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RESEARCH ARTICLE

Prospective Investigation of Glutamate Levels and Percentage Gray Matter in the Medial Prefrontal Cortex in Females at Risk for Postpartum Depression

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Abstract: ***Background*:** The substantial female hormone fluctuations associated with pregnancy and postpartum have been linked to a greater risk of developing depressive symptoms, particularly in high-risk women (HRW), i.e. those with histories of mood sensitivity to female hormone fluctuations. We have shown that glutamate (Glu) levels in the medial prefrontal cortex (MPFC) decrease during perimenopause, a period of increased risk of developing a major depressive episode. Our team has also demonstrated that percentage gray matter (%GM), another neural correlate of maternal brain health, decreases in the MPFC during pregnancy.

***Objective*:** To investigate MPFC Glu levels and %GM from late pregnancy up to 7 weeks postpartum in HRW and healthy pregnant women (HPW).

***Methods*:** Single-voxel spectra were acquired from the MPFC of 41 HPW and 22 HRW using 3-Tesla *in vivo* proton magnetic resonance spectroscopy at five different time points.

***Results*:** We observed a statistically significant interaction between time and group for the metabolite Glu, with Glu levels being lower for HRW during pregnancy and early postpartum ($p<0.05$). MPFC %GM was initially lower during pregnancy and then significantly increased over time in both groups ($p<0.01$).

***Conclusion*:** This investigation suggests that the vulnerability towards PPD is associated with unique fluctuations of MPFC Glu levels during pregnancy and early postpartum period. Our results also suggest that the decline in MPFC %GM associated with pregnancy seems to progressively recover over time. Further investigations are needed to determine the specific role that female hormones play on the physiological changes in %GM during pregnancy and postpartum.

Keywords: Glutamate, pregnancy, postpartum, depression, magnetic resonance spectroscopy, gray matter.

1. INTRODUCTION

The time following childbirth is a time of increased risk for depression, with 10–20% of women experiencing an episode of postpartum depression (PPD). This risk increases up to 65% in women with a history of PPD [1]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), PPD is defined as an episode of major depression (MD) occurring during pregnancy or within the first four weeks following childbirth [2]. However, expert consensus based on recent epidemiological data suggests that this time frame should be expanded to 3 months postpartum [3]. PPD has been divided into early versus late onset, with early-onset PPD occurring within the first 4 weeks following childbirth and late-onset PPD occurring after 5 weeks postpartum [4]. Risk factors for early-onset PPD differ from those of late-onset PPD [5]. Women at high risk of developing early PPD (HRW) are those with a history of major depression (MD), premenstrual dysphoric disorder (PMDD), prior PPD, or any other form of mood sensitivity to female hormone fluctuations [1, 6]. A substantial percentage of women diagnosed with PPD also have an onset of symptoms during pregnancy [7, 8].

Glutamate (Glu) is the major excitatory neurotransmitter in the central nervous system [8]. Dysregulation of the glutamatergic system has been implicated in the pathophysiology of MD [9, 10]. Ketamine, which is a glutamatergic modulator, has been shown to have a rapid-onset antidepressant effect [11-13].

Generally, meta-analyses of MRS investigations have reported that MD patients display decreased levels of Glu or glutamix (Glx), a combined signal of Glu and glutamine (Gln), in the anterior regions of the brains, such as the prefrontal cortex and the anterior cingulate cortex (ACC) [14-

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17]. There are, however, conflicting data. For example, Kantrowitz and colleagues reported elevated Glu and Glx referenced to NAA in the ventromedial prefrontal cortex/ACC of un-medicated MD patients scanned with a 3T magnet [18]. Godlewska's team, on the other hand, performed a well-controlled investigation at high field strength (7T) and found no differences in Glu and Gln levels referenced to water in the ACC of MD patients [19]. 7T MRS investigation provides a clear spectral resolution of Glu and Gln, allowing for their individual measurements [20]. However, our research team has been able to measure Glu levels with minimal contamination of Gln by using a specific technique at 3T [21, 22]. MRS investigations of Glu, Gln and Glx levels in other brain regions of MD patients have also displayed heterogeneous results. For example, Sanacora and colleagues reported increased Glu levels in the occipital cortex of MD patients [23], whereas Truong's team found no differences in occipital Glx concentrations between MD patients and matched controls [24]. Besides magnet strength, MRS technique, the choice of the normalization reference, and the brain region of interest, other factors can impact the direction of the changes in Glx, Gln or Glu levels in MD. In their review of MRS investigations of Glu/Glx in MD patients, Moriguchi and colleagues have identified treatment with antidepressants as a confounding factor [17]. Clinical heterogeneity in terms of psychiatric and physical comorbidities in MD patients may also impact the direction of the alterations of reported Glu or Glx levels [19, 25, 26]. In addition, the duration of illness can influence Glu levels [27]. Thus, while MD is generally associated with decreased levels of Glu or Glx in the prefrontal cortex, contrasting findings have been reported.

It is suspected that hormonal changes have a role in the development of MD during pregnancy and PPD. Levels of estrogen, progesterone, and associated neuroactive steroids (NASs) rise during pregnancy, reaching an apex during the third trimester with a decline following parturition [28-31]. The menstrual cycle exhibits similar fluctuations in female hormones but on a smaller magnitude in contrast to pregnancy and postpartum. Published research from our group has demonstrated a decrease in MPFC Glu levels from the follicular phase to the luteal phase of the menstrual cycle in relation to hormonal fluctuations [32], suggesting that fluctuations of female hormones and their associated NAS impact MPFC Glu levels. The heightened risk of developing a major depressive episode during perimenopause has been linked to the general dynamic decrease in female hormones [33]. Interestingly, we have demonstrated that MPFC Glu levels are decreased during perimenopause [34].

Gray Matter (GM) is another neural correlate of maternal brain health that has been shown to be impacted by gestation. More specifically, our research group showed that near-term pregnant women exhibited a decreased MPFC %GM compared to non-pregnant healthy controls [22], perhaps explaining the subjective cognitive deficits women experience during pregnancy, a phenomenon coined "pregnesia" [35].

The objective of the current study was to evaluate Glu levels and %GM in the MPFC of HRW and healthy pregnant women (HPW) from pregnancy up to 7 weeks postpartum. The purpose is to elucidate the structural and neurochemical changes associated with pregnancy and the early postpartum period, and how these make certain individuals more predisposed to developing PPD.

2. METHODS

2.1. Subjects

41 healthy pregnant women without any current or past psychiatric history (HPW) and 22 healthy high-risk women with a past history of MD, PMDD, or PPD (HRW) were recruited from advertisements and health institutions in Edmonton, Canada. Each woman was recruited according to the guidelines of the Health Research Ethics Board of the University of Alberta. After a description of the study, written informed consent was obtained.

All participants were administered the Structured Clinical Interview for DSM-IV-TR of Axis I disorders [36] to assess any current or lifetime Axis I psychiatric disorders. Participants meeting criteria for current psychiatric disorders were excluded from participation in the study. Participants had neither used any recreational drugs in the previous six months or during the study, nor used hormonal treatment. Participating women were not taking any medications, psychotropic drugs or herbal products with psychotropic activity three months prior to entering the study or at any time during the study, including those with previous histories of PPD. Other confounding factors, such as brain injury or classical contraindications to MRS and any medical conditions that could interfere with the study, including endocrine or neurological disorders, were exclusion factors. The Edinburgh Postpartum Depression Scale (EPDS) was administered to all participants at all visits. Developing PPD was an exclusion criteria. Follow-up continued for at least 13 weeks postpartum to ensure that none of the participants developed late-onset PPD. None of the participants displayed clinically significant depressive symptoms during the investigation.

2.2. ^{1}H-MRS Sessions

The ^{1}H-MRS sessions were scheduled 2–3 weeks prior to delivery, 10 days postpartum, 3 weeks postpartum, 5 weeks postpartum and 7 weeks postpartum.

^{1}H-MRS was performed at the Peter S. Allen MR Research Centre, University of Alberta Hospital, Edmonton, Canada, using a 3T magnet (Magnex Scientific, Concord, California) equipped with a spectrometer (Surrey Medical Imaging System, Surrey, United Kingdom) and a quadrature birdcage resonator. A 2x3x3 cm^3 voxel (for segmentation and spectroscopy) was positioned perpendicular to and centered on the midline. Shimming to ~0.05 p.p.m. was accomplished by using both FASTMAP [37] and an in-house autoshim routine. The optimal in vivo Glu and Gln contrast to background [21], determined using numerical simulation, used an echo time (TE) equal to 240 ms, mixing time (TM) equal to 27 ms, and repetition time (TR) equal to 3 s [38]. The long TE time resulted in minimal macromolecule contamination due to the short T_2 relaxation time [39]. Spectra were the sum of 512 averages, acquired in 16 blocks of 32 averages. This warranted each of the 16 sub-spectra to be analyzed for spectral artifacts because of subject movement or hardware fluctuations before their final summing [40]. The *in vivo* data were analyzed using the LCModel (version 6.0-1) analysis program [41]. The metabolite basis spectra

used in the LCModel analysis were derived by numerical simulation and included N-acetylaspartate (NAA), creatine plus phosphocreatine (t-Cr), myo-inositol, N-acetylaspartylglutamate, taurine, lactate, aspartate, glycine, alanine, gamma-aminobutyric acid, glycerophosphorylcholine plus phosphorylcholine (t-Cho), and Glu. We only report metabolite measures for Glu, NAA, t-Cr, and t-Cho in the MPFC, which were within the reliable threshold for the % standard deviation of the fit (< 15%). The segmentation data were used to scale the water data, used for quantification of established differences in the water content of gray matter (GM) and white matter (WM). In addition, these data allowed us to eliminate the cerebrospinal fluid (CSF) water volume that contributes to the total water signal, so that the quantified metabolite concentrations relate to the tissue space of the GM and WM. The water peak area was utilized as the denominator in concentration calculations after removing the non-brain signal contribution from CSF. A representative spectrum used for analysis is shown in Fig. (**1**).

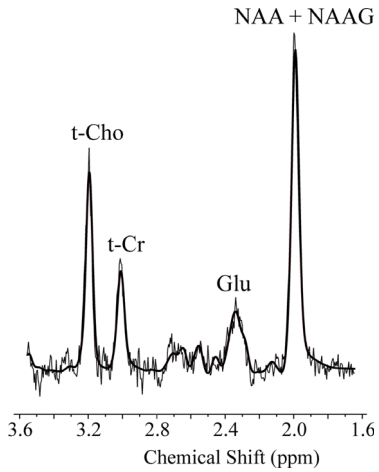

Fig. (1). Sample STEAM localized MRS data acquired from the medial prefrontal cortex and with sequence timings optimized for recovering signal from glutamate (STEAM TE, TM = 240, 27 ms). The spectra illustrate the unfiltered data superimposed with the LCModel fit.

A full description of the methods used to obtain ^{1}H-MRS data in this study can be found in our previous work [21, 22].

2.3. Statistical Analysis

Statistical analyses were performed using SPSS version 19.0. A p-value of less than 0.05 was considered for statistical significance. All statistical testing was performed with two-tailed tests.

Linear mixed (LM) modeling was used to examine the pattern of recovery for metabolites and tissue composition scores over the five time points (2–3 weeks prior to delivery, 10 days postpartum, 21 days postpartum (3 weeks), 35 days postpartum (5 weeks) and 49 days postpartum (7weeks)) because non-linear equations provided the best fit for predicting metabolites and tissue composition scores over the 7 weeks. Another feature of the LM modeling is that it enables us to perform statistical analysis without needing a continuous dataset (*i.e.*, all participants needed to complete their five scheduled successive scans) in comparison to repeated measures in ANOVA analysis. The LM models include parameters that estimate either metabolites or tissue compositions at pregnancy and the rate of change during the pregnancy. The square of time is also included as an estimate of change in the rate for some models because of a quadratic relationship over time for Glu and GM scores. The model had two levels which consisted of one level for the within-individual change over time and the other for between-individual differences in change over time.

In the multivariate models, variables were selected using both forward selection and backward elimination procedures. Forward selection starts with a simple model, then considers all of the reasonable one-step-more-complicated models, and chooses the one with the smallest p-value for the new parameter. This continues until no additional parameters have a significant p-value. Backward selection starts with a complicated model and removes the term with the largest p-value, as long as that p-value is larger than 0.05. All the variables in the base model with $p<0.2$ were included in the forward selection and backward elimination models and only those variables with $p<0.05$ were kept in the final model. Interaction between time and group was included based on $p<0.1$.

3. RESULTS

The number of data points included at each sample time point were as follows: for HPW, 2–3 weeks prior to delivery (21), 10 days postpartum (19), 3 weeks postpartum (31), 5 weeks postpartum (27), and 7 weeks postpartum (16); for HRW, 2–3 weeks prior to delivery (13), 10 days postpartum (8), 3 weeks postpartum (16), 5 weeks postpartum (12), and 7 weeks postpartum (13). Missing data points were due to: 1) women not willing to undergo the initial pregnancy MRS visit, 2) women unable to attend for personal reasons, and 3) poor data quality, which results from movement during the MRS scan. Mean values and standard deviations for metabolites, tissue compositions and EPDS scores are presented in Table **1**.

There was no statistically significant age difference found between HPW and HRW (29.34±4.73 and 30.92±4.31; $p=0.19$, $t=1.33$, $df= 63$). There was a significant interaction between group and time for MPFC Glu levels (Table **2**). The significant interaction indicates that the pattern of change over time is different between the HPW group and the HRW group with MPFC Glu levels being lower during pregnancy and the very early postpartum in the HRW group, returning to HPW levels as the postpartum progresses (Fig. **2a**). Sub-analysis revealed no significant changes in Glu overtime for the HPW group, whereas there was a significant increase in Glu levels over time for the HRW group (Table **2**). This interaction remained statistically significant after correcting for tissue composition changes in %GM (*i.e.*, treating %GM as a covariate) (Table **2**) as %GM was shown to significantly increase over time for both the groups (Table **2** and Fig. **2b**).

Of interest, t-Cho significantly changed over time (Table **2**) with a progressive increase observed for both groups from the pregnancy time point up to 7 weeks postpartum, with no significant differences observed between the groups (Fig. **2c**). There were no significant differences observed in other water quantified brain metabolites (NAA, t-Cr) (Table **2**) or %WM and %CSF (Table **2**).

Table 1. Water-quantified metabolite concentrations and tissue compositions in the MPFC of HPW and HRW groups.

HPW	-	-	-	-	-
	Pregnancy	**10 Days PP**	**3 Weeks PP**	**5 Weeks PP**	**7 Weeks PP**
Metabolites	-	-	-	-	-
Glu	6.74±1.39	6.93±1.74	7.03±1.62	6.51±1.20	6.60±2.00
NAA	8.99±1.44	9.66±1.53	9.00±1.62	8.60±1.25	9.12±1.25
t-Cr	8.65±3.42	9.54±2.91	10.41±3.18	9.60±2.54	9.47±2.99
t-Cho	1.37±0.24	1.79±0.36	1.77±0.36	1.72±0.35	1.89±0.35
Tissue Composition		-	-	-	-
%GM	46.53±10.66	51.59±7.61	51.21±8.59	52.30±9.27	49.34±11.35
%WM	30.94±10.14	26.48±6.99	30.73±6.59	28.89±7.16	31.41±8.17
%CSF	22.54±10.24	21.91±6.88	17.42±6.39	18.19±8.02	18.08±8.81
EPDS Score	2.76±2.59	5.47±3.79	3.84±3.41	3.52±4.39	3.81±3.23
HRW	-	-	-	-	-
	Pregnancy	**10 Days PP**	**3 Weeks PP**	**5 Weeks PP**	**7 Weeks PP**
Metabolites	-	-	-	-	-
Glu	5.56±1.86	6.54±1.79	5.96±0.91	6.88±1.89	6.78±1.48
NAA	9.18±0.99	9.05±1.28	8.69±1.08	8.59±1.46	8.96±1.98
t-Cr	9.27±2.50	10.19±2.99	9.00±1.84	9.31±2.86	9.97±3.31
t-Cho	1.43±0.37	1.46±0.36	1.71±0.28	1.78±0.33	1.82±0.77
Tissue Composition		-	-	-	-
%GM	47.32±7.81	48.30±7.19	54.19±7.97	55.03±13.22	54.89±9.06
%WM	29.88±9.07	26.24±8.30	27.28±6.05	26.23±11.53	28.43±5.29
%CSF	20.47±10.73	25.45±8.72	18.53±9.17	18.76±6.97	16.68±8.10
EPDS Score	4.62±4.84	6.00±3.02	6.50±4.23	5.42±4.60	4.00±3.13

Abbreviations: EPDS, Edinburgh postnatal depression scale; Glu, glutamate; HPW, healthy pregnant women; HRW, high risk women; NAA, N-acetyl-aspartate; PP, postpartum; t-Cho, glycerophosphorylcholine plus phosphorylcholine; t-Cr, creatine plus phosphocreatine; %CSF, % cerebrospinal fluid; %GM, % gray matter; %WM, % white matter.
All values are reported as means ± standard deviation.
Brain metabolites are measured in institutional units.

There were no statistically significant differences in EPDS scores between HPW and HRW groups (Table **2**). The Pearson correlation coefficient was used to assess the association between depressive symptoms (based on scores from the EPDS) and Glu in HPW and HRW at each time point. There were no statistically significant correlations observed between Glu and scores on the EPDS in either group at any of the time points.

4. DISCUSSION

The results of this ^{1}H-MRS investigation suggest that, after adjustment for % in GM, MPFC Glu levels are decreased during pregnancy and the early postpartum period in women at a high risk for PPD, but not in HPW.

The result obtained in HPW is consistent with our previous report of no differences in MPFC Glu levels, after correction for %GM, between healthy pregnant women and healthy controls during the follicular phase of the menstrual cycle [22].

The decrease in MPFC Glu levels in HRW was observed during pregnancy at 10 days and 3 weeks PP but returned to the MPFC Glu levels of HPW at 5 weeks PP (Fig. **2a**). Early-onset PPD (before 5 weeks) has been linked to biological risk factors *versus* late-onset PPD, which has been associated with psychosocial risk factors [4, 5]. This suggests that lower MPFC Glu levels may be a biological contributor to the increased risk of early-onset PPD in biologically vulnerable women (HRW). The 5 weeks PP mark also coincides with the return to normal concentrations of female hormones and associated NASs [29, 42]. Thus, it is reasonable to conjecture that the decrease in MPFC Glu levels may be associated with the early physiological changes in female hormones and NASs in HRW, a significant percentage of them having a history of mood sensitivity to female hormones.

Table 2. Description of trajectories from linear mixed models fitted for water-quantified metabolites: Glu, NAA, t-Cr, t-Cho and tissue compositions: %GM, %WM and %CSF outcomes.

Factor	Coefficient	95% CI	p-value
Outcome: Glu	-	-	-
Intercept	6.833	6.325, 7.340	<0.001
Group (HRW *vs*. HPW)	-1.178	-2.031, -0.326	0.007
Time (in Days)	-0.006	-0.022, 0.010	0.469
Interaction: Time by Group	0.031	0.005, 0.058	0.020
Sub-Analysis[†] within HRW	-	-	-
Intercept	5.679	4.994, 6.365	<0.001
Time (in Days)	0.025	0.003, 0.047	0.025
Sub-Analysis[†] within HPW	-	-	-
Intercept	6.816	6.305, 7.327	<0.001
Time (in Days)	-0.006	-0.022, 0.010	0.468
Outcome: Glu adjusting for %GM	-	-	-
Intercept	6.583	5.226, 7.940	<0.001
Group (HRW *vs*. HPW)	-1.173	-2.026, -0.321	0.007
Time (in Days)	-0.006	-0.022, 0.010	0.459
Interaction: Time by Group	0.031	0.004, 0.057	0.023
Outcome: NAA	-	-	-
Intercept	9.170	8.731, 9.608	<0.001
Group (HRW *vs*. HPW)	-0.221	-0.830, 0.388	0.471
Time (in Days)	-0.006	-0.018, 0.005	0.263
Outcome: t-Cr	-	-	-
Intercept	9.645	8.764, 10.525	<0.001
Group (HRW *vs*. HPW)	-0.280	-1.526, 0.966	0.655
Time (in Days)	0.008	-0.013, 0.030	0.449
Outcome: t-Cho	-	-	-
Intercept	1.546	1.428, 1.663	<0.001
Group (HRW *vs*. HPW)	-0.061	-0.220, 0.098	0.446
Time (in Days)	0.007	-0.004, 0.010	<0.001
Outcome: %GM	-	-	-
Intercept	47.594	44.419, 50.768	<0.001
Group (HRW *vs*. HPW)	2.038	-1.632, 5.708	0.271
Time (in Days)	0.338	0.091, 0.586	0.008
Time-Square	-0.005	-0.010, -0.001	0.028

(Table 2) contd….

Factor	Coefficient	95% CI	p-value
Outcome: %WM	-	-	-
Intercept	29.629	27.404, 31.854	<0.001
Group (HRW *vs*. HPW)	-1.854	-4.553, 0.845	0.174
Time (in Days)	0.0003	-0.069, 0.070	0.993
Outcome: %CSF	-	-	-
Intercept	19.163	16.518, 21.809	<0.001
Group (HRW *vs*. HPW)	-0.451	-4.504, 3.602	0.825
Time (in Days)	-0.030	-0.081, 0.021	0.251
Outcome: EPDS Score	-	-	-
Intercept	4.135	2.970, 10.752	<0.001
Group (HRW *vs*. HPW)	1.580	-0.078, 3.238	0.061
Time (in Days)	-0.015	-0.044, 0.013	0.285

Abbreviations: CI, confidence interval; EPDS, Edinburgh postnatal depression scale; Glu, glutamate; HPW, healthy pregnant women; HRW, high risk women; NAA, N-acetyl-aspartate; t-Cho, glycerophosphorylcholine plus phosphorylcholine; t-Cr, creatine plus phosphocreatine; %CSF, % cerebrospinal fluid; %GM, % gray matter; %WM, % white matter.
† Sub-analysis was conducted to examine the Glu pattern over time within each group (HPW and HRW) because we found the interaction between time and group to be significant. The interaction for time and group for the other outcomes was found to be non-significant.

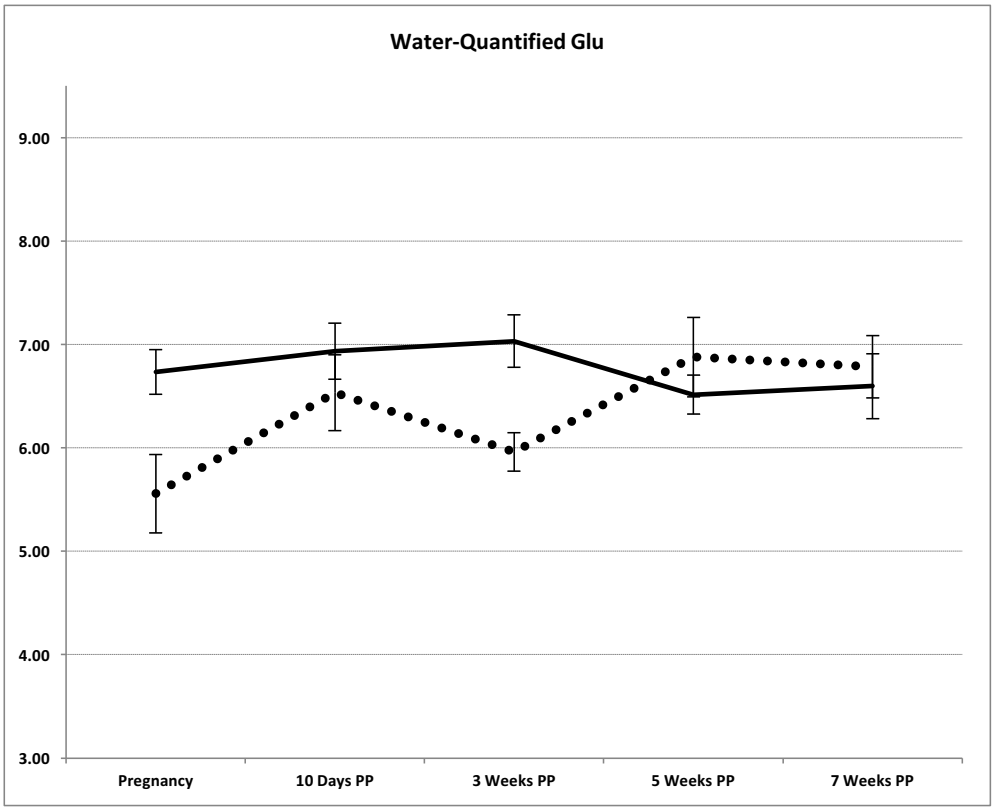

Fig. (2a). Change in Glu from the pregnancy time point up to 7 weeks postpartum.

▬ HPW
▪ ▪ ▪ HRW
Abbreviations: Glu, glutamate; HPW, healthy pregnant women; HRW, high risk women; PP, postpartum.
Values are reported as the mean ± standard error.

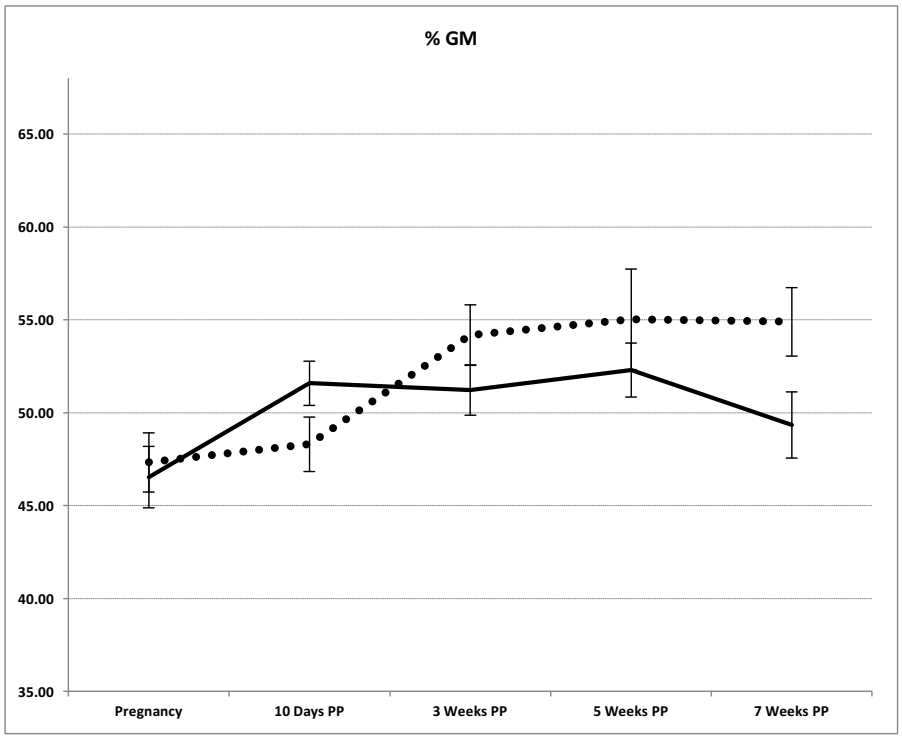

Fig. (2b). Change in %GM from the pregnancy time point up to 7 weeks postpartum.
▬ HPW
▪ ▪ ▪ HRW
Abbreviations: HPW, healthy pregnant women; HRW, high risk women; PP, postpartum; %GM, % gray matter.
Values are reported as the mean ± standard error.

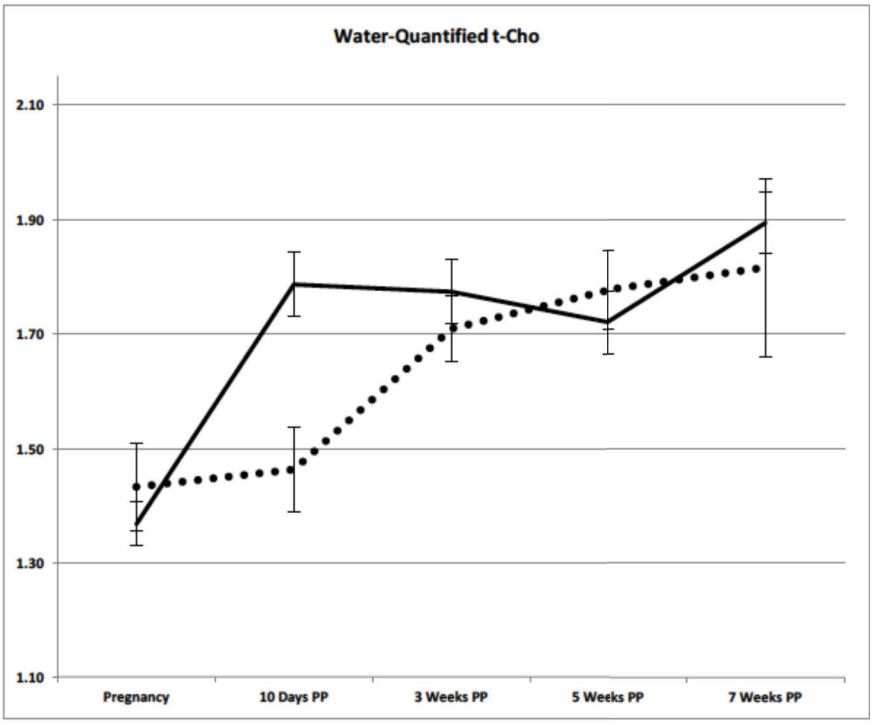

Fig. (2c). Change in t-Cho from the pregnancy time point up to 7 weeks postpartum.
▬ HPW HRW
Abbreviations: HPW, healthy pregnant women; HRW, high risk women; PP, postpartum; t-Cho, glycerophosphorylcholine plus phosphorylcholine.
Values are reported as the mean ± standard error.

We recently found that MPFC Glu levels are decreased in perimenopausal women [34], a phase of women's reproductive life associated with both fluctuations of female hormones (general decrease in estrogen production) and an increased risk for a major depressive episode [33]. Thus, female hormone fluctuations may induce a decrease in MPFC Glu levels that might contribute to the increased risk of a major depressive episode during the early postpartum period and perimenopause.

Allopregnanolone (ALLO), an endogenous neurosteroid, is the major metabolite of progesterone. ALLO's levels rise during pregnancy and decrease abruptly in postpartum. ALLO is a potent positive allosteric modulator at the level of the synaptic and extrasynaptic $GABA_A$ receptors; thus, it contributes to the GABA-mediated inhibitory activity and inherently opposes glutamatergic excitatory activity [43]. Brexanolone, an exogenous formulation of ALLO, is rapidly effective in the treatment of PPD and is FDA approved for the treatment of PPD [44]. HRW are suspected of having maladaptive biological adjustments to physiological fluctuations of female hormones and their metabolites, especially ALLO [45]. The unique decrease in MPFC Glu levels observed during pregnancy and the early postpartum in HRW could be explained by this group's increased sensitivity to the impact of the fluctuations of ALLO concentrations on Glu release.

Animal studies have shown that ALLO administration decreases Glu release in specific brain areas [46]. For example, Hu and colleagues found that ALLO inhibits the release of Glu in rat MPFC, which is our voxel of interest [47]. Interestingly, we have previously shown that PPD is associated with increased MPFC Glu levels [21]. It is possible that the therapeutic impact of brexanolone in PPD may also involve the Glu system and, more particularly, a brexanolone-induced decrease (normalization) of MPFC Glu levels. Further investigations could assess MPFC Glu levels in PPD women after successful treatment with brexanolone.

However, the changes in neurosteroids and sex hormones concentrations during pregnancy and the PP are not limited to ALLO, and there are other neuroactive steroids, such as pregnenolone sulfate, that increase during pregnancy and rapidly decrease postpartum, also affecting glutamatergic transmission at the presynaptic and postsynaptic levels [48, 49]. Estrogen and progesterone, the main ovarian hormones, have also been reported to affect the glutamatergic system, with both stimulatory and inhibitory effects. For example, in cell culture studies, estrogen has been shown to potentiate glutamate transmission [50], while progesterone decreases glutamate-induced excitation in a dose-dependent fashion [51].

Our results of altered MPFC Glu levels in HRW can be discussed in relation to studies on MD patients. A MRS investigation by Taylor and colleagues showed that MPFC Glu levels in un-medicated, remitted MD patients return to healthy control levels between depressive episodes [52]. This observation has been further supported by Price and colleagues, who also found a return to normal Glx levels in the ACC and occipital cortex of remitted MD patients [53]. This suggests patients at risk for MD do not display long-lasting trait-related dysregulation in Glu or Glx levels. These results are consistent with our results as HRW show a return to HPW levels as the postpartum progresses, with only an initial difference in MPFC Glu levels during the early, high-risk postpartum period. Combined, these results suggest no chronic trait-related MPFC Glu level differences in either women at risk for MD or PPD.

Our group's previous work showed that MPFC Glu levels are increased in un-medicated women suffering from PPD [21]. We admit that our findings of lower Glu levels in HRW appear difficult to reconcile with our findings in PPD women. However, discrepancies in the direction of brain Glu levels fluctuations between euthymic high-risk patients for a major depressive episode and patients suffering from an acute depressive episode have been observed by others. For example, Taylor and colleagues found that individuals with a family history of parental depression had higher Glu levels in their parieto-occipital cortex in contrast to healthy controls with no familial risk of depression [54].

It is important to note that our MRS measurements of Glu levels do not allow us to determine its sources. Glutamine is stored in glia (astrocytes) and transported to glutamatergic neurons, where it is converted to Glu. After its release from presynaptic neurons into the synaptic cleft, Glu is taken up by astrocytes and rapidly converted to Gln. Gln is transported back to the presynaptic neuron, where it is recycled into Glu, or to GABAergic neurons, where it is converted into GABA [55]. Glu is, therefore, present in multiple compartments, including synapse, within vesicles, in the extracellular matrix, and within astrocytes. Our findings of decreased MPFC Glu levels during pregnancy and the early postpartum in HRW that we attribute to HRW's sensitivity to sex steroids fluctuations may therefore be metabolic and/or neurotransmission related. Carbon-13 (13C) MRS is the only *in vivo* noninvasive method that allows for the determination of both glutamatergic neurotransmission and cell-specific energetics with signaling and non-signaling purposes [56]. Fluctuations of neurosteroids, especially ALLO, during pregnancy and the pospartum in HRW may affect glutamatergic neurotransmission and/or neuronal metabolism.

Moreover, the activity of Glu and GABA in the brain is inherently entangled and antagonistic since Glu is the main excitatory neurotransmitter and GABA the main inhibitory neurotransmitter [57]. The interpretation of our results would have therefore been facilitated by concomitant measurement of GABA levels.

We did observe a significant increase in %GM in the MPFC from pregnancy up to 7 weeks postpartum. As there were no differences observed between the HPW group and the HRW group, this suggests that the change in %GM is only associated with the physiological changes occurring during pregnancy and postpartum and seems to normalize as the postpartum period progresses. This is supported by our previous publication, which showed a 13.9% decrease in %GM in 21 healthy pregnant women compared to 14 non-pregnant females scanned during the follicular phase [22]. In this investigation, using non pregnant women during the follicular phase as a point of reference, we came to the same conclusion of a decrease in %GM during pregnancy with a greater sample size (n =34), using postpartum %GM as points of reference. Of note, the 21 HPW included in our current study are the same patients from our previous publication [22].

Observed decreases in %GM during pregnancy and the early postpartum with a progressive increase in %GM later during the postpartum are somewhat consistent with previous investigations. An MRI investigation by Oatridge and colleagues compared 9 healthy pregnant women to 5 women with preeclampsia [58]. This is the only other study that directly measured brain volume changes during pregnancy, albeit their focus was on global brain volume measurement [58]. The authors found a decrease in overall brain size in healthy pregnant women and preeclamptic women, with a significant increase in brain size, compared to pregnancy, by 6 weeks postpartum and a return to pre-pregnant levels by 6 months postpartum [58]. These results obtained for the whole brain are consistent with our results of a decrease in % GM in the MPFC during pregnancy with an observed significant increase across the postpartum. However, it is important to note that the results of the study by Oatridge and colleagues are limited due to the small sample size and the lack of consistency in postpartum scanning times. There are also other investigations that have tried to indirectly assess the brain structural changes associated with gestation without actually scanning during pregnancy. Kim and colleagues used voxel-based morphometry to assess GM volume in the prefrontal cortex of mothers at two different time points: 2–4 weeks postpartum and 3–4 months postpartum, and found a significant increase in GM volume from the first measurement to the second [59]. However, as no in between additional time points were measured, it is difficult to determine when the increase in GM volume occurred since, as suggested by our findings, %GM had likely already increased from pregnancy at the 2-4 week postpartum time point. Recently, Hoekzema and colleagues found GM volume to be decreased in multiple brain regions, *i.e.*, medial orbitofrontal cortex, precuneus, superior medial frontal cortex, including brain regions overlapping our MPFC voxel during postpartum compared to pre-pregnancy; they deduced pregnancy to be associated with a decline in GM volume [60]. Contrary to our findings and those of Kim *et al*.'s, Hoekzema's team reported no increase over time in GM in any brain areas, with the exception of partial hippocampal volume recovery [60]. A potential explanation for this discrepancy is that Hoekzema and colleagues only performed two MRI scans following parturition in contrast to our study, which had four, and the gap between the MRI visits was relatively long (73.56 ± 47.83 days for the first post-pregnancy scan and 2.32 ± 0.50 years for the second post-pregnancy scan), whereas our scanning was performed as early as 10 days after parturition [60]. More importantly, from the 25 primiparous women for whom they had one pre-pregnancy scan and one post-pregnancy scan, only 11 came back for a second post-pregnancy scan. In contrast, we have scanned 29 women at the 7-week time point.

A decline in MPFC %GM during pregnancy and early postpartum may be linked to common subjective cognitive complaints experienced during this time, often referred to as pregnesia [21, 35].

From an MRS methodological point of view, it is important to note that the change in %GM seen during pregnancy and postpartum should be taken into consideration for subsequent neuroimaging research, especially MRS investigations looking at brain metabolites during pregnancy and postpartum.

Hormonal fluctuations may be responsible for the changes in %GM during pregnancy [22] and across the postpartum period. Previous work has shown that %GM remained relatively stable in fathers of newborns in contrast to puerperal women, suggesting a biological mechanism for the observed brain structural changes [60]. Furthermore, other researchers have observed decreased %GM in the cortical regions of females undergoing puberty (due to their rising estradiol levels) [61], as well as decreased cortical thickness in transsexual individuals receiving estrogen therapy [62]. Additional research is needed to better understand how hormones affect cortical %GM.

MPFC t-Cho levels significantly increased over time from pregnancy up to 7 weeks postpartum in both the HPW and HRW groups. This has been reported in a previous MRS study comparing pregnant women, with and without preeclampsia, to non-pregnant women. Rutherford and colleagues found an overall decrease in t-Cho levels in pregnant women compared to non-pregnant controls [63]. The demands of the developing fetus have been indicated as a cause for the decreased brain t-Cho levels observed in healthy pregnant women [64]. Our data describing the most significant decrease in t-Cho levels during pregnancy and a gradual return to pre-pregnant levels across the postpartum period were therefore expected in both groups of subjects.

As the impact of pregnancy on brain metabolites is largely unknown, we used water as a reference metabolite. Indeed, in a number of MRS studies, metabolite concentrations are referenced to another metabolite, such as t-Cho [65]; however, the use of a reference metabolite, as seen with the fluctuations of t-Cho in this investigation, can be problematic, producing apparent fluctuations in target metabolites as levels of the reference molecule itself may be fluctuating.

The informal support provided by the research team may explain why surprisingly, none of our HRW developed PPD during the study, which included education related to PPD. Other unmeasured protective factors could have also explained the lack of PPD amongst our HRW.

The relatively small sample size is one limitation of our study. Ideally, all participants would have completed all scans, but the missing data points are unfortunate but inherent to these types of complex MRS investigations in women who are pregnant or have just given birth and are facing complex logistics associated with motherhood. One weakness of this investigation is the monitoring of MPFC Glu levels only up to 7 weeks postpartum, as measurements up to 6 months and a comparison to direct pre-pregnancy levels would have been ideal. However, the cost would have been prohibitive, and it would have been difficult to maintain participation of the subjects for this time duration. A longer follow-up period combined with pre-pregnancy measurements would have allowed us to determine when or if our measurements returned to pre-pregnant levels. It should also be noted that the results of this study are exploratory and should be treated as a driver for future confirmatory work, with a greater sample size.

From an ethical point of view, accumulating evidence exists for the safety of performing MRI and ^{1}H-MRS during pregnancy, especially during late pregnancy when the fetus is fully developed [66-70]. Babies born in women who underwent ^{1}H-MRS during pregnancy in our investigation did not present any postnatal problems.

CONCLUSION

Our study is the first extensive prospective study to measure changes in brain metabolites and %GM directly during pregnancy and across the postpartum period. The observed difference in MPFC Glu levels between HPW and HRW during the early postpartum with a return to normal control levels as the postpartum progresses contributes to the understanding of the biological factors playing a role in the increased risk associated with developing early-onset PPD in HRW. Further investigations are needed to determine the specific role that female hormones play in the physiological changes occurring in %GM during pregnancy and the postpartum. Their role in the unique alterations of MPFC Glu concentrations during that time period in HRW and its relevance to the development of PPD remain to be elucidated.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Health Research Ethics Board of the University of Alberta, Edmonton, Alberta, Canada (Ethics no: Pro00002952).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013 (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/).

CONSENT FOR PUBLICATION

A written informed consent was obtained from the patients.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding authors upon request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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