Author's response to reviews

Title: Urinary Cortisol and Depression in Early Pregnancy: Role of Adiposity and Race

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Author's response to reviews: see over
Response to review.

Reviewer 1.

Comment 1. Overall, this is an interesting study in a specialist area, with some potential to inform the researchers, scientists and clinicians working in this field. The abstract is clear and well-focused and accurately describes the study methodology and results, it is sometimes clearer and better focused than the main report. The title is clear and relates well to the study. The authors have given some valid interpretations of their findings and clearly acknowledged the limitations of the research in terms of sample size and selection, and non-standardization of urinary collection methods. They have provided a rationale for further study in this area with a larger population and greater rigour. The authors acknowledge their debt to previous work.

Author's response: We sincerely thank the reviewer for their positive evaluation of our manuscript.

Comment 2. Discretionary Revision
In the background section, paragraph 1, it is not clear to me whether cortisol levels, which are stated to normally be elevated in response to stress, are also necessarily elevated in depressed individuals. If so, this should be made clearer to provide a stronger rationale the investigation of cortisol levels in pregnant women, in relation to depression. In the background section paragraph 2, inflammatory markers and stress markers are noted to be related to depression and to be more prevalent in black women, however, it should somewhere be stated that cortisol is a stress marker which has been shown to be elevated in depressed individuals, compared to the non-depressed population, if this is something that informed the aims and design of the study.

Author’s response: Thank you for this comment. We have including additional information in the Introduction addressing the item of cortisol levels in depression and pregnancy. We have also addressed the item of cortisol as a stress marker and it’s difference in depressed subjects.

Comment 3: Discretionary Revision
In the background, paragraph 2, the concept of race is introduced, which is a major element of the study. Whilst, this aspect may have been explained at length in other reports, relating to the larger scale longitudinal study, in which this one is nested, here there is no attempt to explain how the different races have been defined. Is there an agreed tool that has been used to define black or white? Race is a complex concept. Have different national and ethnic origins been considered (e.g. black African (born in sub-Saharan Africa, or with parents who are from Sub-Saharan Africa) or black American (where there might be much more ethnic diversity))? Is mixed race categorized differently from black? These aspects may also have a bearing on different socioeconomic profiles within the sample of black women.
Author’s response: This is an important comment, and we agree with the author that race is a complex concept. We have now addressed this comment in the Methods section and included additional information in Table 1. Specifically, the subjects recruited in this study were from the western Pennsylvania region and were all seeking prenatal care at Magee-Womens Hospital of UPMC. The ethnicity of the five county area of western Pennsylvania is primarily African American (~20%) and Caucasian (~70%). By design all of the women in this cohort were self-described as African American. In addition, the women in this study are primarily of low socioeconomic profile. We have published dozens of studies on this relatively stable population, i.e. the population overwhelmingly tends to remain in the western Pennsylvania area. It is beyond the scope of this study to quantify any possible genetic differences in this population, although we acknowledge the shortcomings of self-described ethnicity and the fact that several factors may contribute to differences in racial and ethnic differences in health including genetics, economics and social factors, behavioral factors, and access to care.

Comment 4: Discretionary Revision
In the Background section, paragraph 3 there is a lack of clarity around the aims and objectives of the study. From the way the focus of the study has been written, it is difficult to understand what the null hypotheses are. A clearer statement of aims and objectives will help the reader determine the authors’ reasons for conducting the study and using the selected approaches.

Author’s response: We appreciate this comment and apologize. We have revised the Introduction to more clearly state the objectives of this study.

Comment 5: Discretionary Revision
It is interesting that from Table 1, it appears that the mean BMI for these women is in the obese range. Is there a comparison between this sample’s mean BMI for that for the wider cohort?

Author’s response: The focus of the wider cohort was to investigate differences in pregnancy outcome among primarily obese women. This is in part why the average maternal BMI in this study is obese. However, there are both lean and overweight women in the cohort for this study.

Comment 6: Minor Essential Revision
In Figures 1 and 2, the data points on the graphs need a key, so that readers can distinguish, to which group they are attached (e.g. obese women or non-obese women) them from each other.

Author’s response: We sincerely apologize for this oversight. We have now included a description key for the symbols in Figures 1 and 2, filled symbols are depressed subjects and open symbols are non-depressed subjects.

Reviewer 2.

Major Compulsory Revisions:
Comment 1. Confusing aims and objectives.

Author’s response: We appreciate this comment and apologize. Reviewer 1 also made this comment. We have revised the Introduction to more clearly state the objectives of this study.

Comment 2. Disorderly building up of discussion section.
**Author's response:** We appreciate this comment and apologize. We have revised the organization of the Discussion section.

**Minor Essential revisions:**
Comment 3. Edinburgh Depression Rating Scale is a screening tool hence in my opinion a more robust measure of diagnosis of depression e.g. face-to-face interview or other structured psychiatric interview schedules could be used. This creates a fundamental problem of ascertaining validity of the diagnosis. I would recommend this be mentioned as a major limitation of this study which quite possibly is due to it being part of larger study.

**Author's response:** We agree with the reviewer's comment and have added this as a major limitation of the study design. However, of note, in reviewing the medical records of each patient in this study, 8 of the women with an EDS score of 11 or higher were also noted to have a history of depression or anxiety disorder. This information is now noted in the Methods section.

Comment 4. In discussion section: First paragraph- It wasn’t a stated objective to investigate association of cortisol with PTB and LBW so please mention it afterwards when you have discussed explanations for your main results. It appears to be out of place statement. .......in contrast, plasma cortisol levels were negatively associated with several measures of maternal adiposity, and additional analysis revealed this relationship to be present only in the non-depressed non-obese pregnant black women.... Should it not be just non-depressed women??? Paragraph 1 on page 12 is not well written at all with repetition of facts and lines (last line is repeated twice).

**Author's response:** These corrections have been made to the revised manuscript.

**Reviewer 3.**

Comment 1. I found this a really interesting topic which the authors have clearly thought through a sound rationale for examining the association between cortisol and depression based upon a comprehensive understanding of the previous research base. However there are a number of problems with the analysis and the interpretation that need to be clarified and currently severely affect the interpretation.

**Author's response:** We thank the reviewer for their positive evaluation of our manuscript, and are extremely grateful for their helpful comments which we have addressed below.

**Major Compulsory Revisions**
Comment 2. The description of the timing of the cortisol measurement is severely lacking. It describes that the ‘morning spot urine sample’ was taken and maternal plasma samples were collected between 6-16 weeks gestation. First, the authors need to be explicit whether the urine and blood sample were taken at the same time. Second, and more importantly, far more detail needs to be taken regarding the exact measurement. Due to the diurnal variation in cortisol the timing of these measurements is crucial. The authors comprehensively discuss the findings in relation to cortisol awakening responses in previous cohorts but they are very unclear about when exactly these measurements were taken. A single measurement cannot be seen as indicative of the awakening response and it is the pattern of the response that is more
informative than a single sample. The authors need to explain why Area-under-the-curve (AUC) analysis was not used instead of a single time point. Consequently it is difficult to make some judgments on the paper until this information is transparent.

**Author's response:** We have included additional information in the Methods section detailing the collection of the urine samples. Unfortunately, because of the nature of the urine sample collection only a single sample was collected and therefore we are unable to provide an AUC analysis. We have included this information as a significant limitation of the study, but also hope the reviewer appreciates the effort taken to investigate the possible use of maternal urine samples for this analysis, and the positive correlation between plasma and urine cortisol levels despite a less than ideal collection method. The focus of the cortisol data in the manuscript is more on maternal plasma cortisol values in relation to depression and adiposity since no relationships were observed in urine cortisol measures, despite the positive association with plasma cortisol.

Comment 3. On a similar note, the collection period of 6-16 weeks is quite significant in regards to both the development of the fetus and the mothers’ psychological state. In regards to the first point, how does this difference in time affect rates of cortisol secretion. In regards to the second point, the authors only measure depression but it should be acknowledged that cortisol may be elevated through general stress and anxiety, which may be comorbid to depression. I think this needs some attention as it cannot be presumed that just because the matched controls did not have a diagnosis of depression that they will be unstressed and so have normal cortisol levels. The authors themselves acknowledge that variations in depressive subtype may be influential but it may be more worthwhile to explain the potential influence of comorbid anxiety (http://www.ncbi.nlm.nih.gov/pubmed/18493710)

**Author's response:** We appreciate this point and have expanded the discussion of this topic and included additional information. Specifically, we observe no correlation between maternal plasma or urine cortisol values and gestational age at collection; plasma $r^2=0.03$, $p=0.25$ and urine $r^2=0.02$, $p=0.29$. In reviewing the medical records of the women in this study, 8 of the 25 women with elevated EDS scores had a note of a previous history depression and 1 non-depressed woman had a history of depression without medication.

Comment 4. It appears there are no exclusion criteria for antidepressant medication (I presume the line ‘collagen vascular disease (autoimmune disease) on medication’ relates only to the medication for this specific disorder). This is an important factor due to the associations between several antidepressants with weight gain. This iatrogenic cause of weight gain may interfere with cortisol secretion. Furthermore, evidence has shown that associations between depression and the cortisol awakening response may be principally due to the medication (http://www.ncbi.nlm.nih.gov/pubmed/17855000).

**Author's response:** This is a very important comment. Overall the women in this study were healthy. Despite 8 of the 25 women with an elevated EDS score having a history of depression in their medical record, none of the women with and elevated EDS score were currently being treated with antidepressant medication. We have now included this information in the Methods.

Comment 5. A major concern I have with the study is the limitations of the statistical analysis. The authors provide a power calculation to explain the number of 50 women but they do not explain the analysis this applies for. Is this to see a difference between the depressed and non-
depressed? This needs to be explicit. If this is the case then it really needs to be made explicit that the study is not powered to detect differences when the groups are further divided by whether obese or not obese and this is purely exploratory. A more general point is that I find the matching of depressed to non-depressed a very crude division and may overlook the subtleties in how differences within group affect the findings. For example there is a very large outlier in figure 2. I think a hierarchal regression model would be far more robust with depression as a categorical variable entered after controlling other variables such as weight. I appreciate some of the data may be non-parametric but then bootstrapping techniques would compensate for this.

Author’s response: We apologize for this oversight. We have now clarified the power calculation in the Methods. In addition, when the outlier in Figure 2 is removed from the analysis of maternal plasma cortisol to percent body fat among non-depressed women the significant negative correlation persists, $r^2=0.32$, $p<0.01$. This additional information is now included in the manuscript.

Minor Essential Revisions
Comment 6. Keep it simple for the reader by not removing the acronyms of PTB and LBW for Preterm birth and low birth weight. These terms are not used enough in the text to necessitate an acronym.

Author’s response: Thank you for this comment. We agree and have removed PTB and LBW as abbreviations.

Comment 7. The figures cannot be interpreted as there is no legend describing what data points and lines represent each group. Furthermore, 3 lines exist in Figure 1 so I’m unclear what group other variable is represented.

Author’s response: We apologize for this oversight, which was also mentioned by the other reviews. We have now included a description key for the symbols in Figures 1 and 2, filled symbols are depressed subjects and open symbols are non-depressed subjects. The description of the trend lines in Figure 1 are included in the figure legend.

Discretionary Revisions
Comment 8. Line numbers would be appreciated for future revisions to isolate where changes are needed. The majority of the first paragraph in the Discussion is fairly obsolete. Opening with a summary of the findings would be far more useful to the reader and highlight the importance of the study. In the discussion it states ‘we did not observe a relationship between early pregnancy cortisol (plasma or urine) and PTB or LBW’ but this finding is barely mentioned in the results. The study is very underpowered to detect such a difference between groups with these outcomes. I suggest to not make this the focus of your opening paragraph of the discussion.

Author’s response: We have now included line numbers in the manuscript, and have revised the Discussion section as suggested.

Comment 9. There are a lot of interesting findings in this study but as a reader they sometimes get lost as it is hard to discern what is the primary outcome, secondary outcome, post hoc analysis. I suggest breaking this down more clearly. For example, ethnicity comes across the priority, then the association between plasma and urine, then the association between adiposity
and cortisol, then the influence of depression. I think if the authors are very clear about what they see as the most important finding it will make it easier to recognize the ‘take home’ message.

Author’s response: We appreciate this comment and have revised the manuscript to try and make the primary and secondary findings more clear.

Comment 10. Despite describing the importance of examining ethnicity throughout the introduction, the fact that the sample is entirely black almost becomes obsolete. The demographics should give some clearer idea of the ethnic origin and with socio-economic status being an influential factor when examining racial disparities, this information would be useful. Even if as simple as education levels. I think the authors can be fully justified in saying they chose to look at exclusively black women as these are an underrepresented sample at potentially higher risk but I think they need to describe it as a limitation in the Discussion that socio-demographic factors were not fully explored.

Author’s response: We appreciate this comment which was also made by the other reviewers, and have now included additional information about the socio-demographics of the women in this analysis. Please note that the majority of the black women in this study were similarly low income.