Reviewer’s report

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

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Reviewer: Nicholas Cauwenberghs

Reviewer's report:

I've read with great interest the manuscript entitled "Long-term exposure to particulate air pollution and brachial artery flow-mediated dilation in the old order Amish" by Salami et al. Although I acknowledge the relevance of their work, several shortcomings in the reporting currently hamper publication of these findings.

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.

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.

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

- 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).
- Try to avoid imprecise wordings such as "about one-fifth" or "about one-fourth".

- 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).

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