Author’s response to reviews

Title: Long-term Exposure to Particulate Air Pollution and Brachial Artery Flow-Mediated Dilation in the Old Order Amish

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Tim Nawrot

Environmental Health

Dear Editor-in-chief,

Enclosed please find our responses to the reviewer’s comments and revised original research submission “Long-term Exposure to Particulate Air Pollution and Brachial Artery Flow-Mediated Dilation in the Old Order Amish” for consideration of publication in Environmental Health. The manuscript contains important results relevant to the environmental health issue of air pollution health effects. Strikingly, our results are novel as it presents association of air pollution and endothelial function in clinically healthy individuals residing in rural areas. We also applied more a more accurate exposure assessment method. The manuscript is not under consideration for publication elsewhere. The author’s names are listed below and their contact information is listed on the cover page of the manuscript; also, their contributions are listed in the Acknowledgements section. Upon acceptance for publication, we will gladly make any formatting changes to the References section or other sections deemed necessary.
Thank you for your time and effort in consideration of publication of this important work; we look forward to your response.

Respectfully,

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Author responses to reviewer comments:

We thank the reviewers for their careful reading of our paper and thoughtful comments.

Reviewer #1:

My main concern is the scarce amount of information the authors provide on how the flow-mediated dilation (FMD, the main study outcome) was performed. FMD can be a valid and reliable tool to assess endothelial function, but only if applied in a standardized way. The authors should therefore provide way more information in the Methods section on the practical considerations they implemented in the FMD protocol to guarantee standard testing conditions (see Harris et al, Hypertension 2010 and Thijssen et al, Eur Heart J 2019 for recommendations). This includes information on the subject preparation (pre-test resting time, fasted state, prior use of tobacco/alcohol/caffeine, .. ), the ultrasound technology used (device, probe, .. ), the vascular occlusion (esp. occlusion duration) and the FMD analysis (manual measurements or edge detection software, summary statistics on reproducibility of the measurements, .. ). Any deviation to the standardized protocol should be mentioned in limitations, as they limit comparison between FMD studies.

Response: Our BART protocol did indeed follow standardized guidelines as recommended by the International Brachial Artery Task Force. In fact, Dr. Mary Corretti and co-author Dr. Robert Vogel were major contributors to these guidelines (see Corretti reference). In this revised version we have provided more details about the protocol as requested by both reviewers (see page 7, paragraph 3). We have also added the suggested references.

Other comments:

- I'm concerned that the manuscript is not voluminous and innovative enough and that it does not hold enough novelty beyond the population studies done by Krishnan et al 2012 and Wilker et al. 2014 as referred to by the authors.
Response: In response to the reviewer’s point, we have more clearly stated the novelty of our work (see paragraph 3, page 16, and paragraph 1 page 17). In brief, we used a different exposure assessment methodology than prior work, and one that provides greater temporal resolution by accounting for the date of the FMD exam rather than using the calendar year average. Moreover, most previous study populations were heterogeneous. Considering that FMD is influenced by genetics, lifestyle, and other environmental factors, using a homogenous study population such as the Old Order Amish minimizes the impact of potential unmeasured covariates. Yet another distinctive feature of our study population is that study subjects were clinically healthy and recruited on the basis of elevated cardiovascular risk. Finally, our study addresses the lack of information in the literature regarding association of air pollution and FMD in population residing in rural area.

- Lack of other FMD measurements such as changes in blood velocities and shear stress during reactive hyperemia.

Response: We thank the reviewer for this comment. We have now added the shear stress and response to shear stress as alternative outcomes. Neither PM2.5 nor PM10 were associated with shear stress. Shear stress induces nitric oxide release from endothelial cells which results in post-ischemic hyperemia and vasodilation (response to shear stress) The vasodilation depends on the nitric oxide bioavailability. We assessed the associations of PM2.5 and PM10 with the response to shear stress after adjustment for base brachial diameter and other covariates. The method quantifying shear stress and response to shear stress are explained in the Methods section, page 8.

- To me, the regression models should not be adjusted for brachial artery diameter (BAD). %FMD is already standardized by BAD and thus already accounts for BAD. In fact, inserting two highly correlated predictors such as BAD and FMD in the same model could disrupt this model. Alternatively, BAD and absolute change in BAD could be implemented in the same model (or %FMD only).

Response: We agree with the underlying concern expressed by the reviewer. To the reviewer’s suggestion, we have repeated our analyses using models that do not include BAD as a covariate. These new analyses did not substantially change our results. We have addressed this in the Methods section page 10, paragraph 1; Results section, page 12, paragraph 2.
- Try to avoid imprecise wordings such as "about one-fifth" or "about one-fourth".

Response: We thank the reviewer for this comment and have revised and clarified the language in this regard.

- You claim that the association between PM10 and FMD is stronger than that between PM2.5 and FMD. In order to adequately compare effect sizes, one should standardize them by the distribution of the predictors you compare (here PM2.5 and PM10). This can for example be done by calculating effect sizes per standard deviation increase in PM2.5 and PM10 (SD derived from PM2.5 and PM10 distributions, respectively).

Response: We thank the reviewer for the suggestions. All results now are based on standardized exposures.

Reviewer #2:

1. FMD measurements: the paper should document more into detail the conditions of the measurements (time of the day, instructions about food/beverage intake) as well as about the machines used for the measurements (is there an automatic detection of the maximal dilatation?). In addition, the authors should provide data on the intra-observer variability of the measurements.

Response: See response to Reviewer #1, Item 1. Our BART protocol did indeed follow standardized guidelines as recommended by the International Brachial Artery Task Force. In fact, Dr. Mary Corretti and co-author Dr. Robert Vogel were major contributors to these guidelines (see Corretti reference). In this revised version we have provided more details about the protocol as requested by both reviewers (see page7, paragraph 3).

2. As FMD is very sensitive to short term variations in the environment, authors should correct for acute effect of air pollution on FMD. They should determine the association between FMD and the level of PM in the days before the FMD measurement and include this information in the multivariate analysis for assessing the longterm effect of air pollution exposure on FMD.
Response: Unfortunately, daily PM2.5 and PM10 levels were not available at the subject’s residences during the time period of our study. We have added this point as a limitation in page 17, paragraph 2.

3. The finding that the association was more prominent in male than in female is intriguing, which might suggest a protective effect of estrogen on this relationship. Could you do a subgroup analysis focusing only on healthy people below 50 y in order to exclude menopausal women. The interactive effect could become significant in this subgroup.

Response: We thank the reviewer for the suggestion. We have repeated these analyses stratified by age (younger than 50, 50 years and older) by sex.

In the sex stratified analyses, the magnitude of association between PM2.5 and FMD was very similar between men (β=-0.09; 95% CI: -0.16, -0.01; p=0.04) and women (β=-0.07; 95% CI: -0.17, 0.03; p=0.2) (Table 2). In secondary analyses, we performed sex and age-stratified analyses. Effect sizes remained moderately larger in men than in women and were larger in older (≥ 50 years) compared to younger (< 50 years) individuals, although none of these associations were statistically significant in these subgroups (Table S2) (page 12, paragraph 3).

In age- and sex-stratified analyses, PM10, was more strongly associated with FMD in men than in women in both younger and older individuals. Only in the subgroup of men younger than age 50 years did the association between PM10 and FMD achieve statistical significance (β=-0.16; p=0.005) (Table S2) (page 13, paragraph 2).

4. Do we have information on other pollutants (nitrogen dioxide, sulfur dioxide, carbon monoxide).

Response: Unfortunately, we do not. As of the time of writing, exposure models for gases such as NO2, SO2 and CO were not currently available, though for some of these pollutant models are under development and thus estimates may be available for use in the future.