Reviewer’s report

Title: The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes

Version: 1 Date: 29 Jan 2019

Reviewer: Marloes Dekker Nitert

Reviewer’s report:

PRCH-D-18-00165R1 The effects of Mg-Zn-Ca-Vit D co-supplementation of biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes

This manuscript describes an interesting study of supplementation to reduce oxidative stress in gestational diabetes. The study overall is well-performed but the data analysis and presentation need to be improved.

1. Methods, randomization and treatment allocation: To what extent were the women compliant with the standard pregnancy supplementation protocol where they already were taking 1000 IU of vitamin D3? If they were compliant, what was the expectation that an additional 400 IU of Vit D daily would contribute?

2. Methods, randomization and treatment allocation: how was the blinding performed?

3. Methods: how was blood glucose control monitored? Did any of the women need medication to control their blood glucose levels? The blood glucose data needs to be supplied both at baseline and after 6 weeks.

4. Methods, outcomes: Please define what the specific primary outcomes were. The current statement is very underspecified and is not useful.

5. Methods, biochemical measures: the Griess method measures nitrite/nitrate and differences in the levels are quite difficult to determine. What was the level of sensitivity for these assays?

6. Methods, biochemical measures: given that ferric reduction antioxidant power was used in the determination of TAC, were these measures adjusted for the Hb content of the blood?

7. Methods, sample size calculation: Normal hs-CRP values in pregnancy in the third trimester are around 300 umol/L or 300ng/mL. A value of >3000 ng/mL would be very high: where were these values obtained?

8. Results, compliance: the compliance is rated at 100%. Given that this is determined by return of the bottles, it could also be that the women just returned empty bottles to make
it look like they were compliant. Given the relative small changes in biochemical levels, it appears that some may have done this. Please tone the statement down somewhat.

9. Results, table 1: please include blood glucose levels at the start of the study to this table. And to table 2 as well.

10. Results, figure 2: from the text, it looks like the figure should show change from baseline, is this really fold change or just change from baseline? Check the y-axis label and adjust. In addition, this information should also be provided as absolute values: currently, the reader cannot deduce whether an increase by 0.3 mg/dl in Mg or of 0.4 vs 4 mg/dL in Zn is clinically meaningful. So a table with the concentrations at baseline and the end of the study should be provided. In addition, the figures should be a boxplot with the mean and standard deviation rather than a bar graph. Currently, from the figure it is unclear how such large standard deviations can result in such significant p-values. Especially for the vit D: how can this difference be significant?

11. Comment 10 also applies to figure 3 and 4.

12. The reduction in birth weight and macrosomia is very large: it is unclear whether this is just beneficial or could also have negative effects.

13. Discussion: The statement that this trial reduces inflammatory and oxidative stress markers is an overinterpretation of the data as presented in this study. Please tone this down. This needs to be done for the conclusion and the abstract as well.

Minor comments

P7, l158: please replace nomograms with normograms.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review? If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal