Author’s response to reviews

Title: Exposure to Ambient Air Pollution and Calcification of the Mitral Annulus and Aortic Valve: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Version: 1 Date: 28 Nov 2017

Author’s response to reviews:

Dr. Francesco Forastiere,
Associate Editor,
Environmental Health.

November 27, 2017

AUTHORS RESPONSE TO REVIEW

Ms. Ref. No. ENHE-D-17-00243

Exposure to Ambient Air Pollution and Calcification of the Mitral Annulus and Aortic Valve: The Multi-Ethnic Study of Atherosclerosis (MESA).
Dear Dr. Forastiere:

We thank the Reviewers for their thoughtful comments, which have allowed us to significantly improve our manuscript. Please see below for the responses to each of the comments. In addition to this Response letter, we have uploaded to Environmental Health a marked-up copy of our manuscript that highlights changes made to the original version and a clean version. We hope that we now have satisfactorily addressed all of the Reviewers’ concerns.

Reviewer #1:

The authors present interesting results from a longitudinal assessment of the association between ambient air pollution (specifically PM2.5, PM2.5 weighted and NOx) and prevalence and progression of cardiac calcification (specifically mitral annulus and aortic valve calcification) in the well-characterized MESA cohort. Although primarily null findings, these data are novel in that they look at progression of calcification in vascular beds previously not extensively studied in a large population-based cohort free of CVD. The associations of these vascular markers (MAC and AVC) with heart failure highlight the potential significance of these data. Major strengths of this study include the large sample size and population-based design, thus adding to the generalizability of findings, the comprehensive measurement and modeling of the exposures PM2.5 and NOx and the availability of extensive data on potential confounders. The major limitation, as the authors indicate, is the short follow-up (only 2.5 years on average) and the age of the population that may limit their power to detect effects.

Authors: Many thanks for your positive comments. Indeed, these results provide potential novel mechanistic insights into the association between ambient air pollution and CVD. We have outlined the stated limitation in our discussion section.

Significant emphasis is placed on the confirmatory CAC progression results throughout the manuscripts (abstract, results and discussion). This emphasis detracts somewhat from the conclusion of this paper. The rationale for including CAC progression during the same time frame as the AVC and MAC progression as a comparison appears reasonable but the emphasis on the these results is somewhat distracting.

Authors: Thank you for this thoughtful comment. We have now scaled back on the emphasis on the confirmatory CAC progression results throughout the manuscript. For example, we no longer present the adjusted prevalence ratios or beta-coefficients for annual change for CAC in the main results text, but simply refer the reader to the Tables where this CAC data can be found for comparison. We have also removed discussion of the clinical implication of air pollution with CAC in our Discussion section, since Kaufman et al covered this discussion in their prior Lancet paper. We removed mention of CAC in our Conclusion sections, both in the abstract, and in the manuscript text.

It is not clear in the presentation of the progression analyses whether the authors used baseline, follow-up or cumulative exposure levels of air pollutants. Please clarify in tables/figures.
Authors: Many thanks for this comment. For progression analysis, we used averages of fortnightly residence-specific predictions of pollutants from baseline CT scan to follow-up CT scan dates, rounded to the nearest whole year to account for potential seasonal effects. To address the Reviewer’s comments and for more clarification, we have revised the methods section of the manuscript:

“For progression analysis, averages of fortnightly residence-specific predictions of pollutants from baseline CT to follow-up CT scan dates, rounded to the nearest whole year were used to account for potential seasonal effects. Year 2000 concentrations were used in our baseline prevalence analysis”

We have also clarified this in Table 3 title and Figure 3 legend:

“Table 3. Adjusted associations of averages of PM2.5 and NOx over follow-up with annual change in AVC and MAC, compared to CAC.”

“Figure 3: Adjusted associations (with 95% CI) of annual averages of PM2.5 and NOx over follow-up with annual change in AVC and MAC, compared to CAC.

I recommend that some additional data with respect to the distribution of the outcomes be presented in order that the reader may be able to assess the appropriateness of the use of modified Poisson regression. Are there too many zeroes to fit the Modified Poisson regression model with robust variance estimation?

Authors: Thanks for this comment/question. Please find below the distribution of the outcomes from our Stata output. As shown below and in both our Table 1 as well as Figure 1 of the manuscript, majority of the participants had AVC/MAC=0 at baseline.

<table>
<thead>
<tr>
<th>avcagat1</th>
<th>aortic valve calcium (Agatston)</th>
</tr>
</thead>
<tbody>
<tr>
<td>type: numeric (double)</td>
<td></td>
</tr>
<tr>
<td>range: [0,7672.3]</td>
<td>units: .01</td>
</tr>
<tr>
<td>unique values: 469</td>
<td>missing : 2/6814</td>
</tr>
<tr>
<td>mean: 26.5106</td>
<td></td>
</tr>
<tr>
<td>std. dev: 208.645</td>
<td></td>
</tr>
<tr>
<td>percentiles: 10% 25% 50% 75% 90%</td>
<td></td>
</tr>
<tr>
<td>0 0 0 20.6</td>
<td></td>
</tr>
</tbody>
</table>
Moreover, do the annual progression data meet a normal distribution that meet the assumptions of linear regression?

Authors: Thanks for this question. Yes, the annual progression data for both AVC and MAC did approximate a normal distribution. These were visualized using histograms.

Did the authors consider whether there was a significant effect of PM2.5 when NOx was also in the model without the interaction term?

Authors: Thanks for this question. Yes, in our sensitivity analysis, we also examined the effect of each pollutant as adjusted for the other co-pollutant (without interaction term) on AVC and MAC. The results remained largely unchanged from the primary analysis of single exposure modules.

We have now added that to our results as follows:

“In sensitivity analysis, we also examined the effect of each pollutant as adjusted for the other co-pollutant on AVC and MAC, and the results remained largely unchanged from the primary analysis of single exposure modules.”

We previously also described our interaction testing as follows:

“Also, there was no evidence for effect modification when pollutants were combined in the same model.”

Minor comments:

* Introduction, last paragraph - State the specific measures of ambient air pollution assessed.
Authors: Thanks for this suggestion. In response to your comment, we have now updated the last paragraph of the Introduction to include the specific measures of ambient air pollution assessed:

“We sought to examine associations of household-level concentrations of particulate matter less than 2.5 microns in diameter (PM2.5) and oxides of nitrogen (NOx) with the prevalence and progression of AVC and MAC, measured by cardiac computed tomography (CT), and compared to CAC, in a well-characterized cohort from six metropolitan areas in the United States (U.S.).”

* Methods, Figure 1 - It is a little confusing as to why the number of participants with missing F/U CT scans vary by cardiac calcification measure. Are not the same CT scans used for all 3 measures considered in this manuscript?

Authors: Thanks for this question. Yes, all 3 measures of cardiac calcification were read from the same CT. However, in MESA, the cardiac CT were first performed and interpreted for CAC. Years later, a new grant was acquired to facilitate a re-analysis of the scans - the investigators went back to the original scans and newly read them for AVC and MAC. There were a few missing or non-interpretable scans on the re-read for these new calcification measures. This explains why there is a slight difference between the number of participants with missing values for valvular calcium score compared to CAC.

* Results - It may be helpful to reader to present percentages for AVC/MAC in Table 1. Can present it as part of header.

Authors: Thanks for your suggestion. We have now included percentages for AVC/MAC in Table 1.

* It is interesting that there were more smokers in the group with AVC and MAC (Table 1). Is this surprising? There should be some mention of this in the first paragraph of the Results section where the population is described by AVC/MAC status.

Authors: Thanks so much for this thoughtful comment/Question. Indeed, it was initially surprising to us that there were more current smokers in the group without prevalent AVC and MAC. However, upon further examination of the data, we found that this is most likely explained by the fact that participants with prevalent AVC and MAC were older, with multiple comorbidities and often on cardiac medication which could have triggered them to quit smoking. Thus, there were more former smokers among those with prevalent AVC and MAC compared to those free of calcification at baseline. We have now included the distribution of former smokers to our smoking variable in Table 1 to help convey this point. Also, per the reviewer’s recommendation, we have included a statement to the population description in the first paragraph of the Results section to explain this finding:

“Of note, participants with prevalent AVC and MAC at baseline were less likely to be current smokers, but on the other hand, these participants were older, more likely to be on cardiac medications and thus more likely to have quit smoking.”
From Table 1:

<table>
<thead>
<tr>
<th>Smoking status (%)</th>
<th>All</th>
<th>AVC</th>
<th>No</th>
<th>MAC</th>
<th>Yes</th>
<th>MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>AVC</td>
<td>36.6</td>
<td>46.6</td>
<td>35.1</td>
<td>40.0</td>
<td>36.3</td>
</tr>
<tr>
<td>No</td>
<td>MAC</td>
<td>12.8</td>
<td>10.1</td>
<td>13.3†</td>
<td>9.8</td>
<td>13.1*</td>
</tr>
</tbody>
</table>

* What is the overlap between AVC/MAC? Consider adding to text or Table 1 distribution of each in the other group.

Authors: Many thanks for this question and suggestion. Among those with prevalent AVC, 28.7% had MAC>0 at baseline. Conversely, among those with prevalent MAC, 40% had AVC>0 at baseline. Since recent epidemiologic studies have suggested separate biological mechanisms may lead to AVC and MAC, we opted to model these as separate outcomes in this study. Per the reviewer’s suggestion, we have included this in text in the Methods and Results sections, respectively, of the manuscript:

Methods:

“Since recent epidemiologic studies have suggested that separate biological mechanisms may lead to AVC and MAC, we opted to model these as separate outcomes in this study”.

Results

“Among those with prevalent AVC, 28.7% had MAC>0 at baseline. Conversely, among those with prevalent MAC, 40% had AVC>0 at baseline. Only 3.7% of the participants had both AVC and MAC at baseline.

Reviewer #2:

The analysis presented in the paper tests a hypothesis about the relation of the degree of calcification of mitral annulus (MAC) and of aortic valve (AVC) with the level of exposure to particulate matter (PM2.5) and nitrogen dioxide in ambient air. The authors compare the results
concerning MAC and VAC with associations of a progression of coronary artery calcification (CAC) with the exposure, analyzed and published previously. The data have been collected in a framework of well documented study MESA, applying well established methods and using thorough quality assurance protocols. Though the analysis suggests an association of the prevalent MAC and AVC, as well as of the changes in MAC with PM2.5, all associations are statistically nonsignificant. The authors suggest that this is the result of small sample size and low frequency of MAC and AVC, and propose to test their hypothesis in a larger sample. While this conclusion might be correct, its strength would increase and further studies would be facilitated if some additional data are shown or further analyses are performed in the available data set.

Authors: Thank you for your positive comments. Unfortunately, as acknowledged in our limitation section, although we had a relatively large sample of over 6000 adults free of clinical CVD, we were limited by the low frequency of the outcome (MAC/AVC) in this cohort, and there is no additional data in the available dataset to present at this time. However, our work is intended to be exploratory and to encourage replication of our findings by other investigators. Additionally, in the future, if successful funding is obtained, the MESA Exam 5 CTs could be potentially re-interpreted for MAC and AVC to provide 10-year change, similar to CAC. However, at this time, those latter 10-year scans have not been interpreted for MAC/AVC (so longer term data is not available) and there is no plans to have that in near future, as funding has not been acquired to facilitate that work. Nonetheless, other publications in MESA have utilized the short term (2.5 year) MAC/AVC progression data.

- Is there any tendency for higher adjPRR and stronger relation of annual change in MAC and AVC with air pollution in older subjects? Results in Table 1 suggest that the calcification process is age-related and it might be that the influence of air pollution is restricted to older people.

Authors: Thanks for this question. In our sensitivity analysis, we tested for effect modification by age, race/ethnicity, sex, study site and co-pollutants. No significant interactions were found due to insufficient power. Furthermore, we restricted our analysis to participants aged above 62 years (mean age) and found that the strength of associations between pollutants and valvular calcium were generally attenuated.

- What is the impact of MAC and AVC assessment error on the results? This could be indicated by the proportion of people with MAC or AVC found in the first study but not in the follow up.

Authors: Overall, there were minimal “AVC and MAC assessment error” on the results. Among those with AVC at baseline, only 1.2% did not have AVC on follow-up CT. Likewise, among those with MAC present at baseline, only 2.1% were found not to have MAC on follow up CT. It is however unclear whether these could all be explained by assessment error of these outcomes.

- Would the relation with the exposure be stronger if the analysis would be restricted to subjects (close to 50% of the study group) with CAC>0?
Authors: Thanks for this comment. Yes, those with prevalent calcification are perhaps more likely to progress. As mentioned above, we were already limited by the relatively low frequency of prevalent AVC/MAC when the whole cohort was considered, and those numbers would be further reduced if we dropped half of the study sample (i.e. include only those with CAC>0). We anticipate that this would further reduce our statistical power for detecting associations, rather than strengthen it. Furthermore, MESA is representative of a community-based cohort free of CVD. To be more generalizable, we were interested in calcification progression among all individuals (including progressing from non-zero to >0 scores), and not only just progression among those with prevalent CAC already. Also, the CAC data from MESA Air has been extensively published by Kaufman et al in their Lancet paper. To avoid overlap, the purpose here was to include CAC data in the same short time frame for comparison with AVC and MAC. In our revisions, we are downplaying the results for CAC as suggested by Reviewer 1.

- Prevalence of smoking is lower in groups with AVC and MAC. These subjects are also older and more often under medication. What is the proportion of ex-smokers in these groups? Have this category been included in the "smoking status" variable?

Authors: Thanks so much for these questions. Please refer to responses to Reviewer 1 above for a response to same question.

- What was the rate of change in AVC and MAC in subjects with various AVC / MAC status at the baseline? This should be included in Table 1.

Authors: Thanks so much for this questions and thoughtful comment. Rather than include that information in Table 1, we had included the crude 2.5-year rate of change of AVC/MAC in our Figure 1 of the manuscript, so the requested information is found there.

- Has the baseline status been included in the regression models for AVC / MAC change?

Authors: Thanks for this question. We subtracted the baseline Agatston score from follow-up Agatston score to obtain a change from baseline in our models for AVC/MAC progression. We chose this method over linear mixed effect models because we only had two data points for each participant.

- Individually-weighted exposure data were defined in the Methods section but the results of the analysis using this indicator are not mentioned. Have they been similar to the main analysis?

Authors: Thank you for this question. A strength of the MESA Air data is the ability to obtain individually-weighted exposure concentrations that take into account indoor concentrations and time in the indoor and outdoor space. However, since Air Quality Standards are set based on outdoor concentrations of pollutants, we usually include the individually-weighted exposure data as secondary analysis. Also, these predictions may present additional error to our estimation of exposures and may not represent the best approach to use in epidemiological study. As demonstrated by the results, the individually-weighted PM2.5 results were generally weaker for the progression analysis. We did include these PM2.5iwa results in our tables in our original
submission, but we previously were remiss to mention them in the results text section. In this revised, we have now mentioned the secondary analyses in the results section as follows:

“In secondary analyses, we also present the results of the individually-weighted PM2.5 exposure (PM2.5iwa) for cross-sectional (Table 2) and longitudinal (Table 3) analyses. Associations were somewhat stronger for AVC prevalence (Table 2) but weaker for AVC/MAC/CAC progression analysis (Table 3).”

Editor Comment:

Please also ensure that your revised manuscript conforms to the journal style, which can be found in the Instructions for Authors on the journal homepage. Please make sure that the title page, the tables and all of the main text are in accordance with our style requirements. We discourage the use of subheadings in the Results section. You may want to compare with a recently accepted manuscript for illustration.

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