Author’s response to reviews

Title: Susceptibility of Prediabetes to the Health Effect of Air Pollution: a Community-Based Panel Study with a Nested Case-Control Design

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Response to reviewers’ comments

Reviewer #1: General Comment:
This is a very well written manuscript. It is the first to directly compare a group of prediabetic individuals with a group of individuals without this condition for PM2.5 effects. The study used an appropriate panel design and approach to evaluate relationships between a large suite of pathophysiological endpoints and PM2.5 exposures in the preceding 1-14 days. Statistical analyses are appropriate and the interpretation of the results are reasonably adequate to support the conclusions. The study found that prediabetic individuals were more susceptible in certain specific pathophysiologic pathways of PM2.5 enhanced effects. This finding has important public health implications, as clearly elaborated in the manuscript. I only made the following suggested edits for the authors to consider.
General Response: We appreciate the reviewer’s affirmation on the importance of these findings, and we also thank for the comments to improve the manuscript with accurate expression. We have corrected the raised points accordingly. Please find the detailed response as followed.

Abstract:
1. Background - add "than in healthy individuals" to the last sentence following "… in PreDM individuals".
Response: Corrected correspondingly.

2. Conclusions - Replace "PreDM population" with "Individuals of prediabetes". Similarly replace "the healthy population" with "healthy individuals”. I don't think the study subjects (60 PreDM vs. 60 healthy controls" are representative of a population. Same comment to the use of 'population" in "5. Conclusions".
Response: Thanks for pointing out. We have replaced “populations” with “individuals” correspondingly for abstract and conclusion section.

Introduction:
1. First page last sentence of the 2nd paragraph - replace "… characterised by pathological chronic inflammation" with "…characterised pathologically by chronic inflammation"
Response: Corrected correspondingly.

2. Last paragraph before the heading of Materials and methods (line 14-15) - replace "…potentially bias the effects…” with "potentially mask the effects".
Response: Corrected correspondingly.

Discussion:
1. First paragraph last sentence: do you mean significantly enhanced (by PM2.5) systemic inflammation, elevated …? This sentence now may read like that PreDM subjects had higher levels of inflammation, BP, etc, than healthy subjects (not related to PM2.5 effects).
Response: Thanks for pointing out the potential ambiguity sentence. We have rephrased the sentence to “Compared to healthy subjects, preDM subjects showed significantly enhanced PM2.5-associated systemic inflammation (increased serum IL-2 and IL-8)…”

2. Last sentence on the 3rd page of Discussion - replace "insignificant changes" with "statistically non-significant changes" or just "non-significant changes". "insignificant" has a different meaning from "not statistically significant'. The word "insignificant" was misused in several other places including figure legends.

Response: Corrected correspondingly.

Tables and Figures
1. Figure 1 and 3: legends should be "Healthy" (not Heath) and "PreDM".

Response: Corrected correspondingly.

Reviewer #2:

The study addresses an important question as investigates if changes in cardiovascular, pulmonary, and inflammatory biomarkers and in BP triggered by air pollution are larger in prediabetic population than in non-diabetic population. Overall results show air pollution related changes in BP and in inflammation and metabolic biomarkers are larger in prediabetic rather than in non-diabetic.

The study explores cardiovascular outcomes (BP) and biomarkers in prediabetic patient. In the cohort description there is no mention about participant being normotensive or not but it seems that a considerable amount of the population is taking antihypertensive drugs so it is likely that they may have been diagnosed suffering from hypertension. Sensitivity analysis excluding subject suffering from hypertension should be performed in order to evaluate the effects of PM on subjects with exclusive glicemic control impairment.

The paper is well written and easy to follow. The report of the results could benefit addressing the following comments.
General Response: We appreciate the reviewer’s affirmation on the importance of these findings and thank for the detailed comments and suggestions. Regarding the reviewer’s concern on the multiple testing correction and confounding effect of hypertensive status of subjects, we have added secondary analysis with detailed results in the supplemental materials. Please find the detailed response as followed.

Title

The title leads the reader imagine that in the paper "Health Effect of Air Pollution" (eg cardiovascular or pulmonary diseases) are assessed. However, apart from BP the other study outcomes are all biomarkers.

Response: We understand in the environmental epidemiological study area, the most concerned health outcomes include mortality, morbidity and some outcomes (e.g. BP measurements) because they are most clinically relevant. However, we reckon a lot more people may have already suffered from the adverse effect of air pollution before any clinical diagnosis. Some biomarkers measured in this study have been used for clinical diagnosis as key standards (such as blood cells, glucose, insulin), and other biomarkers (such as FENO, some cytokines) are proposed as clinical relevant/surrogate biomarkers which might become important diagnostic standard with more supportive evidence. In that sense, it should be fair to include subclinical changes reflected from biomarker measurements in the spectrum of the health effect of air pollution.

Abstract

In the methods authors first state they are going to look at "20 pulmonary and cardiometabolic outcomes" and in the following line they mentioned they are going to estimate "associated effect on multiple biomarkers". I suggest to be more detailed and to declare immediately which are the outcomes under study (both xx biomarkers and xx health effect) and maybe mention the 4 groups described in the methods (xx cardiopulmonary, xx inflammation and xx glucose metabolism biomarkers, and xx measurements of BP).

Response: Thanks for the suggestions to clarify the biomarker category. We have revised the related content throughout the whole manuscript. For this part in abstract, we rephrased as “... and arranged each subject to complete up to seven repeated clinical visits with measures of 6 cardiopulmonary biomarkers, 6 cytokines, 4 blood pressure and endothelial function outcomes and 4 glucose metabolism biomarkers.”
In the results are presented as "significant" also changes in the diastolic blood pressure and augmentation pressure that are not significant judging from the confidence intervals (1.2% [CI: -0.1%-2.4%], 5.7% [CI: -0.1%-11.8%]).

Response: Actually, in this sentence we delivered two messages. The first half sentence stressed that PreDM subjects had significant stronger PM2.5-associated adverse changes than healthy subjects in 6 cardiometabolic biomarkers; the second half sentence took preDM as an example to quantify the maximum changes of the 6 markers. Therefore, the numbers that the reviewer mentioned (i.e. the changes in DBP and AP (1.2% [CI: -0.1%-2.4%]) and 5.7% [CI: -0.1%-11.8%])) are the estimated changes in PreDM subjects, instead of the difference between the two groups. Although PM2.5-associated changes in these two biomarkers are marginal significant in PreDM subjects, we did observe a significant difference when comparing between the two groups. Here, we listed the maximum effect in PreDM subjects mainly for the ease of comparison with other studies which interested in similar health outcomes.

Regarding the ambiguity of this long sentence, we divide it into two shorter ones, which is rephrased as below:

“PreDM subjects had significant stronger adverse changes compared to healthy subjects in 6 cardiometabolic biomarkers, namely, interleukin-2, interleukin-8, systolic and diastolic blood pressure, augmentation pressure, and glucose. The maximum elevation of these 6 biomarkers in PreDM subjects were 8.6% [CI: 4.1%-13.3%], 10.0% [CI: 3.9%-16.4%], 1.9% [CI: 0.2%-3.6%], 1.2% [CI: -0.1%-2.4%], 5.7% [CI: -0.1%-11.8%], 2.4% [CI: 0.7%-4.2%], respectively, per an interquartile increase of ambient PM2.5 (61.4 μg m−3) throughout the exposure window of the preceding 1–14 days.”

Methods

Description of the health outcomes at page 10 is not consistent with ST1. Outcomes are grouped in 4 groups in the text and in 3 groups in the ST1.

Response: Thanks again for the suggestions to clarify the biomarker category. We have revised the descriptions in ST1 according to the 4 groups mentioned in the manuscript. Please find the revised version of table 1 in the supplement.

P values <0.05 were considered significant. Multiple testing adjusted p values should also be added.

Response: Thanks for the suggestion on the multiple testing adjustment. We have added a secondary analysis false discovery rate (FDR) correction on top of the main analysis. The corrections in the manuscript include:
1. In the statistical analysis section: “A two-tailed P-value < 0.05 was considered statistically significant in the main context. Considering the multiple health outcomes in this study, we also adjusted P-value based on the Benjamini-Hochberg method as a secondary analysis false discovery rate correction (Benjamini, Y., and Hochberg, Y. (1995)).”

2. In the last paragraph of result section, add FDR correction analysis (right above Discussion) and in Fig S7-8: ”After the false discovery rate correction, the PM2.5-associated effect on the biomarkers among all the subjects were basically unchanged with similar lag pattern (Figures S7-8).”


Results

At page 13 authors decide to show only 12 cardiopulmonary and metabolic outcomes. It is not clear why and how these outcomes have been selected. Also, note that in the methods at line 15 it seems that cardiopulmonary outcomes measured are only 6 and metabolic are 4 for a total of 10.

Response: Considering one plot is too busy to represent the total of 20 biomarker, we chose three biomarkers from each outcome categories as representatives. The result of the other 8 biomarkers are listed in the supplement and were also reported and discussed in the manuscript. We chose the 12 biomarkers for two main reasons. First, we decided to show the biomarkers with the most significant changes among each group and left in the supplement the biomarkers with similar effect pattern (such as monocyte and lymphocyte are similar to WBC and neutrophil). Second, the key issue for this paper is to compare the health changes between preDM and healthy subjects, so we chose the biomarkers in each group that shows the significant difference, and left those biomarkers with non-significant difference between two groups in the supplement.

Regarding the second part of reviewer’s comment for cardiopulmonary and metabolic outcomes, as the 12 biomarkers are from all the four categories, we rewrite this part as below:

“Figure 2 shows the biological changes in 12 exemplary biomarkers per an interquartile range (IQR) increase in the Avg. 1- to 14-day exposure to ambient PM2.5 within all subjects. Each horizontal panel of Figure 2 displays the changes of three biomarkers from each of the four outcome categories as representatives. The results of the other biomarkers were listed in Figure S1.”

At page 14 line 12 WBC and neutrophils are mentioned among the inflammatory biomarkers while in methods are included among the cardiopulmonary biomarkers.
Response: WBC and neutrophils are referred as “respiratory and cardiovascular inflammatory biomarkers” in the method section, which means they reflect the cardiopulmonary effect and meanwhile relate to inflammation. To be noted, the second group of biomarkers are also related to inflammation but limited to cytokines/molecules level (“systemic inflammatory cytokines”) compared to WBC and neutrophils in the first groups as cell levels.

For clarity, we have rephrased the sentence at page 14 line 12 as below:

A significant increase was also observed in inflammatory cells and cytokines, with maximum elevation of…

At page 16 line 12 authors state the no significant difference was found in pulmonary biomarkers. Please specify which are these biomarkers.

Response: The only pulmonary biomarker measured in this study is FeNO which showed a non-significant difference in the PM2.5-associated effect between the two groups. We have added this information in the sentence as followed:

No significant difference was observed for the changes in the pulmonary biomarker (i.e. FENO) between the two groups.

At page 16 Figure 3 is presented. In the legend there is no explanation of the meaning of the grey background (that should identify the significant difference between prediabetic and non-diabetic). Also, from the moment 3 out of the 3 BP outcomes resulted significantly different in prediabetic and non-diabetic it would be interesting to show results also excluding subjects actually suffering from hypertension (if not available this information use of anti-hypertensive drug could be used as proxy). Results from this analysis may be useful in interpretation of the findings as it is expected that the effect of air pollution on BP is larger in pre-diabetic that suffer from hypertension.

Response: As the reviewer expected, the grey background/shaded area refers to the significant difference between preDM and no-diabetic subjects. We added this information at Page 15 Line 50 as below:

“The shaded box identifies the significant difference between preDM and healthy subjects.”
Regarding the second point, thanks to pointing out the potential confounding factor of hypertensive status/medication. As we have this information in the baseline questionnaire recoding the disease history from subject’s most recent medical record (less than 1 year from the study). We rerun the examination excluding the subjects with high blood pressure (PreDM =18, Healthy=4), and found the results were robust and significant with similar lag pattern. We modified the content in the manuscript in the discussion (page 19 line 15) and supplement as below:

“Considering the potential confounding effect of hypertensive status and medication, we further run the examinations excluding subjects with high blood pressure (PreDM =18, Healthy=4). The differences in the PM2.5-associated effect on BP elevation between the two preDM and healthy subjects remains significant with similar lag pattern (Fig S9).”

Discussion

At page 17 it is not clear if the sentence starting at line 19 refers to baseline difference between prediabetic and non-diabetic or to the difference between the two groups with increased exposure to PM2.5?

Response: Thanks for pointing out the potential ambiguity sentence. We have rephrased the sentence to “Compared to healthy subjects, preDM subjects showed significantly enhanced PM2.5-associated systemic inflammation (increased serum IL-2 and IL-8).”

At page 19 line 15 the authors state that results support that the progression of glucose metabolic disorder may aggravate the PM-induced elevation in arterial BP. It is of importance that in the analysis almost half of the prediabetic population takes drugs (including anti-hypertensive). It could be of interest to explore BP elevation in prediabetic normal tensive patients vs prediabetic patients suffering from hypertension in order to support the sentence above. Also, more elaborate discussion on the possible mechanisms underlying this association is needed (also based on the results of the present paper).

Response: Similar to the last question for result section, we have added an additional analysis on normal hypertensive subjects and modified the content in the manuscript in the discussion and in the supplement (page 19 line 15).

We also added a bit more discussion at the end of this paragraph on the potential mechanisms, although the current evidence from both experimental and epidemiological studies are largely lacking for a deep discussion. We are also very interested in this topic and believe this potential interaction/susceptibility is related to some mutual biological pathways of hypertension and
metabolic disorder, e.g. systemic inflammation, oxidative stress, and imbalanced central nervous system. However, these speculation needs well designed investigations in further studies.

The discussion on the mechanisms at the end of this paragraph as below:

“Although the underlying mechanism remains unclear so far, based on the emerging evidence from both epidemiologic and experimental studies, the interaction of glucose metabolic disorder and hypertension may relate to their mutual biological pathways, such as systemic inflammation, oxidative stress, and imbalanced central nervous system.”

At page 20 line 31 authors state that the study suggests that elevated glucose and HOMA-IR occur in preDM but not in not diabetic subjects. However, looking at the results (Fig 3) it seem that no significant difference was detected for HOMA-IR.

Response: Thanks for pointing out the ambiguity. We used “elevated glucose in HOMA-IR” in the manuscript instead of “significant elevated” as we also noticed that the increase in HOMA-IR is only marginally significant in preDM subjects. To clarify, we rephrase the sentence as below:

Our study further supports the potential effects of short-term PM2.5 exposure on glucose metabolic disorder but suggests that significant elevated glucose and marginally significant elevated HOMA-IR occur in preDM but not healthy subjects.

In the discussion there is no mention of pulmonary biomarker results. Please, elaborate more on this negative finding.

Response: For the biomarkers in this group including FENO and blood cells, we only observe a significant increase in the whole subjects but no difference between the two groups. Here, our findings of the increase in FENO and WBC have simply confirmed the classic mechanism of immune activation in both respiratory and cardiovascular system. As this study focuses on the difference between the two groups, we did not expand too much discussion on this point. To response the reviewer’s comment, we have revised the paragraph with the stress on those biomarkers in the first categories (Page 20) as below: The significant increases in FENO, WBCs and neutrophils in both groups after acute exposure to ambient PM2.5 observed in this study confirm this classic mechanism of immune activation in both respiratory and cardiovascular system, which may also be associated with alterations in BP, endothelial function, and insulin resistance.
Conclusions

Only cardiometabolic is mentioned while from the results also inflammation biomarkers seem to be relevant.

Response: The conclusion is revised as followed:

Our study provides evidence that the pre-diabetic population is susceptible to the cardiometabolic and inflammatory impacts of air pollution.