Author’s response to reviews

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

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Author’s response to reviews:

Dear Dr Aronin,

Thank you so much for your letter informing us of your decision regarding the Manuscript ID PRCH-D-18-00165 entitled as "The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes". The manuscript has been revised according to the referees’ comments. Responses to the reviewers’ comments have been provided below.

Yours Sincerely,

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First of all, we would like to thank the reviewers for their great comments to improve the quality of the manuscript. We tried our best to revise the manuscript according to the reviewers' comments.

Comments from the Editors and Reviewers:

Reviewer reports:

Marloes Dekker Nitert (Reviewer 3): 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 vitamin D daily would contribute?

Authors: Thank you for this important comment. This point clarified in the revised version:

“All pregnant women in Iran have a monthly check-up meanwhile they are monitored for vitamin D supplements compliant and whether they have had any side effect with this supplement. In this study women were compliant with the standard protocol of pregnancy for vitamin D. Since vitamin D requirement is increased in pregnancy, we added 400 IU to the combination of other elements and we did not add more because of risk of side effects as well as we believe calcium might have synergistic effect with vitamin D. Observing significant effects in this study moreover clarifies that although 1,000 + 400 IU/day vitamin D might not be much different from 1,000 IU/day in placebo group, however combination of other helpful nutrients with vitamin D can differentiate the impact of 1,000 + 400 IU/day from 1,000 IU/day” (Lines 250-8).

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

Authors: This point has been clarified in the revised version:

“Study participants were randomized using computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants until the final analyses were completed. The randomized allocation sequence, enrolling participants and allocating them to intervention groups were conducted by a trained midwife at the gynecology clinic. Another person, who was not involved in the trial and not aware of random sequences, assigned the subjects to the numbered bottles of capsules” (lines 134-9).
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.

Authors: These points have been clarified in the revised version:

“among women with GDM who were not on oral hypoglycemic agents” (Lines 49 and 94-5).

“Patients were requested to come back in order to check their blood glucose levels weekly (besides their daily self-monitoring) during the study” (Lines 127-8).

“To determine FPG, we used enzymatic kits (Pars Azmun, Tehran, Iran)” (Line 170).

Data for FPG (baseline and week 6) has been added to the revised version. Please, see Table 2.

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

Authors: This comment has been addressed:

“In the present study, the primary outcomes were serum hs-CRP and plasma total nitrite levels” (Line 145).

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?

Authors: This question has been answered:

“The plasma total nitrite concentrations were measured using Griess method” (Line 177).

“In addition, we did not evaluate the sensitivity of plasma total nitrite concentrations using Griess method” (Lines 336-7).

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?

Authors: As we did not evaluate circulating hemoglobin levels, we were not able to adjust these measures for the Hb content. This point has been added as one of the limitations of this study:

“However, TAC levels are evaluated using FRAP assays. Since hemoglobin data were not available in this study, we were not able to adjust our results based on circulating hemoglobin levels. This should be considered in the interpretation of our findings” (Lines 333-6).
7. Methods, sample size calculation: Normal hs-CRP values in pregnancy in the third trimester are around 300 umol/L or 300 ng/mL. A value of >3000 ng/mL would be very high: where were these values obtained?

Authors: I apologize for this mistake. This point has been clarified in the revised version:

“Applying hs-CRP as a primary outcome with a mean distinction of 3.2 mg/L and a SD of 4.0 mg/L, we used the standard formula for sample size calculation in clinical trials. Considering a type one error (α) of 0.05 and type two error (β) of 0.20 with the power of 80%, the calculated sample size was 25 subjects in each treatment group [24]. Assuming 5 dropouts in each group, the final sample size was determined to be 30 subjects in each group” (Lines 187-91).

“Elevated hs-CRP is more common among women with GDM as compared to women with normal pregnancy. A reference value of hs-CRP levels below 3 mg/L is considered normal [29], and there are a few studies have reported circulating levels of hs-CRP higher than 3 mg/L in women with GDM [30, 31]” (Lines 274-7).

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.

Authors: This point has been addressed in the revised version:

“Sending daily reminders for supplements consumption, relying on participants’ responds and by considering that higher than 90% of capsules were consumed throughout the trial in both groups, assessed through empty bottles back, we assume that the compliance rate in the current study was acceptable” (Lines 207-10).

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

Authors: Data for FPG has been added to Table 2.

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 vitamin D: how can this difference be significant?
Authors: Data for biomarkers of inflammation and oxidative stress has been presented in Table.

Please, see Table 2.

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

Authors: Please, see Table 2.

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.

Authors: Thank you for this important comment. This point clarified in the revised version as follows:

“Following supplementation, the difference between birth weights was large and non-significant between intervention groups (3089.8 vs. 3346.3, P=0.05). This result has been presented as mean values, however when we assessed data of birth weight individually we did not see any birth weight below normal value. On the other hand, the reduction in macrosomia rate was one of the goals of this study. Overall, based on current findings we did not consider these reductions as negative effects” (Lines 306-11).

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.

Authors: This point has been addressed:

“Overall, the findings of this study has demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation for 6 weeks to women with GDM may reduce biomarkers of inflammation and oxidative stress” (Lines 60-2, 246-8 and 340-2).

Minor comments

P7, 1158: please replace nomograms with normograms.

Authors: This point has been addressed:

“the latest normograms based on gender and gestational age” (Line 164).