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 "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:

Editor:

Thank you for your submission to BMC Pregnancy and Childbirth. I am sorry for the delay in informing you of our decision.

In addition to addressing the reviewers' comments below, please address the following editorial points:

1. In the Ethics approval and consent to participate statement in the Declarations, please include the name of the committee that approved the study, along with the reference number, and a summary of the informed consent procedure.

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

“Ethics approval and consent to participate

All study procedures performed were in accordance with the ethical standards of the institutional and national research committee and followed 1964 Helsinki declaration and its later amendments. This study was approved by the Ethics Committee of the Arak University of Medical Sciences (AUMS) (no. IR.ARAKMU.REC.1395.408). All participants provided written and informed consent” (Lines 319-24).

2. In the Funding statement, please specify what role the funder had in the study, if any.

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

“Funding

The research grant (no.1395.408) was provided by Research Deputy of Arak University of Medical Sciences (AUMS) for providing laboratory kits and staff costs” (Lines 335-7).

3. Please do not repeat the funding information in the Acknowledgements. If you have no other Acknowledgements, please write "not applicable."
Authors: This point has been addressed.

“Acknowledgements

Not applicable” (Lines 344-5).

Victoriano Pérez-Vázquez, Ph.D. (Reviewer 1): The paper presented here by Mehri and co-authors entitled: "The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes" aims to determine the effects of magnesium-zinc-calcium-vitamin D co-supplementation on parameters of inflammation and oxidative stress, and pregnancy outcomes among women with GDM, conducted an adequate approach and this paper is well written and clear.

Authors: Thank you.

Lucyna Alicja Wozniak (Reviewer 2): The statistical analysis requires few additional data provided to conclude. Data in Table 2 in the presented form are fragmented and difficult to interpret whether the given values and their significance are indeed the result of supplementation.

1. In particular, magnesium concentration is only given as a change within 6 weeks of trial (P value for supplementation/time); providing information that within a 6 weeks’ trial there are different changes in Mg concentration. However, crucial for analysis of the influence of supplementation would be differences in concentration of Mg between within the two groups (at start and end of the trial) as measured by P-values.

Authors: As we mentioned in the paper in the randomization and treatment allocation and biochemical measure sections, we measured serum magnesium, zinc, calcium and vitamin D at both baseline (week 0) and end of the trial (week 6). In the revised version, we presented results as figures for being more understandable also we have added a supplementary table summarizing serum levels of different nutrients at baseline and follow-up in both case and control group (your comment 5).

2. Whether the concentration of magnesium was significantly different between groups is one thing, but was the change a result of supplementation - in other words the influence of time - important? This can be questioned at this stage. In the group with supplementation at the beginning was 1.8 mg/dL and after 6 weeks was 1.8 mg/dL. Even for the 0.1 increase reported, this increase in relation to the average 1.8, and SD 0.1 and 0.2, for 30 patients this effect is definitely not important.

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

“However, in the current study, magnesium levels increased by 0.1 mg/dL in the intervention group, which was statistically significant although might not be clinically significant. The
important point that should be considered is that serum magnesium concentrations do not thoroughly reflect dietary or supplemental magnesium intake. Although serum magnesium levels are dependent on dietary intake, due to difference in intestinal absorption and kidney function, urinary magnesium excretion and intracellular magnesium concentrations are better indicators for magnesium status than serum magnesium levels. These parameters are also more sensitive to oral supplementation than serum magnesium concentrations. However, we were not able to assess intracellular magnesium concentrations in the current study due to funding limitations. Some investigators have also recommended applying erythrocyte magnesium content to assess dietary intake [28]. Others have shown that the magnesium content of white blood cells is a better index of intracellular magnesium in skeletal and cardiac muscle [28]. Overall, due to low sensitivity of serum magnesium for assessing magnesium status, our results were not clinically significant” (Lines 241-53).

This point has been added into the limitation of the study.

“Finally, we were not able to assess intracellular magnesium concentrations in the current study due to funding limitations” (Lines 306-7).

3. Moreover, analogous data for zinc and calcium are lack in manuscript, so in conclusion, Table 2 is not valid for the given conclusions without further information (presented in tables or diagrams with P-values for placebo and supplementation for major measured biomarkers).

Authors: This comment is not very clear. In Table 2 we have shown that compared to placebo, the combination of nutrients led to significant increase serum nutrient levels which demonstrate the compliance of supplementation and we also have shown significant decrease in CRP and MDA and increase in TAC in supplemented group. Based on these results, we concluded that magnesium-zinc-calcium-vitamin D co-supplementation for 6 weeks had beneficial effects on inflammatory and oxidative stress markers in women with GDM. For more clarification, results of zinc and calcium have also been presented as figure in the revised version and in the supplementary table in detailed.

“After the 6-week treatment, compared with the placebo, co-supplementation with magnesium-zinc-calcium-vitamin D significantly increased serum magnesium (+0.1±0.2 vs. -0.05±0.1 mg/dL, P=0.002), zinc (+4.1±1.8 vs. +0.4±2.6 mg/dL, P<0.001), calcium (+0.3±0.4 vs. +0.1±0.1 mg/dL, P=0.001) and 25-OH-vitamin D (+6.1±3.5 vs. +3.8±1.2 ng/mL, P=0.001) (Fig. 2).

The co-supplementation of magnesium-zinc-calcium-vitamin D resulted in a significant reduction in serum hs-CRP (-1.2±3.5 vs. +0.8±2.0 mg/L, P=0.01) and plasma MDA concentrations (-0.3±0.3 vs. +0.3±1.1 µmol/L, P=0.003), and a remarkable increase in TAC levels (+38.2±76.5 vs. -16.3±93.5 mmol/L, P=0.01), compared to placebo (Fig.3 &4)” (Lines 211-20).

Please, see Figures 2-4.
4. Tests post-hoc for ANNOVA analysis in Table is recommended which would demonstrate correlations and differences between subgroups.

Authors: This comment has not been clearly explained as well. We had two comparison groups and not any subgroups, and we believe post-hoc tests are usually used when we have more than two comparison groups or subgroup analysis. Please explain in detail what you exactly mean by post-hoc test here. Thanks.

5. Additionally, graphical presentation (Figures) of results (Boxplots, median; interquartile range; ranges (excluding outliers) would give deeper inside and support conclusions.

Authors: We created the figures as requested. Please, see Figures 2-4).