Author's response to reviews

Title: Smoking during Pregnancy and Risk of Abnormal Glucose Tolerance: A Prospective Cohort Study

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Author's response to reviews: see over
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Melissa Norton, MD
Editor-in-Chief
BMC Pregnancy and Childbirth

MS: 1778665802333399

Dear Dr. Norton:

Thank you for considering our manuscript “Smoking during Pregnancy and Risk of Abnormal Glucose Tolerance: a Prospective Cohort Study” for publication. We have altered the manuscript in accordance with reviewers’ comments using underlining. Below, please find our point-by-point response. We would also like to thank the editor and reviewers for their helpful comments on our manuscript.

Sincerely,

Lisa Chasan-Taber, ScD
Associate Professor of Epidemiology
Response to Reviewers’ Comments

MS: 1778665802333399
“Smoking during Pregnancy and Risk of Abnormal Glucose Tolerance: a Prospective Cohort Study”

Reviewer #1
Reviewer: Emily Oken
Overall:
This is a well-written paper examining associations of smoking before & during pregnancy with risk for GDM. Exposures and outcome are measured reasonably well, though not via gold standard techniques (e.g. cotinine, fasting GTT). Power is a major issue with this study, as the wide confidence intervals include effect sizes that could be important. In addition, the authors need to justify better why and how smoking might lead to glucose intolerance.

Major compulsory revisions
1. Page 7: “those factors that changed the association between smoking and preterm birth by 10%”. I assume the authors meant AGT, not preterm birth?

We apologize for the typographical error and have revised the term to state “AGT”.

2. In the introduction the authors should provide some justification or potential mechanism for why smoking should predispose a woman to develop AGT or GDM. Is the interesting period of exposure before pregnancy or during pregnancy? If both, why? A statement of hypothesis would be extremely useful.

We have revised the Introduction (page 3, para 2) to now propose a potential mechanism for why smoking would predispose a woman to develop AGT or GDM. We have also added a statement of hypothesis (page 4, last para).

3. In the analysis of quitters, adjustment for gestational weight gain mainly resulted in a widening of the confidence limits, but not much of a change in the point estimate. The effect may be real, but obscured by the low power. Overall, most of the confidence limits are quite broad and inclusive of effects that may be important. I am not convinced this is an informative null study. The authors should discuss the issue of power/sample size.

The reviewer raises an important point. The concern regarding the low power for the subanalysis among quitters was also raised by other reviewers. In light of this concern, we have removed this subanalysis from the manuscript.

4. Given this issue of power, which appears to be a major limitation, did the authors consider using the outcome as a continuous variable?

This is an excellent point and we have revised the manuscript to now include an analysis of the outcome as a continuous variable (page 10, last para; and Table 3). Indeed we have excellent
power (>99%) to detect clinically significant differences in screening glucose levels using a two
group t-test with a 0.050 two-sided significance level. We also now comment on the issue of
limited power in the Discussion section (page 13, last para).

Minor essential revisions

5. The beginning of the introduction is somewhat misleading, as it is written as though AGT and GDM are two distinct conditions; for example the statement statement ‘ranging from abnormal glucose tolerance (AGT) to gestational diabetes mellitus’. I suggest the authors clearly define AGT from the start and make it clearer that AGT includes GDM as well as milder degrees of glucose intolerance.

We have revised the Introduction (page 3, para 1) to now clearly define AGT and its relation to GDM.

6. “Women who quit smoking gained significantly more weight up to the time of GDM screen as compared to continued smokers (p=0.02).” Please provide the amounts of weight gain in each group, or the difference, not just the p value.

Because this subanalysis has been removed (please see response to comment #3 above), this sentence is no longer in the manuscript.

7. Although the authors state that they included 1006 women in this analysis, Table 2 suggests that a smaller number was included in each analysis. Please report somewhere the total N available for each exposure.

We have revised the Methods section (page 6, last para; page 7, 1st para) and Table 2 to clarify the total N available for each exposure.

Minor discretionary revisions

8. It would be helpful if the authors would clarify throughout what is the reference group. That information appears in the table but should also appear in the text.

We have revised the Results section to consistently clarify the reference group.

9. Please include the sample size in the abstract.

We have revised the abstract to include the sample size.

Reviewer 2

Reviewer: Magnus Fasting

The authors have written a paper on the association between smoking in different stages of pregnancy and the occurrence of abnormal glucose tolerance (AGT) among a cohort of Hispanic mother-child pairs in Western Massachusetts. Their main conclusions were that light pre-pregnancy smoking and pregnancy awareness smoking cessation may be associated with increased occurrence of AGT. A secondary conclusion was that smoking during pregnancy was not associated with the development of AGT. The paper is in general
well written, and the data supports the main conclusions drawn. However, I have some issues of concern.

Major compulsory revisions:
1. The authors cite several previous studies assessing the research question, as well as a recent review. These studies mainly conclude that there is no association between maternal smoking in pregnancy and AGT. They also argue that the study has merit because the population under study is Hispanic. What I miss is a clear research hypothesis stated in the introduction, and a discussion of the results according to this hypothesis. Would you believe that the association between maternal smoking and AGT would be different between other ethnicities and Hispanics? And in that case, why?

We have revised the Introduction (page 4, last para) to present a clear research hypothesis and now address whether the association would differ by ethnic group (page 3, last para). We have also revised the discussion (page 12, last para; page 13, 1st para) to interpret results according to these hypotheses.

2. The authors state in the methods section that the final sample size is 1006. However, they operate with different numbers of subjects in all the tables. I would like to know how many of these they had exposure data, i.e. smoking status at different stages of pregnancy.

We have revised the Methods section (page 6, last para; page 7, 1st para) and Table 2 to clarify the total N available for each exposure.

3. The authors elaborate on how the confounders for the statistical analyses were chosen in the methods section, and I have some comments to this section. First, there is clearly a typographical error, where the authors state: “To assess confounding we independently included each potential confounder in the model: those factors that changed the association between smoking and preterm birth by more than 10% were included…” Second, the authors do not state which variables that became confounders after this test, which they should. Third, the authors also state that some variables were chosen as confounders a priori without stating why, they should do that as well. The authors could do these changes, and it would be okay by me. However, I personally, have some problems using the 10%-cutoff method to arbitrarily choose confounders. In my opinion, a better method, is to just choose confounders a priori, based on knowledge of the subject at hand.

We have corrected the typographical error noted by the reviewer. In addition, we have revised the Methods section (page 8, last para) to now justify that age, BMI, and gestational weight gain were selected a priori for inclusion in multivariate models based on their established associations with AGT. We also now clarify (page 8, last para) which factors were included based on the 10% cutoff method (i.e., education and parity).

4. The authors report in the beginning of the results section and in Table 1 several associations between various covariates and abnormal glucose tolerance. I count 13 statistical tests, suggesting that: higher age, higher education, higher household income,
higher prepregnancy BMI, family history of diabetes, personal history of GDM and current history of hypertension is associated with AGT. Some of these results, that higher education and higher household income is associated with AGT is surprising to me. However, the authors do not discuss these findings at all, and they do not focus on these associations as a research questions. I suggest removing these statistical tests from the table, and use table 1 as a purely descriptive table of the population. However, if the authors disagree, at least they should discuss these results in the discussion.

We agree with the reviewer and have removed these statistical tests from the table and Results section.

5. Table 2 is the main table of the study, and I have some questions about the number of mother-child pairs in this table. When I sum the non-smoker and smoker categories for pre-pregnancy, early pregnancy and mid-pregnancy smoking, I get 912, 847 and 697 subjects, respectively. Additionally from table 1, the number of mother-child pairs with information on age, prepregnancy BMI, parity, weight gain and education (variables in the multivariable model) is 1006, 986, 1003, 805 and 909, that is, 20% missing in one category. This brings me to the question: how many mother-child pairs were included in the statistical analyses in the different steps? Did the authors restrict the analyses to only those with complete information in the unadjusted analyses? Often, small changes in odds-ratios observed like this are simply due to subjects falling out of the statistical analyses because of missing variables. I would like the authors to clarify this.

We have revised the Methods section (page 6, last para; page 7, 1st para) and Table 2 to now clarify the final N included for each exposure. To examine the potential effect of missing data, as the reviewer has suggested, we have conducted an additional analysis comparing the characteristics of women who were missing pre-, early-, and mid-pregnancy smoking information to those with complete smoking information during these time periods, as well as comparing the missing groups to each other (Results: page 11, para 2). We have also revised the Discussion section (page 14, para 2) to comment on the implications of this missing data.

We have revised the Methods section (page 6, last para; page 7, 1st para) to clarify that we limited our regression analyses to women with complete information on smoking and AGT.

In order to retain a consistent sample size between unadjusted and adjusted analyses, women with missing information on any covariate (i.e., age, BMI, weight gain, parity, and education) were retained in the model by assigning their covariate category to ‘missing’ for that specific covariate. We have clarified this in the Methods section (page 8, last para).

6. Despite that the authors do not state this as a research object in the background section, the main conclusion of this paper is that maternal smoking cessation at pregnancy awareness may be associated with the development of AGT, possibly through increased pregnancy weight gain. The authors support this idea by the finding that those mothers who were light smokers before pregnancy had a possible elevated odds ratio for AGT compared to never-smoking mother. I find this conclusion a bit too speculative based on
the data and literature presented in this study. I suggest that this finding is reduced to a secondary finding, and that the sequence of the discussion is changed accordingly.

In light of this concern and in response to concerns raised by the other reviewers, we have removed the subanalysis evaluating smoking cessation.

7. In the literature referenced in this paper, only human epidemiological studies are presented. Does it exist any animal studies on this subject, possibly shedding light on a putative biological basis of the associations presented?

We have added a discussion of the potential biological mechanism to the Introduction (page 3, para 2) and to the Discussion (page 12, last para; page 13, 1st para).

Minor compulsory revisions
1. The authors state that the mean weeks of gestation at inclusion was 15 weeks. I would like them to also report some measure of the spread of this variable, e.g. standard deviation or min-max. The same is for the mid-pregnancy interview.

We have revised the Methods section (page 2, para 1&2) to clarify the standard deviation of the weeks of gestation at the early- and mid-pregnancy interview.

2. The authors wanted to examine the effect of quitting smoking with the onset of pregnancy in relation to the risk of AGT. They state that they studied 316 women who smoked at pregnancy awareness, of whom 45% quit at pregnancy awareness. However, in table 3, I miss the total number of subjects in the cont. smoking and the quit smoking categories. Is the 16 and 7 the number of subjects with AGT, or is it a typographical error and was it supposed to be the total number of subjects?

A concern regarding low power for this subanalysis among quitters was raised by other reviewers. In light of this concern, we have removed this subanalysis and the corresponding Table 3 from the manuscript.

Minor discretionary revisions
1. The authors use, as many others also do, the wording “statistically significant association” several times in the papers. However, this wording is unnecessary and puts too much focus on the p-value. If there is an association between two variables, it is implied that this association is statistically significant. If it wasn’t, there would be no association. For example, in the first paragraph the authors write: “… however, this result was attenuated and no longer statistically significant after adjusting for weight gain…” A better way to word this, in my opinion, would be: “… however, this result was attenuated after adjusting for weight gain …”

We have revised the Results to avoid use of the term “statistically significant”.

Reviewer #3
Reviewer: ELIANA WENDLAND
Minor Essential Revisions
1. In page 4, paragraph 1, line 3: replace “non-methanol” by “non-menthol”.

We have made the suggested correction.

Major Compulsory Revisions
2. In general, the article is well written and the research question is interesting and of public health significance. However, there are some considerations to do:
   • This is not the first time that the association between smoking and glucose intolerance was studied in women from Latino America. Wendland et al. 2008 (Arq Bras Endocrinol Metab 2008;52/6) have studied over 4000 Brazilian women.

   We apologize for the oversight and have revised the Introduction (page 3, last para) and Discussion (page 12, para 2) to cite the Wendland article (citation #21).

3. Many previous studies that explore this association between smoking and gestational diabetes and AGT have not found any association. Wendland et al. showed that parity is an important confounding factor in the association. After controlling for maternal age, BMI weight gain, study center, and race, they found an inverse association between smoking and GDM in nulliparous women that smoke during pregnancy. After this publication, it is important to explore the effect of parity in the Puerto Rico Latin women.

   We agree with the reviewer and, in our data, found parity to be an important confounder of the association between smoking and GDM and included parity in our adjusted analyses. Our sample size was too limited to evaluate parity as a potential effect modifier and we have added this suggestion for future research to the Discussion section (page 15, 1st full para).

4. Although not statistically significant, the measure of association between smoking and AGT was less than 1 and the association is increased after adjustments. It would be interesting to explore at greater length the reasons for these findings in the discussion.

   The reviewer raises an important point. We have added a column to Table 2 showing the age- and weight-gain adjusted results such that the reviewer can see that adjustment for weight gain was largely responsible for the strengthening of associations. We have revised the Discussion (page 12, 1st para) to explore this finding.

Reviewer #4
Reviewer: J Liu

The authors used the data from a prospective cohort of 1,006 Hispanic pregnant women to examine the association between smoking during pregnancy and risk of abnormal glucose tolerance (AGT). The smoking status was self-reported by the respondents at recruitment (mean=15 weeks) and mid-pregnancy (mean=28 weeks). The outcome AGT was defined as >135 mg/dL on the routine 1-hour glucose tolerance test. The study found that women who smoked 0-9 cigarettes/day before pregnancy had an increased risk of AGT compared to the non-smokers after adjusting for age. The relationship was insignificant after additional
adjustment of BMI, gestational weight gain (GWG), parity and education. Smoking in early and mid pregnancy was not associated with AGT. Quitters were about twice as likely to develop AGT compared to continued smokers but this association became insignificant after adjusting for GWG and other risk factors. The authors concluded that their findings do not support an association between smoking during pregnancy and AGT. Pre-pregnancy smokers who quit during pregnancy may have increased risk of AGT. Here are some comments and suggestions for its further improvement.

1. It would be useful to provide a flow chart to describe sample attrition process for the study. It is not clear why sample sizes for Table 2 were different from the statement "...a final sample size of 1,006 participants" (p5). If this is due to missing measurements at different visits, please specify. It is not clear why the sample size for pre-pregnancy and early pregnancy were different given that the information was collected at one time (first study visit).

A similar concern was raised by the prior reviewers and we have revised the Results section (page 6, last para; page 7, 1st para) and Table 2 to now clarify the final N for each of the exposure variables. The reviewer is correct that the information on pre-pregnancy and early pregnancy smoking were collected at the same study visit. However, because this visit took place at the time of a prenatal exam, women were often called into their exam in the middle of the interview and were unable to complete the questionnaire. We have added a comparison of those women missing data at pre-, early-, and mid-pregnancy with those women not missing this information to the Results section (page 11, para 2) and comment upon this in the Discussion section (page 14, para 2). These women were similar to those with complete smoking data.

2. Related to comment 1, due to various sample sizes for the analysis, it is hard to make comparison about the effects of smoking status on AGT at different time points. The authors only compared the characteristics of the sample of 1006 women who were screened for GDM with those who did not screened for GDM. In fact, due to the different sample sizes for different analysis, it is necessary to compare the differences between analytical samples as well.

We agree with the reviewer, and we now compare the characteristics of women who were missing pre-, early-, and mid-pregnancy smoking information to those with complete smoking information during these time periods, as well as comparing the missing groups to each other (page 11, para 2). We now comment on this in the Discussion section (page 14, para 2).

3. I also noticed that the study may have limited power to detect the effect of light (0-9 cigarettes) and heavy smoking (>=10 cigarettes/day) on AGT (<10 AGT women in those categories per Table 2). I wonder whether the authors have considered to categorize the smoking patterns in the following way: 1) non-smokers (never smoked in pre, early-, mid-pregnancy); 2) persistent smokers (smoked in all periods), 3) quitters (those who quitted smoking after pregnancy). This coding might be useful for you to do a unified analysis using all information available to you (~1,006 women). If a woman who has missing values in smoking status in early or mid-pregnancy, you will use whatever information is available to you to determine whether she is a smoker or quitters... You may need to consider how to
code those weird smoking patterns (such as women might start to smoking during pregnancy) based on their answer etc.. The similar method of studying smoking patterns using PRAMS-questionnaire was used in prior study (Liu J. 2006, Am J of Public Health). If you have adequate sample size, you might consider to split category 3 into (early quitters, those who quit in early preg, mid quitters, those who quit in mid-pregnancy).

This is an interesting suggestion, and we performed an analysis replicating the categorization used in the Liu et al. AJPH manuscript. However, our study design differed in several important ways from the AJPH manuscript. For example, we had 2 as opposed to 3 exposure assessment periods during pregnancy and also did not have information on postpartum smoking. This smaller number of exposure assessment periods limited the opportunity for women to be classified as quitters or relapsers. In addition, the number of persistent smokers (pre, early, and mid pregnancy) in our study was too small to constitute a category. Because we observed that our findings using this new categorization scheme were not substantively different from those presented in the original manuscript, we retained our original categorization. However, in response to concerns regarding statistical power, we have added an analysis of 1-hr OGTT plasma glucose values as a continuous outcome measure.

4. Re. the analysis, the authors stated the attenuation of the effects might be caused by gestational weight gain. This was not fully supported by the results presented because you did age-adjusted analysis first, then you adjusted multiple variables (GWG, parity, BMI, education) simultaneously. Thus, it would be useful to present a separate model to show the estimate was significant after adjusting for parity, BMI, education and it became insignificant after adjusting for GWG... This applied to all types of models presented in Table 2 and 3. Per your results, GWG seems not different by AGT status. However, is GWG different by smoking status?

The reviewer raises an important point. We have added a column to Table 2 and Table 3 showing the age- and weight-gain adjusted results and now describe these results in the text (page 10, para 2) such that the readers can now see that adjustment for weight gain was largely responsible for the strengthening of associations. We have revised the Discussion (page 12, 1st para) to comment upon the reasons for this finding.

Although non-smokers tended to gain slightly more weight on average during pregnancy as compared to smokers, these differences were not statistically significant.

5. Authors may consider adding justification on why they selected AGT as the outcome instead of GDM, given that GDM is the focus of the main study. How did they decide the cut-off points of 135? Did the authors consider to use glucose as a continuous variable instead of a categorical AGT?

We have added a justification to the Introduction explaining why we chose AGT as the outcome (page 3, para 1). We now more clearly emphasize that recent large studies have found a consistent, linear increase in risk of adverse perinatal outcomes over the entire range of maternal blood glucose levels, not just with GDM, suggesting that identifying risk factors for milder degrees of disturbances in glucose metabolism is critical for maternal and offspring health.
In response to the reviewer’s suggestion we have revised the manuscript to now include an analysis of the outcome as a continuous variable (page 10, last para; and Table 3). We also now comment on the issue of limited power to evaluate frank GDM in the Discussion section (page 13, last para).

We also now justify the use of the cutpoint of 135 mg/dL in the Methods section (page 7, para 2).

6. No information was provided on how quartiles of pregnancy physical activity were developed.

We have updated the Methods section (page 7, para 3) to provide information on how quartiles of pregnancy physical activity were developed.

7. According to citation 9 (review article), it seems that more studies were published on this association in addition to what were cited in this article (#10-15).

We have updated the Introduction and Discussion to cite the full breadth of articles mentioned in the review.

Editorial Comments

1. Please add additional information on the study question/aims to the background section of the abstract.

We have revised the Introduction to now add additional information on the study question/aims (page 4, last para).

2. Please clarify the ethics declaration in the methods section to state whether the study was approved by the institutional review board.

We have revised the Methods section to state that the study was approved by the institutional review board (page 5, para 1).

3. Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.

We have highlighted with underlining all changes made in this revision.