Author's response to reviews

Title: Inadequate glucose control in type 2 diabetes is associated with impaired lung function and with systemic inflammation.

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Author’s response to reviews.

Title: Inadequate glucose control in type 2 diabetes is associated with impaired lung function and with systemic inflammation.

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Dear Editors:

Please find attached our revised manuscript entitled “Inadequate glucose control in type 2 diabetes is associated with impaired lung function and with systemic inflammation” for consideration of publication in *BMC Pulmonary Medicine*.

We would like to thank both reviewers for their comments and critique. The revised manuscript attempts to address all the reviewer’s comments. We think that the revised manuscript is now much improved in clarity due to their observations. Below please find our point by point answers to the observations.

This study was made possible by funding from COLCIENCIAS, Colombia, Code 2239-04-16300. No part of this work has been previously published; an e-presentation was presented at the European Respiratory Society 19th annual congress, Vienna, September 2009. No part of this manuscript is under consideration in any other journals. All of the authors have reviewed the manuscript and approve of its content. As described in the manuscript, all authors have contributed significantly to the manuscript. No author discloses any conflict of interest that relates to this work.

We thank you in advance for your consideration of this revised manuscript and for the length of time provided to us to revise it.

Respectfully,

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Reviewer Prof. Davis:

Abstract:

- **Results** - Second sentence: Those with inadequate control had lower (not higher) mean residuals for FEV1 and FVC and higher (not lower) residuals for FEV1/FVC.

  Reply: This has been corrected now, thanks.

- **Results**: - Final sentence: Please specify the P value more accurately (i.e., P<= 0.039).

  Reply: We think that it is equally clear to show P values that are less than 0,01 or 0,001 as “P < 0,01” or “P<0,001” respectively, given the very low probability of chance explaining the results under the null hypothesis of no differences between groups. We showed the P value for the inflammation markers as “P < 0,05” because it refers to all the P values in table 3 with the exception of IL6. The exact P values are shown in Table 3. We suggest leave as currently is in the abstract.

- **Methods**: The authors did not exclude people with asthma or emphysema or other COPD. How many of the study subjects fitted into one or more of these categories?

  Reply: All subjects were asked for prior diagnoses of emphysema, chronic bronchitis, and asthma. No other COPD conditions were enquired. There were 2 subjects with prior diagnosis of emphysema (both in the inadequate glucose control group), 11 with chronic bronchitis, 8 in the inadequate control group (2.2%) and 3 in the adequate control group (2.1%), and 12 with asthma, 8 in the inadequate control group (2.2%) and 4 in the adequate control group (2.8%). These differences were not statistically significant. We now include these frequencies in Table 1.

- **Sample size**: “y” should be replaced by “and”.

  Reply: This has been corrected now, thanks.

- **Statistical Analysis**: “ACCESS” has double S.

  Reply: This has been corrected now, thanks.
• The authors state that the prediction equations by Hankinson et al for Mexican-Americans provide a good fit for Colombian subjects based on previous studies by their group. Can the authors cite a reference for this work?

Reply: The citation has been included now. The reference is: Rojas MX, Dennis RJ. Valores de referencia para parámetros espirométricos en la población adulta residente en Bogotá D.C., Colombia. Biomédica. 2010; 30(1): 82-94.

• The statistical analysis mentions adjustment for exposure to wood smoke. The results include lifetime exposure to dusts and gases as well as wood smoke. How were these environmental factors estimated?

Reply: We used a respiratory questionnaire: “Epidemiology Standardization Project (American Thoracic Society. Ferris BG. Am Rev Respir Dis. 1978 Dec;118(6 Pt 2):1-120”, which had been translated into Colombian Spanish, adapted, and previously used in other published projects. We have now added a new sentence under “Methods” (data collection section) clarifying this, and added the proper citation.

Adjustment for exposure to wood smoke was based on a yes/no response to the question “Have you regularly cooked or worked in a kitchen using a woodstove?” Although we also collected data on years of exposure, we did not use this variable in the analysis.

• Results: Subject characteristics: How was past clinical history of cardiovascular and pulmonary co-morbidity defined and ascertained?

Reply: Please see above as well. We used standardized questions on history of hypertension and medication use, coronary heart disease, heart failure, stroke, asthma, CB, emphysema, symptoms of cough, sputum, wheezing, and dyspnea.

• Table 2 – Typos in the variable names.

Reply: This has been corrected now, thanks.

• In the text the authors state that the higher FEV1/FVC in those with inadequate glycaemic control did not reach statistical significance, but it did according to table 2 (P=0,0447). Which is correct?

Reply: Table 2 is correct. The reviewer is correct in pointing out the statistical significance. The text has been corrected now, thanks.

• Likewise, in the text it is stated that FVC % predicted <70% was not statistically significant by glycaemic control status, but in table 2 the P-value for FVC % predicted <70% is 0,0200, which is significant. Which is correct?
Reply: Table 2 is correct. The reviewer is correct in pointing out the statistical significance. The text has been corrected now, thanks.

- **Relationship between glucose control and pulmonary function:** First sentence: there needs to be a minus sign in front of the lower CI.

  Reply: This has been corrected now, thanks.

- **Please state what the “minimally” increased values of FEV1/FVC and their differences were.**

  Reply: This has been stated now, thanks.

- **Table 4:** In the text it states that the mean residual values are stratified by BMI and woodsmoke exposure history as well as smoking history, but the table shows only stratification by smoking history. Please amend appropriately.

  Reply: This has been corrected now. In a previous version we had data in the table stratified as well by BMI and woodsmoke exposure history, but it was deleted for the current version.

- **As table 4 stands, since smoking history is stratified, the mean residuals were not adjusted for smoking history. This should be deleted from the title and the foot note.**

  Reply: Values in bold are mean residuals for FEV1, FVC and FEV1/FVC adjusted for differences in age, height, sex, and smoking history. Values not in bold stratified by smoking history are mean residuals adjusted for differences in age, height, and sex. This has been made clear now in the title with reference to the footnote.

- **Please can the authors add the inflammation markers to the multiple linear regression models to see if, after adjusting for age, sex, height, smoking history, and HbA1c, any of them add significantly to the models?**

  Reply: While this is a very good comment, it was not in our *a priori* objectives and would constitute an *a posteriori* analysis difficult to put into context in the current manuscript. Besides, one of the current hypotheses we discuss is that inflammation may be in the causal pathway between inadequate glucose control and lung involvement, so we may be over-adjusting by having HbA1c and the markers simultaneously in the model. We will certainly test the hypothesis suggested by the reviewer but we beg to disagree that it should be part of this publication.

- **Discussion:** 3rd paragraph first sentence: “and” instead of “y”.

  Reply: this has been corrected now, thanks.
• Discussion: 3rd paragraph, second sentence: Since the study is cross-sectional, the authors might want to temper their statement that their results suggest a causal pathway.

Reply: Agreed. We have now changed this to “...suggesting a potential association”.

Additionally, in our conclusions, we also state that: “...our results need to be confirmed by longitudinal studies (given that diabetic control is a time-dependent variable)...”

• 3rd paragraph, last sentence: “cytokines” not “citokines”.

Reply: This has been corrected now, thanks.

Reviewer Prof. Savage:

• This report indicates that there is an association between levels of HbA1c of 7% and below vs. higher levels with measures of decreased pulmonary function and some laboratory measures of inflammation. The authors should explain why they chose this “cutpoint” for the primary analysis. Do they have any evidence to expect a change in risk over a narrow range? How do they integrate these analyses with results of the quintile analyses in table 5?

Reply: Our a priori hypothesis was related with the potential impact of adequate versus inadequate glucose control on lung function and the association with makers of inflammation. We did not want to first look at the data and then as a posteriori hypothesis, select a “cut point” for HbA1c values that would maximize differences in further analysis. We believe that from a clinical point of view our primary analysis has merit, and an HbA1c “cut point” of 7% is used often in clinical practice to discriminate between adequate or inadequate control (although we do acknowledge in the Discussion that only over the past few months, as well as the limitations of the cross-sectional design approach). We now explain this in the text in the Methods section, and add a reference to support this “cutpoint”, the consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes (Diabetes Care. 2009 Jan;32(1):193-203).

Of secondary interest and more exploratory in nature was to evaluate, for the inflammation markers and for lung parameters, what shape would a “risk” function take over quintiles of HbA1c values, as shown on table 5.

• There is a difference in reported duration of diabetes between the “adequate” and “inadequate” control groups. The authors should comment on the potential implications of this difference. Despite the well known problems with estimating the duration of diabetic hyperglycemia in a patient with type 2 diabetes, this difference could explain at least part of the difference in observed lung function and inflammatory marker levels
that the authors ascribe to differences in glycemic control. In other published studies, both duration of diabetes and degree of control have been linked to impaired lung function.

Reply: We totally agree with this comment and we are aware that previous studies have shown that diabetes duration impacts on lung function. Given that we found disease duration to be associated with degree of control, the issue we see in our study is if disease duration is confounding the association between degree of control and lung function, as well as the association with inflammatory markers. We conducted our analysis adjusting as well for disease duration, and the results did not change significantly. We mention this in the text in the Discussion section. However, because of the limitations in the assessment of diabetes duration by patient history and/or chart review (please see below), and the possibility of residual confounding, we now acknowledge this in the discussion:

“Although those diabetic subjects with poorer control also had longer disease duration, adjustment for time since diagnosis did not change results; given that we assessed diabetes duration by patient history, however, we can’t exclude residual confounding and non-differential misclassification as a possible explanation for this finding. “

- Information on existing chronic or recent pulmonary disease should be included in table 1.

Reply: This has now been done.

- Speculation on mechanisms for which relevant data and analyses are not provided can be minimized or deleted.

Reply: This has now been done, specifically in the Discussion section.

Minor Essential Revisions:

- The revision should also include more data on the specific lab tests and information re quality control of the assays, both general and when measures where in different batches, comparability of control data over time.

Reply: Blood samples were taken after a minimum of 6 hours fasting with the exception of those diabetics dependent on insulin, using evacuating tubes. Samples were centrifuged and processed on a daily basis for glucose fasting levels, HBA1c, fibrinogen and ferritin, following standard daily laboratory procedures. A set of serum samples were stored at -20°C for a maximum of two months for other analyses. TNF-\(\alpha\), IL-6, and high sensitivity C-RP were measured by a solid phase, enzyme labeled, chemiluminescent sequential immunometric assay method, using the Immulite 1000 analyzer (EURO/DPC Ltd, Llanberis, UK). A total of seven runs in batches were conducted during the course of the study. Each run for C-RP, TNF-\(\alpha\) and IL-6 was
processed in duplicate, and met standard specific control assays provided by the manufacturer, two for TNF-α and IL-6 (high and low), and three for C-RP (high, intermediate and low).

This has now been included in the text, in the Methods section.

- **Indicate units of measure for all variables in table 1.**

  Reply: This has now been done.

- **There is no information on the clinical significance of the abnormalities found. Brief comments on the clinical importance of the magnitude of changes observed may be helpful to readers.**

  Reply: We do agree with the reviewer on this point, although it is difficult to justice how important is the magnitude of change from the clinical perspective given the cross-sectional approach of our study; ie, the importance of changes in lung function has usually been judged as the rate of change overtime derived from observational cohorts or RCTs. We have now included a sentence on this in the Discussion section: “From a clinical perspective, the importance of changes in lung function has usually been judged as the rate of change overtime derived from observational cohorts or clinical trials, thus, it is difficult to justice how important is the magnitude of change observed in this study, given the cross-sectional approach.”

Discretionary Revisions:

- **P4, p1, l2: What data do you have on the case mix of patients in your study sample relative to cases seen in the area and among other patients attending your center – how representative of the local diabetic population is your sample? How is the cost of care at your institution covered?**

  Reply: We do not have comparative data relative to cases attended elsewhere; but based on the fact that this centre offers standard ambulatory care for diabetic patients of all socio-economic strata (by contracts with most health maintenance organizations in the city that provide managed care to which about 70% of the population is affiliated or by out of pocket payments), we think these patients are locally representative of the local diabetic population with no relevant filters for case-mix or a particular severity of disease. We suggest leave the same description as is now: “The sampling frame from which patients were selected was that of the Colombian Association of Diabetes (ACD), a private institution in Bogota, Colombia, that provides basic and specialized ambulatory care for diabetic patients of all socio-
economic strata; patients need not be referred with any special degree of severity or case-mix. “

- **P4, p1, l8:** Do you have verified data on the duration of diabetes among study participants? If so, what type of data—patient history, chart review, sequential glucose documentation, etc.?

  The patient was the primary source of information with regards to disease duration, and this was validated with chart review. We have now included this in the text. In case of discrepancy, we selected the one provided by the patient, given the standardized nature of our data collection strategy. We have now acknowledged as well the limitations of this strategy in the Discussion section (please see below).

- **P4,p2,l4:** what was the total number of volunteers excluded? Can you provide more information about exclusions for known chronic or recent acute inflammatory lung disease?

  Reply: We contacted 1888 subjects with a past history of Diabetes type 2. The most frequent cause for exclusion was age outside the stipulated limits (about 65%). Past history of TB, lung fibrosis, lung cancer, and thoracic trauma or surgery accounted for close to 21% of exclusions. A history of rheumatoid arthritis was next. We have included this information now in the results section: “We contacted 1888 subjects with a past history of Diabetes type 2. The most frequent cause for exclusion was age outside the stipulated limits and past history of TB, lung fibrosis, lung cancer, and thoracic trauma or surgery.”

- **P4,p3,l3:** Pulmonary function averages may vary with racial/ethnic group. What do you know about the comparability of lung function measures in the two referenced studies to the group you studied?

  Reply: The second reference is a study in our own population here in Colombia by our group. Additionally, and as mentioned, the reference equations we used had been previously validated locally, reference of which is now included.

- **P5,p2,l3:** Please provide more information on laboratory methods used and quality control information for your labs for each of the tests. How were samples processed, stored? If all samples were not run in the same assay, include information re stability of samples and control results for assays run over time.

  This has been provided now, with emphasis on C-RP, TNF-α and IL-6 (please see above).

- **P6,p2,l3:** See earlier question as to how diabetes duration was determined. Because of the possible long time between development of diabetic hyperglycemia and the
diagnosis of diabetes in type 2 diabetes patients, duration data derived from patient history or chart review is known to contain considerable error. This should be acknowledged.

Reply: We agree with this comment. As mentioned in the text now, disease duration data was derived by patient history and validated by chart review. We now acknowledge the limitations this may have, in the Discussion section (please see below).

• P6,p2,l12: define GOLD stage 1.

Reply: GOLD stage 1 (as different from stage 0) has a FEV1/FVC ratio below 70%. We show this in the text. We believe it should be sufficiently clear as is.

• P7,p2,l2: please clarify. Are you presenting results for both diabetics with a adequate and inadequate control versus results from your reference population? Did the reference population include patients with diabetes? Do your data indicate that results in your adequate control group are still lower that the predicted value?

Reply: As mentioned in the Methods section here, we used reference equations validated previously by our group in Colombia (we have now included the reference), and originally derived in Latino populations in the US. We are not aware that this reference population systematically excluded subjects with diabetes. Our present study also had a control arm of subjects without diabetes, results of which have been published locally here in Colombia (Dennis R, Maldonado D, Rojas MX, et al. Diabetes Mellitus tipo 2 y deterioro de la función pulmonar. Acta Med Colomb 2008; 33: 105-110). With a similar analysis as that presented here, we showed lower predicted lung function between diabetics in general and controls that validate the results presented here.

• P7,p3,l3: Diabetes (not diabetics).

Reply: This has been corrected now, thanks.

• P8,p1,l2: Comment more on results in table 5 and address question of whether this data support a dose effect in the Discussion.

Reply: This has been done now. The phrase now reads as: “Similarly, those subjects with poor control also had significantly increased levels of inflammation markers (TNF-α, ferritin, fibrinogen, and C-RP), although, as presented in table 5, there is no clear, relatively linear dose-effect relation.”

• P8,p1,l2: Did the authors look at possible associations of type of diabetes treatment with lung function and inflammatory markers? Could other drugs, such as statins, have altered inflammation?
Reply: This is an excellent comment, but beyond our current study objectives. We are currently finishing data collection on medication exposure on these subjects, but it will be the basis of a different publication.

• **P8,p3,l5:** The comment on smoking is speculative. The data on pack years are not available.

Reply: We agree that our comment is not strictly based on our observed results, and that is why we acknowledge this: “Persistent confounding by smoking may be possible given that we did not quantify the intensity of lifetime exposure (i.e. pack-years)”, but we still believe it is very important to comment that “…we think that the likelihood of this is low, however, because the intensity of current cigarette exposure seemed to be lower in those with inadequate control.” We would ask to be able to keep this additional comment.

• **P8,p3,l8:** The duration issue is a problem because of the well known difficulty of clearly establishing the duration of type 2 diabetes. Failure of adjustment to change results may be due to accuracy of the duration data. If, as table 5 suggests, there is not a strong, relatively linear relationship with lung function abnormalities, inflammatory markers and control, it is even more difficult to interpret these data (HbA1c gives an estimate of glucose control over several weeks, not the long run). Comment on the possibility that both duration and degree of glycemic control may alter pulmonary function abnormalities.

Reply: We agree with this comment, as mentioned before. The phrase has now been modified as follows: “Although those diabetic subjects with poorer control also had longer disease duration, adjustment for time since diagnosis did not change results; given that we assessed diabetes duration by patient history, however, we can’t exclude residual confounding and non-differential misclassification as a possible explanation for this finding.”