] and depressed patients show lower 5α-reductase and 11ß-HSD-2 activity suggesting less clearance of cortisol[24]. Pharmacological treatment of depression for 4 weeks results in increases in 5α-reductase activity to levels comparable to a non-depressed control group[24].

Previously, we have described postpartum corticosteroid metabolism in this cohort of women[27]. 11β-HSD-1 and 11β-HSD-2 activity did not show significant changes with time. 5α-reductase activity was low in the immediate post-partum period, increased over the initial 3 months and then gradually stabilised, returning to levels comparable to a comparison female cohort who had not recently been pregnant. However, there has been no longitudinal assessment of the relationship between weight change and steroid metabolism postpartum. Neither has the role of steroid metabolism in maternal postnatal depression been previously examined. Therefore, this exploratory study aimed to describe the maternal postpartum weight change trajectory and associated pattern of glucocorticoid metabolism in a sample of UK women across the first postnatal year; examine the concurrent relationships between maternal glucocorticoid metabolism and maternal symptoms of postnatal depression; and examine the relationships between weight change and maternal glucocorticoid metabolism in the postpartum year independent of confounds of breastfeeding, demographics and maternal depression. We hypothesised that greater weight loss would be associated with lower 11β-HSD-1 and 5α-reductase activity, increased 11β-HSD-2 activity and with a reduced total F metabolite production rate. We

hypothesised that higher depression scores would be associated with greater weight retention as well as lower 5α-reductase and 11β-HSD-2 activity. We also predicted that higher 11β-HSD-1 activity and total F metabolite production rate would be related to poorer mental health.

Method

Materials and Methods

The protocol received ethical approval from Birmingham East, North and Solihull Research Ethics Committee, UK (ref. 10/H1206/67). All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Birmingham Women's NHS Foundation Trust granted research and development approval (ref. 10/BWH/NO95). Women without complications during pregnancy/labour on low risk maternity units in Birmingham, UK were approached after labour and gave fully informed written consent. Mothers were visited at 1-week, 1-month, 3-, 6- and 12-months post-partum to collect urine samples and were weighed wearing light indoor clothing without shoes at 1-week, 1-, 6- and 12-months. Maternal height was measured at 1-week postpartum using a stadiometer. Maternal weight and height were converted into BMI (kg/m^2).

During the 1-week home visit, mothers reported their pre-pregnancy weight, pregnancy medical history and provided demographic information. At each time point, mothers gave a single urine sample and reported whether they were still breastfeeding. Mothers completed the Edinburgh Postnatal Depression Scale[28] (EPDS), a widely used and well-validated screening tool for postnatal depression, at

1, 6, and 12 months postpartum. Higher scores indicate greater symptoms and scores of 13 or above indicate that participants are probably suffering from clinical depression.

Urinary steroid metabolite analysis

Spot urine samples were analysed; absolute corticosteroid concentrations were corrected per g of urinary creatinine to assess absolute production rates. Previous work has demonstrated that spot urine samples yield measures of corticosteroid activity that are very highly correlated with measures from 24 hour samples (r=.959) and thus spot urine samples accurately reflect daily corticosteroid activity[29]. Gas chromatography / mass spectroscopy (GC/MS) was used to analyse urinary corticosteroid metabolites as described elsewhere[16,30]. Solid-phase extraction was used to extract free and conjugated steroids. Steroids were enzymatically released from conjugation and then re-extracted. Methyloxime-trimethylsilyethers (MO-TMS) were formed by adding internal standard (stigmasterol) prior to derivitization. The final derivative was dissolved in cyclohexane and transferred to a GC/MS autosampler vial. An Agilent 5973 instrument in selected-ion-monitoring (SIM) mode was used to run the samples.

The sum of total cortisol metabolites (THF (tetrahydrocortisol), THE (tetrahydrocortisone), 5α-THF, α-cortolone, cortisone (E), cortisol (F), β-cortolone, β-cortol, α-cortol) was used to measure cortisol secretion rate (total F metabolites). Here, the term total F metabolites means the total of measured metabolites, not the complete excretion of all metabolites. The ratio of tetrahydro-metabolites of cortisol (THF + 5αTHF) to those of cortisone (THE) was used to measure 11β-HSD-1

activity. The ratio of urinary cortisol (F) to cortisone (E) accurately reflects renal 11β-HSD-2 activity (with higher F/E ratios indicating decreased 11β-HSD-2 activity). The activity of 5α-reductase was inferred from measuring the ratio of 5αTHF/THF.

Participants

59 mothers (mean age 29±5years, mean BMI @ 1 week postpartum 26.9±3.9kg/m^2) of 36 male and 23 female infants, who had spontaneous normal vaginal deliveries without complications, provided informed written consent to participate and gave a series of spot urine samples in the postpartum year. None had any significant past medical history or were taking any regular medication. Mothers with gestational diabetes, preeclampsia or pregnancy induced hypertension were excluded. Infants born prior to 36-weeks gestation or who were small for gestational age were not eligible for participation. Two mothers were pregnant at the 12-month visit, so their data were removed from all analyses. Urine samples and sufficient data for calculation of BMI change was available for 49 of the women who gave consent to take part in the study.

Statistical approach

Statistical analysis was undertaken using IBM SPSS v22. Repeated Measures ANOVAs were used to examine changes across time in BMI, postnatal depression and cortisol metabolism. Observed power was reported. Posthoc pairwise comparisons identified the source of significant effects. Maternal BMI change scores (ΔBMI) at each stage were calculated. Positive ΔBMI implies reductions in BMI. For parsimony, total postpartum ΔBMI score was calculated from 1 week to 12-months postpartum and used in all analyses of relationships between weight change and

cortisol metabolism. Pearson's correlation coefficients between demographics, breastfeeding duration, symptoms of depression and maternal steroid metabolism determined the necessity to control for these covariates in subsequent analyses. Post hoc power estimates were calculated. Bonferroni corrections were not applied given their unnecessarily stringent effects because we did not want to increase the risk of type II error in this relatively small sample, given the exploratory nature of the work. Postnatal depression symptoms at 1, 6 and 12 months and overall ΔBMI across the postnatal year were then correlated with glucocorticoid metabolism at each data collection point, using one-tailed partial correlation analyses controlling for maternal age, education, income, smoking, alcohol consumption and total breastfeeding duration. Additionally, analysis of ΔBMI glucocorticoid metabolism relationships were adjusted for depression.

Results

Table 1 illustrates the descriptive and demographic information of participants providing weight and urine measures at each time point.

Table 1 about here

The sample were affluent, well educated, relatively healthy weight, predominantly White British, with high initial breastfeeding rates and low levels of pregnancy smoking.

BMI change across the postpartum year

Table 2 about here.

Table 2 illustrates the pattern of BMI and BMI change from pre-pregnancy to 1 year postpartum. BMI increased during pregnancy (3.2kg/m^2 gained from pre-pregnancy to 1 week postpartum). Women lost some of this weight across the postpartum year, with a mean BMI loss of 1.7kg/m^2 from 1 week to 12 months. However, overall, women retained an average of 1.5kg/m^2 at 12 months in comparison to their pre-pregnancy weight.

Repeated Measures ANOVA demonstrated that BMI reduced significantly across the postnatal year (Pillai's Trace $F(4,45)=28.99$, $p<.0001$, observed power =1). Posthoc pairwise comparisons showed significant postnatal reductions in BMI at all time points except between 6 and 12 months. All postnatal BMI measures were significantly higher than pre-pregnancy BMI ($p<.0001$). Measures at one week were greater than BMI at any other time point ($p<.0001$). BMI at one month was greater than BMI at 6 and 12 months ($p<.0001$). BMI at 6 and 12 months were not significantly different (see Figure 1a).

Maternal symptoms of postnatal depression across the postpartum year

Repeated Measures ANOVA demonstrated that there was no significant change in postnatal depression symptoms across the postnatal year although there was a trend towards fewer symptoms with time (Pillai's Trace $F(2,47)=2.71$, $p<.077$; observed power .51). Posthoc pairwise comparisons showed a significant decrease in postnatal depression symptoms between 1 month and 12 months only ($p<.023$) (see Figure 1b).

Maternal glucocorticoid metabolism across the postpartum year

Table 3 illustrates the pattern of cortisol metabolism from pre-pregnancy to 1 year postpartum. Steroid metabolite analysis from some of these individuals have been reported elsewhere in the context of infant growth and development across the first year of life[27]. Presented in Table 3 are the urinary steroid metabolite ratios reflective of specific enzyme activities for mothers where detailed pre and post partum weight measurements are available.

Repeated Measures ANOVAs demonstrated that there was no statistically significant change in 11ß-HSD-2 activity ($F(4,22)=2$, $p=.13$, observed power= .51) or Total F metabolites ($F(4,22)= 1.6$, $p=.21$, observed power =.41) across the year. There was a significant increase in both 5α-reductase activity ($F(4,22)= 44.5$, $p=.0001$; observed power =1.0) and 11β-HSD-1 activity ($F(4,22)= 7.1$, $p=.001$; observed power =.98). Post hoc pairwise comparisons showed a significant difference between 5α-reductase activity at 1 week and all other time points ($p<.0001$) and at 1 month and all other time points ($p<.0001$). Activity was higher at 3 months than at 1 week and 1 month and lower than at 6 and 12 months ($p<.05$). There were no significant differences between 6 and 12-month 5α-reductase activity. Differences in 11β-HSD-1 were less profound but post hoc comparisons showed a significant difference in 11β-HSD-1 activity between 1 week and 1 month, and between 1 week and 6 months ($p<.05$). 11β-HSD-1 activity at 1 month was also lower than that at 3 and 6 months ($p<.05$). 11β-HSD-1 at 3 months was lower than at 6 months ($p<.05$). Finally, 11β-HSD-1 activity at 6 months was significantly higher than at 12 months ($p<.05$) (see Figure 1 c-f).

Figure 1 about here

To examine which variables were required as covariates in subsequent analyses, one tailed Pearson's correlation coefficients between demographics, breastfeeding, symptoms of depression, postpartum ΔBMI and cortisol metabolism were calculated (Table 4).

Table 4 about here

Table 4 demonstrates that postpartum ΔBMI showed no correlation with postnatal depression and few significant correlations with demographics. Women who smoked less during pregnancy had greater postpartum ΔBMI. Symptoms of postnatal depression were not correlated with demographics or breastfeeding duration. Postnatal depression symptoms at 12 months were associated with greater total F metabolites at 12 months. Maternal age, income, education, smoking and alcohol consumption during pregnancy showed significant relationships with a number of indices of cortisol metabolism across the first year, and with 5α-reductase activity in particular. Longer breastfeeding was associated with lower activity of 11βHSD-2 and 5α-reductase at 1 month, and greater total F metabolites at 12 months. Before adjustment for covariates, women who showed greatest ΔBMI in the postpartum year had significantly lower 11β-HSD-1 activity at 12 months postpartum. Women with greater 11ß-HSD-2 activity at 1 week showed more weight loss in the postpartum year. Lower 5α-reductase activity at all time points was associated with greater postpartum ΔBMI. Lower total F metabolites at 6 months were associated with greater weight loss. Post hoc power estimates for these correlation analyses suggest the sample was sufficient to detect meaningful relationships, i.e. where effect sizes were moderate (.3) to large (.5). At n=40, power to detect a correlation of .3 was .62; to detect a correlation of .5, power was .97.

To examine the relationships between symptoms of postnatal depression with cortisol metabolism, and BMI change with cortisol metabolism, a series of partial correlations coefficients were calculated controlling for covariates (**Table 5**). Correlations between BMI change and cortisol metabolism were additionally adjusted for symptoms of depression at 12 months.

Adjusted Relationships between symptoms of postnatal depression and maternal glucocorticoid metabolism across the postpartum year

After adjusting for demographics and breastfeeding, the pattern of relationships between symptoms of postnatal depression and concurrent cortisol metabolism remained the same: women with poorer mental health at 12 months had greater concurrent total cortisol secretion rates. There were no other relationships between measures of depression and cortisol metabolism at any other time points.

Adjusted Relationships between ΔBMI and maternal glucocorticoid metabolism across the postpartum year

11ß-HSD activity

After adjusting for covariates, greater ΔBMI was associated with significantly lower 11β-HSD-1 activity at 1 week and 12 months postpartum. Women with greater 11ß-HSD-2 activity at 1 week showed more weight loss in the postpartum year.

5α-reductase activity

Adjusting for covariates removed the significance of 5α-reductase activity at one week and one month, and reduced the relationship between 6-month 5α-reductase activity and ΔBMI to a trend. Lower 5α-reductase activity at 3 months and 1 year remained significantly associated with greater postpartum ΔBMI.

Total F Metabolites

After adjusting for covariates, there were no significant associations between total F metabolites and ΔBMI.

Discussion

This study described maternal postpartum weight change, mental health and cortisol metabolism in a sample of healthy UK women across the postnatal year. Women experienced a mean BMI loss of 1.7kg/m^2 from 1 week to 12 months postpartum and retained +1.5kg/m^2 at 12 months postpartum in comparison to their pre-pregnancy weight with notably large variability in postpartum weight change. Postnatal depression levels were as expected, showing slight decline across the year. Levels of 11ß-HSD-2 and Total F metabolites did not show significant change, but levels of 11ß-HSD-1 and 5α-reductase significantly increased between 1 week and 6 months postpartum, consistent with our previous analyses of a larger sample of this cohort[24]. However, there were few relationships between weight change and depression, demographics or breastfeeding duration. Indices of cortisol metabolism showed significant relationships with postnatal depression symptoms and postpartum weight change, underlining the importance of disturbances in the HPA axis for the prediction of postnatal weight change and mental health. However, the relationship between weight change and cortisol metabolism was independent of any relationship with depression.

Over the post-partum year, we have identified changes in steroid hormone metabolism that are associated with maternal weight loss. Most notably, in line with our hypotheses, lower 11β-HSD-1 and 5α-reductase activity was associated with enhanced weight loss when adjusted for covariates including depression. The role of steroid hormone metabolism in the regulation of weight has been extensively studied in the context of simple obesity and type 2 diabetes[31], but has not previously been examined in maternal post-partum weight.

11β-HSD-1 generates active glucocorticoid, cortisol, in metabolic target tissues including adipose, liver and muscle[32]. Interestingly, all clinical studies that have used selective 11β-HSD-1 inhibitors have demonstrated reproducible weight loss[18,19,21] and in our cohort, lower activity (at one week and 12 months) was associated with enhanced weight loss, which may reflect decreased adipocyte differentiation and lipid accumulation as a consequence of decreased tissue specific cortisol availability[33]. Although 11βHSD-2 is not thought to be expressed at significant levels in adipose tissue, the global increase in activity that we observed may also contribute to decreased tissue glucocorticoid availability. Only 11ßHSD-2 activity at 1 week was associated with greater weight loss in the postnatal year, suggesting that urinary measures of this enzyme are not key determinants of postnatal weight loss nor key correlates of postnatal depression in healthy participants.

Lower 5α-reductase activity at 3 and 12 months postpartum was associated with increased weight loss. There are 2 isoforms of 5α-reductase (type 1 and type 2) that have differential tissue specific expression profiles and both contribute to steroid hormone metabolism.[34] Cross-sectional studies have demonstrated the association between increased 5α-reductase activity and adverse metabolic phenotype[16,17,35].

This extends to simple obesity, non-alcoholic fatty liver disease and the adverse metabolic phenotype associated with polycystic ovary syndrome. Weight loss is associated with a reduction of 5α-reductase activity[22]. 5α-reductase clears glucocorticoids to their inactive dihydro-metabolites (subsequently converted to tetrahydro-metabolites by the action of 3α-hydroxysteroid dehydrogenase) and therefore limiting glucocorticoid availability to bind and activate the glucocorticoid receptor. In addition, they have a role in activating androgens, converting testosterone to the more potent androgen dihydrotestosterone. Lowering activity has the potential to decrease androgen availability whilst simultaneously reducing glucocorticoid clearance, and both androgens and glucocorticoids regulate fat mass. What remains unclear is whether changes in 5α-reductase reflect cause or consequence of changes in postpartum BMI; the associations that we have described cannot infer causality. Rodent models with genetic deletion of 5α-reductase type 1 have an adverse metabolic phenotype, largely confined to the liver without alteration in fat mass[36]. Clinical studies in patients have shown that non-selective 5α-reductase inhibition (type 1 and 2), but not exclusive type 2 inhibition, is associated with systemic and hepatic insulin resistance and with hepatic lipid accumulation[37]. Taken together, it seems likely that the changes that we have observed in 5α-reductase activity reflect a compensatory response such that with weight gain, glucocorticoid availability to bind and activate its receptor is limited. This may occur in response to the increased adrenal glucocorticoid output that is seen with increased weight [38]. As weight falls, adrenal glucocorticoid output falls and as a result there is no longer the need to repress local glucocorticoid availability and in parallel therefore, 5α-reductase activity decreases. Therefore, patients with the largest weight loss display greatest reduction in 5α-reductase activity.

Total glucocorticoid production was significantly correlated with depression symptoms at 12 months, but there were no relationships between postnatal depression at any other time point or any other measures of cortisol metabolism. However, 6% of the women in the sample had EPDS scores indicating probable major depression at 12 months and mean EPDS score was 5, comparable to other relatively mentally healthy UK postnatal samples[39]. Despite this, women with more depression symptoms at 12 months had greater total cortisol metabolite production, previously associated with greater BMI and abnormal glucose tolerance[17]. The causal nature and long term implications of this relationship is impossible to determine from our data, but are consistent with the suggestion that women with greater, and/or more persistent, symptoms of postnatal depression may be at greater risk for the development of metabolic disorders.

The clinical implications of these findings are limited at this stage given the healthy nature of our sample. However, for women with a problematic postnatal weight loss trajectory, selective 11β-HSD-1 inhibitors could be investigated as an adjunct to diet and lifestyle intervention to aid weight loss. Furthermore, examination of total glucocorticoid production using spot urine samples has the potential to offer a more personalised approach to the evaluation of future metabolic and mental health risk.

In general, our findings regarding links between maternal postnatal mental health, weight retention and cortisol metabolism in this healthy sample show similarities to the patterns found in severely obese women, when serum cortisol levels were measured[13]; mental health was related to cortisol level, cortisol level was related to postnatal weight retention but cortisol level did not mediate the relationship between weight and mental health. Together, these studies provide evidence to support that weight and mental health are both associated with dysregulation of glucocorticoid

secretion and metabolism, but that there may be different pathways to depression and obesity. There are other important contributors to/moderators of the development of each phenotype that were not examined in this study, such as early biological programming, gene expression or experience of stress. Further work is required to better understand these pathways and their contributors to both physical and psychological postpartum morbidity.

There are a number of limitations of this study. This was a relatively healthy weight sample with low levels of depression and our conclusions cannot be extrapolated to obese or depressed populations. The absence of expected relationships, such as that between weight change and depression, may be a result of this limitation. Furthermore, the sample was predominantly White, well educated, affluent, with high levels of breastfeeding. There are likely to be important demographic patterns in the relationships between weight retention, depression and cortisol metabolism that this study could not capture. In addition, we did not collect data on stressors such as sleep deprivation, which may have an impact on both mental health and cortisol metabolism[40]. Finally, we did not correct for multiple testing to reduce the risk of a type II error in this small exploratory study, which naturally increases risk of a type I error. Therefore these findings, whilst important for future hypothesis generation, require replication.

In summary, this study significantly expands our understanding of the relationships between cortisol metabolism, mental health and weight change postpartum. Lower activity of 11β-HSD-1 and 5α-reductase was associated with greater postpartum weight loss in healthy women, independent of demographics and depression. More depressed women also demonstrated higher cortisol secretion rates, independent of demographics and breastfeeding, but depression was unrelated to BMI change. In

healthy samples, cortisol metabolism is related to both BMI and symptoms of depression, but weight and depressed mood have independent pathways that link them to HPA activity.

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Table 1. Demographics (%), concurrent BMI measures and symptoms of depression (Mean, SD) at each time point for the whole sample (N=49; column 2) and for all mothers providing urine samples at each time point (columns 3-7).

	Whole sample (n=49)	1 week (n=40)	1 month (n=46)	3 months (n=42)	6 months (n=45)	12 months (n=39)
Maternal age (years) Mean (SD)	30.8 (5.5)	31.4 (5.0)	30.9 (5.6)	30.9 (5.4)	31.0 (5.3)	30.9 (5.6)
Maternal concurrent BMI (kg/m^2) Mean (SD)	27.0 (3.7) @1 wk 26.5 (3.6) @1 mo 25.7 (3.8) @6 mo 25.3 (3.7) @12 mo	26.7 (3.7)	26.2 (3.6)	-	25.6 (3.9)	25.3 (3.9)
EPDS score Mean (SD)	6.3 (4.6) @1 mo 5.7 (4.5) @6 mo 4.9 (4.4) @12 mo	-	6.1 (4.6)	-	5.4 (4.4)	4.9 (4.7)
Percent (n) depressed (EPDS ≥13)	8% (4) @1 mo 4% (2) @6 mo 6% (3) @12 mo	-	8.7% (4)	-	4.4% (2)	7.7% (3)
Percent (n) some breastfeeding	82% (40) @1 wk 71% (35) @1 mo 63% (31) @3 mo 59% (29) @6 mo 33% (16) @12 mo	85% (34)	70% (32)	69% (29)	62% (28)	33% (13)
Percent (n) Infant gender	55% (27) male	65% (26) male	58.7% (27) male	54.8% (23) male	57.8% (26) male	56.4% (22) male
Percent (n) Degree educated	41% (20)	45% (18)	43.5% (20)	45% (19)	42.2% (19)	41% (16)
Percent (n) In highest	65% (32)	77.5% (31)	67.4% (31)	69% (29)	68.9% (31)	66.7% (26)

income bracket						
Percent (n) Smoked in pregnancy	10% (5)	7.5% (3)	6.5% (3)	9.5% (4)	8.9% (4)	10% (4)
Percent (n) Consumed alcohol in pregnancy	43% (21)	50% (20)	43.5% (20)	45.2% (19)	44.4% (20)	43.6% (17)
Percent (n) Primary ethnic groups	57% (28) White British 12% (6) White Other 12% (6) Asian Pakistani	57.5% (23) White British 12.5% (5) White Other 10% (4) Asian Pakistani	58.7% (27) White British 13% (6) White Other 10.9% (5) Asian Pakistani	54.8% (23) White British 14.3% (6) White Other 9.5% (4) Asian Pakistani	57.8% (26) White British 13.3% (6) White Other 8.9% (4) Asian Pakistani	61.5% (24) White British 15.4% (6) White Other 7.7% Asian Pakistani (3) 7.7% (3) Black Caribbean

N sizes for each time point vary because some mothers did not produce urine samples for all visits. Percentages in columns 3-7 are expressed as a function of the number of women providing urine samples at each time point.

Table 2. BMI and ΔBMI from pre-pregnancy to 1 year postpartum (N=49). BMI expressed as (kg/m^2)

	Minimum	Maximum	Mean	Std. Deviation
Pre-pregnancy BMI	18.8	31.9	23.8	3.1
1-week maternal BMI	19.2	35.7	27.0	3.7
1 month maternal BMI	18.8	35.9	26.5	3.6
6 month maternal BMI	19.5	35.2	25.7	3.8
12 month maternal BMI	18.9	36.4	25.3	3.8
ΔBMI pre-pregnancy to 1 week postpartum	-8.1	.7	-3.2	2.3
ΔBMI 1 week postpartum to 1 month postpartum	-.4	3.3	.5	.7
ΔBMI 1 month postpartum to 6 months postpartum	-2.5	4.7	.8	1.6
ΔBMI 6 months postpartum to 12 months postpartum	-5.4	3.1	.4	1.5
ΔBMI pre-pregnancy to 12 months postpartum	-7.6	3.0	-1.5	2.4
ΔBMI 1 week postpartum to 12 months postpartum	-4.6	7.8	1.7	2.3

ΔBMI = change in BMI (kg/m^2). Positive ΔBMI is indicative of weight loss.

Table 3. Urinary steroid metabolite ratios as measured by gas chromatography / mass spectrometry across the first post-partum year.

Steroid ratio	Time point	N	Min.	Max.	Mean	SD
THF+5αTHF/ THE	1-week	40	0.45	1.31	0.86	0.2
	1 month	46	0.48	1.21	0.83	0.18
(11β-HSD-1 activity;	3 months	42	0.51	1.55	0.89	0.24
conversion of cortisone to	6 months	45	0.56	1.47	0.99	0.21
cortisol)	12 months	39	0.5	1.3	0.84	0.20
F/E	1-week	40	0.39	1.69	0.8	0.26
	1 month	46	0.33	2.19	0.67	0.29
(11-βHSD-2 activity;	3 months	42	0.36	1.39	0.65	0.23
conversion of cortisol to	6 months	45	0.36	1.93	0.65	0.25
cortisone)	12 months	39	0.29	1.33	0.63	0.24
5αTHF/THF	1-week	40	0.08	0.46	0.2	0.09
	1 month	46	0.11	1.32	0.47	0.29
(5α-reductase activity ;	3 months	42	0.24	2.32	0.85	0.43
clearance of cortisol to	6 months	45	0.18	3.17	1.00	0.59
tetrahydrometabolites)	12 months	39	0.35	2.5	0.99	0.43
Total F Metabolites	1-week	40	1123.0	17489.0	6346.6	4277.7
	1 month	46	728.0	19811.0	7784.9	5091.3
(μg/g urinary creatinine;	3 months	42	1515.0	31204.0	7472.8	6496.3
total cortisol metabolite	6 months	45	1023.0	27826.0	9849.1	8278.4
production)	12 months	39	1374.0	28322.0	8990.8	6942.1

THF =tetrahydrocortisol, THE =tetrahydrocortisone, E=cortisone, F=cortisol

Table 4. Unadjusted one tailed Pearson's correlation coefficients between demographics, pregnancy smoking, pregnancy alcohol consumption, breastfeeding duration, with ΔBMI and cortisol metabolism.

	Time	ΔBMI	Concurrent EPDS	Maternal age	Income	Education	Smoking	Alcohol	Total Breast - feeding Duration
ΔBMI	1 week-12 months	-	-	.17	.18	-.01	-.32*	.10	.03
Depression (EPDS)	1month	-.13	-	-.01	.05	-.05	.15	.01	.11
	6 months	-.02	-	.05	.08	-.01	.04	.07	.11
	12 months	-.05	-	-.03	.10	-.07	.06	.13	.02
11β-HSD-1	1 week	-.23t	-	.04	.17	.25	-.24	.13	-.11
THF+5aTHF/ THE	1 month	-.16	-.15	.22	-.25*	.16	-.22	-.01	.15
	3 months	-.21t	-	.09	-.03	.06	.29*	-.14	-.02
	6 months	.06	.02	-.03	.02	.06	-.03	.02	-.18
	12 months	-.32*	-.16	-.01	-.10	-.10	.18	-.24	-.06
11β-HSD-2†	1 week	-.35*	-	-.24	-.22	-.38**	.10	-.12	-.08
F/E	1 month	.04	-.02	.19	.09	.02	-.03	.30*	.28*
	3 months	-.06	-	.04	.02	-.13	.27*	-.22	.25
	6 months	.03	.01	-.20	-.03	.07	.05	.17	-.04
	12 months	.09	.09	-.20	-.11	-.30*	.18	.01	-.04
5α-reductase	1 week	-.29*	-	-.28*	-.41**	-.18	.58***	-.23	-.06
5aTHF/THF	1 month	-.31*	.12	-.28*	-.45**	-.29*	.27*	-.18	-.28*
	3 months	-.45**	-	-.38**	-.29*	-.24	.50***	-.21	-.22
	6 months	-.40**	.08	-.29*	-.20	-.17	.54***	-.29*	-.14
	12 months	-.39**	.00	-.19	-.28*	-.29*	.28*	-.26	.13
Total F Metabolites	1 week	-.03	-	.02	-.05	-.19	.25	-.23	.12
	1 month	-.14	-.13	-.06	-.02	.03	.17	.01	-.19
	3 months	-.07	-	-.20	-.24	-.06	-.12	-.08	-.04
	6 months	-.26*	-.01	-.26*	-.23	-.13	.45**	-.25*	.09
	12 months	.03	.43**	-.06	-.18	-.19	.33*	-.12	.43**

**p<.05, **p<.01, ***p<.001* † greater F/E ratios indicate lower 11βHSD-2 activity.

THF =tetrahydrocortisol, THE =tetrahydrocortisone, E=cortisone, F=cortisol

Table 5: 1 Tailed partial correlation coefficients between cortisol metabolism and maternal symptoms of depression at 1, 6 and 12 months and ΔBMI.

	Time	EPDS§	ΔBMI 1 week to 12 months§
11β-HSD-1	1 week	-	-.39*
	1 month	-.10	-.20
conversion of cortisone to cortisol; THF+5aTHF/THE	3 months	-	-.17
	6 months	.05	.05
	12 months	-.14	-.30*
11β-HSD-2†	1 week	-	-.36*
	1 month	-.09	.01
conversion of cortisol to cortisone; F/E	3 months	-	-.05
	6 months	.01	.07
	12 months	.03	.15
5α-reductase	1 week	-	-.01
	1 month	.17	-.18
clearance of cortisol to tetrahydrometabolites; 5aTHF/THF	3 months	-	-.33*
	6 months	.13	-.25t
	12 months	-.01	-.33*
Total F Metabolites	1 week	-	.07
	1 month	-.16	-.08
Total cortisol secretion rate; µg/g urinary creatinine	3 months	-	.10
	6 months	-.01	-.01
	12 months	.50**	.28t

t=trend: p<.1; *p<.05; **p<.01 † greater F/E ratios indicate lower 11βHSD-2 activity. THF =tetrahydrocortisol, THE =tetrahydrocortisone, E=cortisone, F=cortisol.

§ Analyses adjusted for demographics and breastfeeding duration. ΔBMI and cortisol metabolism analyses adjusted for demographics, breastfeeding duration and symptoms of postnatal depression at 12 months.