Reviewer’s report

Title: Effect of Prenatal EPA and DHA on Maternal and Cord Blood Insulin Sensitivity: A secondary analysis of the mothers, omega 3, and mental health study

Version: 0 Date: 19 Jul 2019

Reviewer: Stephen Contag

Reviewer's report:

1. Summarize the manuscript's content.

This manuscript is a secondary analysis of "The Mothers, Omega-3, and Mental Health Study," using the same group randomization of primarily EPA, DHA or placebo. The authors also included 25-hydroxycholecalciferol levels as an independent variable. Samples were taken at study entry in the second trimester prior to supplementation and in the third trimester to evaluate maternal effect of supplementation. Samples were also taken from cord blood to measure outcome in cord blood. Outcomes measured were surrogates for insulin sensitivity: 1) adiponectin levels, 2) leptin levels, and 3) adiponectin leptin ratio (ALR), levels. Increasing levels of outcomes 1, 3 and 4 are associated with increased sensitivity and increased levels of outcome 2 with decreased sensitivity.

Authors compared baseline levels to third trimester levels and compared the amount of change between groups. The also compared absolute levels between groups, and compared levels in cord blood between groups. They performed a multivariable regression analysis to identify factors that could co-modify the amount of change between groups. Analyses were performed on raw data and after square root transformation for non-normal distribution.

There were 113 pre supplementation samples, 109 post supplementation samples and 98 cord blood samples. There was no difference in the outcome levels pre and post supplementation between groups. There was a significant decrease in adiponectin and ALR over the period of supplementation but no difference between groups. Leptin increased but not significantly in the three groups.

The regression analysis used the pre supplementation levels, or the post supplementation levels as the dependent variable, and the 4 intervention variables, BMI, and maternal age as the predictors. In this model, the concentration of serum DHA was associated with higher ALR in the pre supplementation and post supplementation samples. DHA in maternal serum was associated with adiponectin in the post-supplementation samples. Vitamin D was associated with adiponectin in pre but not in post supplementation samples. BMI was positively associated with leptin and inversely associated with ALR. Maternal weight gain was inversely associated with adiponectin and ALR. The magnitude of the effect of supplementation with DHA is approximately 2-10 greater than the effect of weight and BMI on ALR.
Group allocation had did not affect outcomes in cord blood and on multivariable regression, no differences noted after adjusting for birthweight, gestational age or mode of delivery. The DHA level and birthweight were significantly associated with cord blood leptin.

Study concludes that supplementation did not modify outcomes compared with placebo. Insulin sensitivity decreases with advancing gestation and this is correlated with BMI and weight gain.

2. List the manuscript's overall strengths and weaknesses.

The strengths of the manuscript are evaluation of omega acid supplementation on surrogate markers of insulin sensitivity. The main results are that supplementation did not modify levels. The authors went on to evaluate relationships between the actual serum concentrations of omega acids and vitamin D with the outcomes. There is a positive association between DHA and all markers of insulin sensitivity in maternal serum, and a positive association of DHA with cord blood leptin. The discussion regarding increased BMI and weight gain with increasing insulin resistance is not novel.

The perceived limitations are:

1. The initial hypothesis was that supplementation of omega acids would increase insulin sensitivity. A second hypothesis states that DHA and vitamin D concentrations in maternal and cord serum would be directly proportional to the selected markers of insulin sensitivity. The introduction focuses on the supplementation and increasing insulin sensitivity, it does not discuss serum DHA and Vitamin D levels and their correlation with insulin sensitivity. This may have to be readdressed.

2. The differences in the levels of adiponectin, leptin and ALR are relatively small in the regression analysis. This could mean that a larger number of women might need to be included in order to attain a significant difference. There is no power analysis to see how many women would be needed to establish a significant difference in these markers with supplementation.

3. The regression analysis dos not account for the effect that mood stabilizing medications may have on insulin sensitivity.

3. Is the work of potential clinical importance?

Based on the data presented there is evidently no benefit from DHA or EPA supplementation to increase insulin sensitivity in pregnancy. There is limited information on Vitamin D levels and insulin sensitivity, suggesting that higher levels are associated with higher sensitivity, but this study is not designed to evaluate Vitamin D supplementation.
4. Provide specific comments on individual sections (see below).

Title

Does the title accurately convey the message of the paper? The title suggests that the paper is about EPA and DHA supplementation. The manuscript also discusses relationship between levels and insulin sensitivity as well as introducing Vitamin D. I think it would strengthen this manuscript to focus on what is proposed in the title and in the first part of the hypothesis. The regressions can be a secondary aspect and should not be the main focus of the manuscript as it is proposed.

Abstract or Summary:

Is the abstract/summary a faithful outline of the paper? I would remove the vitamin D data. It is distracting to the reader as it has no relationship with the main theme.

Can the abstract be understood without reading the manuscript? Yes

Do any discrepancies exist between the abstract/summary and the remainder of the manuscript? No

Introduction

Does the introduction succinctly lay the groundwork for what was done and why it was worth doing? As noted above, need to address the literature regarding omega acid serum concentrations and Vitamin D serum concentrations and insulin sensitivity.

Materials and Methods

For original research papers, is the hypothesis clearly stated? Yes.

Is the study design appropriate to allow their hypothesis to be tested? This is a secondary analysis of a previous study analyzing the effects of omega acid supplementation. The authors had limited input on study design.

Could another investigator reproduce the study using the methods as outlined? Yes

Are the data collected and analyzed properly? The main analysis is the effect of supplementation of insulin sensitivity. The authors performed multivariate regression using maternal weight gain,
BMI. These are both related and can confound both predictors and outcomes causing significant positive bias in results.

Results

Are the results valid based on the methods used? The results are valid. I would recommend additional analysis or reporting the regression using the delta adiponectin, leptin and ALR. Also would see if information is available regarding what medications the women were exposed to in pregnancy as these can influence outcomes.

Page 12 lines 16-26: This statement was not clear in results, where the authors did not state the direction of the association based on the regression.

Discussion

Does the discussion adequately compare and contrast the results with those of other papers that have previously been published?

The discussion might benefit from focusing on the main outcome: supplementation did not affect insulin sensitivity as assessed by using surrogates. Authors should focus on this outcome and how it could be affected by sample size, medications, timing of second maternal blood sample.

Is there a paragraph that acknowledges study limitations?

Yes

Does the conclusion of the article give a clear "take-home message"? This message should be adjusted to reflect what was proposed and done.

Tables: Table 2: not clear what numbers 1 and 3 mean in second column..

References

Are the references current and comprehensive?

Yes.

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

Yes

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

Yes

**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.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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