Reviewer’s report

Title: Maternal cortisol and stress are associated with birth outcomes, but are not affected by lipid-based nutrient supplements during pregnancy: an analysis of data from a randomized controlled trial in rural Malawi

Version: 1 Date: 6 September 2015

Reviewer: lieven Huybregts

Reviewer’s report:

This study assesses the effect of prenatal nutrition supplementation on maternal cortisol levels and, as secondary analysis, the association between maternal cortisol and birth size and gestational length. I read the paper with great interest as it aids in entangling the complex hormonal mechanism of nutrition, regulation and birth outcomes in a LMIC setting. There are some problems with the analysis that might lead (or not lead) to erroneous conclusions. Also, I feel that part of the general mechanism could be moved from discussion to introduction to leave more space to discuss what these finding add to the evidence on HPA and birth outcome.

Major

1. The introduction does not really hypothesize a mechanism for the 2 main research questions to underline why maternal cortisol was measured in relation to birth outcome. The part that explains this better is now situated in the discussion and parts of it could be moved to the introduction. This is especially interesting for readers without endocrinologic expertise.

2. I believed that the essence of the HPA mechanism is situated at the placental level. Maternal cortisol gets converted to inactive cortisone by 11β-HSD2. Poor performance of that 11β-HSD2 system (because of eg. malnutrition) can lead to increased exposure of the fetus to cortisol which in turn could affect growth and/or gestational duration.

The negative association between maternal cortisol and birth size is clearly stronger at 36 weeks compared to 28 weeks, which happens to be the timing when also 11β-HSD2 levels are getting lower. So might this imply that ‘damaging’ levels of maternal cortisol only then exert an influence on fetal size trough increasing fetal cortisol levels? However, papers from our group (cfr bottom) from West-Africa did not really demonstrate strong associations with birth size (at least not when adjusting for IGF-1/2 levels). One wonders if another mechanism can explain the association between late gestation maternal cortisol and birth size. It would be interesting to add this aspect to the discussions so it expands the chain of observations.

3. Throughout the discussion authors need to clarify if it concerns maternal or fetal cortisol levels. Despite some publications reporting on positive correlations, it would conceptually be better to treat them separated given the role of the
placental interface/barrier function.

4. Table3/4: There is a clear imbalance in cortisol levels at baseline. Why wasn’t an overall analysis conducted including all data? It would be interested to see the impact of adjusting for baseline cortisol levels on the overall trend.

5. Table 3/4: It also seems that at every time point a different subsample of the data is analyzed so comparing means between time points is problematic as we can’t really tell if differences are related to time points or different subjects. Either consider an overall analysis, or make sure we are looking at the results of the same sample.

Minor

1. L121: to what extent are nurses able to conduct complex ultrasound measurements? Was there a data quality control conducted by an obstetrician (X% of ultrasound pictures verified by an expert). Is there any data available on intra and inter-rater coefficient of variation of these measurements of these nurses?

2. L161: this needs to be specified better. What was the proportion measured within 24hrs, 48hrs, 72 hrs and was it different between intervention groups? Measuring birth weight within 24 hr is a quality criterion. Between 24-48hr birth weight can decrease with 50-75 g already. If there is a substantial spread in the timing when BW was recorded, it is recommended to adjust for this variable as it can enhance your precision.

3. L174: were there any values below the limit of detection of the analytical tool, if yes, what strategy was followed to deal with these values?

4. Add sample size calculation to situate what significant differences one is able to detect with these samples?

5. L198: why parity and not primiparity, the latter constituting of a particular sub-population. Both Huybregts et al and Roberfroid et reported higher cortisol concentrations in primiparous or primigravidae.

6. L205: add “with robust estimation of SE”. Poisson distribution has the assumption that E(X)=Var(X) which is theoretically impossible for a binomial distribution.

7. L206: add the analysis of SGA to be consistent with literature on prenatal interventions. Strangely SGA is mentioned in the discussion

8. L206: add that SGA was also analyzed

9. L208: repetition of line 198

10. L215: add which imputation method was used

11. L246: crude and adjusted analyses were conducted, it is unclear what results are shown in table 3.

12. There is a clear imbalance in cortisol levels at baseline. Could an overall analysis be conducted? I was thinking of a mixed effects model with mother as random intercept (and if needed random slope) that adjusts for baseline
imbalances.

13. Table 3: tables should be stand alone: add below table what analysis (model, covariates etc) was used

14. Table 4: For coefficients (=point estimates), 95% confidence intervals are required (=interval estimates).

15. L262 and L273: a p-value cannot be statistically significant, rather a difference

16. L290: Please report coefficients that were 10% different from the limited data analysis. If missingness is substantial so will be the uncertainty of the estimations at the expense of the gain in statistical power.

17. L316 Compared to other two trials. Authors first mention that this study did not find a difference in maternal cortisol, so L336 is a little contradictory?

18. Discussion part L345- 379 gives an overall explanation of which part could be moved to the introduction as it is not directly linked to the observations. I recommend you go through this part and filter out a hypothesized mechanism model that made you decide to undertake this study on prenatal nutrition, maternal cortisol and birth outcome.

Refs

Note that Robefroid et al. reported increased cord cortisol concentration as a consequence of prenatal MMN in primiparous.


**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests