Author’s response to reviews

Title: Does the oxidative stress play a role in the associations between outdoor air pollution and persistent asthma in adults? Findings from the EGEA study.

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Author’s response to reviews:

Dear Mrs Havet,

Your manuscript "Does the oxidative stress play a role in the associations between outdoor air pollution and persistent asthma in adults? Findings from the EGEA study." (ENHE-D-19-00218) has been assessed by our reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in Environmental Health, once you have carried out some essential revisions suggested by our reviewers.

Their reports, together with any other comments, are below. Please also take a moment to check our website at https://www.editorialmanager.com/enhe/ for any additional comments that were saved as attachments.
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We look forward to receiving your revised manuscript soon.

Best wishes,
Dear editor,

We thank you and the reviewers for your helpful comments. We have clarified all the points and improved the manuscript accordingly.

The revised version has been modified as follows: we have re-written the results and the conclusion in the abstract, and added some explanations about the public health implications in the introduction. We have detailed the associations between air pollution and persistent asthma, and have explained the negative associations between PM and persistent asthma. We also added discussion points on the associations previously observed in the same study between exhaled 8-iso level with PM, on the association reported in literature between the telomere length - another biomarker related to oxidative stress - and persistent asthma, and finally on the caution with which the results of the mediation analysis should be interpreted.

We believe that we have addressed all the points and that the manuscript has been strengthened by the revision. We hope that this revised version is now suitable for publication in Environmental Health.

Please find below our point-by-point responses.

Best regards,

Anaïs HAVET

Reviewer #1: This study of Havet et al uses the EGEA study to examine whether oxidative stress explains the association between residential air pollution exposure and persistent asthma in adults. The study is of interest because there are not many studies assessing this relationship. Hereby I include some comments:

Abstract:

- In the abstract, please include the year when air pollution levels were estimated and also the years of EGEA2 and EGEA3 follow-ups.

As suggested by the reviewer, we have included the years in the abstract as follows: « Persistent asthma was defined as having current asthma at EGEA2 (baseline, 2003-2007) and EGEA3..."
(follow-up, 2011-2013). Exposures to nitrogen dioxide, nitrogen oxides, road traffic, particulate matter with a diameter ≤10 μm (PM10) and ≤2.5 μm were estimated by ESCAPE models (2009-2010), and ozone (O3) by IFEN models (2004). ».

We removed « 11 years later » to reduce the number of words.

- The results section of the abstract needs to be rewritten. The last two sentences are not clear. Also, I think it would be nice to first show the total effect of O3 on persistent asthma (OR=2.16) and then the effect of FlOPs on this association. The results of PM10 in relation to asthma and FIOPS should be shown in the abstract as well as that no association was found with NO2 and PM2.5.

We rewrote the results section of the abstract as follows «FIOPs levels increased with PM10 and O3 (adjusted ß=0.04 (95%CI 0.001–0.08), aß=0.04 (95%CI 0.009–0.07), respectively), and the risk of persistent asthma increased with FIOPs levels (aOR=1.81 (95%CI 1.08–3.02)). The risk of persistent asthma decreased with exposures to NO2, NOx and PM2.5 (aOR ranging from 0.62 to 0.94), and increased with exposures to PM10, O3, O3-summer and road traffic, the greater effect being observed for O3 (aOR= 1.78, 95% CI 0.73–4.37, per 10 µg/m3). Using mediation analysis, we observed a positive total effect (aOR=2.16, 95%CI 0.70–11.9), a positive direct effect of O3 on persistent asthma (OR=1.68, 95%CI 0.57–7.25), and a positive indirect effect mediated by plasma FIOPs levels (OR=1.28, 95%CI 1.01–2.29) accounting for 41% of the total effect. »

Introduction:

- I miss some explanation in the introduction on the public health implications of the present study. Filling a gap in knowledge is enough to justify it but it would be nice to describe the potential future interventions. Also, it would be nice to mention the different mechanisms linking air pollution and persistent asthma (a part of oxidative stress).

As suggested by the reviewer:

1) we mentioned other mechanisms linking air pollution and persistent asthma, as follows (line 117): « The underlying biological mechanisms by which outdoor air pollution may affect respiratory health include inflammatory processes, immune response modulations, genetic modifications and oxidative stress damages, which are increasingly suggested. »

2) we added some explanations on the public health implications, line 120 « Studying biomarkers is a useful approach to provide new insights into the biological mechanisms that drive the disease process, to predict the development and progression of a disease and to personalize intervention strategies (Margaritelis et al. 2016 ; Guerra et al. 2014). In prospective studies, high plasma FIOPs levels were positively associated with the incidence of coronary heart diseases (CHD) among men without previous cardiovascular events [9], and with the risk of future CHD in women [10]. »
We added line 129 « Overall, better understanding the underlying biological mechanisms related to asthma, and discovering novel biomarkers is the first step towards improving asthma management. »

Methods:

- The years between EGEA follow-ups differ between the abstract (11 years) and methods (12 years) - please revise.

Thank you for notifying us this typographical error. The duration of the follow-up in the methods (« 12 years ») is correct. In line with your first comment on the abstract, the duration of the follow-up has been removed from the abstract.

- Please, report only one number here; it is confusing: 1602 participants (n=1571 adults aged ≥16 years) - also in the supplementary material.

As suggested, we have removed the number « 1602 » in the main text and in the supplementary material.

- In methods, indicate when blood sample was collected (in EGEA2 follow-up between 2003 and 2006) in the main text and not in the supplementary material; otherwise, is not clear the temporality of exposure - mediator - outcome.

We clarified this point in the methods of the main text as follows « Plasma samples were collected in EGEA2 between 2003 and 2006 and stored immediately at -80°C during 5.0 to 8.0 years until FIOPs measurements. »

- A brief explanation of the other covariates such as familial dependence and smoking will be thankful.

- In the mediation analysis, models did or did not include random effects on center and familial dependence?

As suggested, we added the definition of the current smoking status (line 238) as follows: « We defined never-smoker as a participant who have never smoked in their life, ex-smoker as a participant who quit smoking for at least 4 weeks at EGEA2 and current smoker as a participant who was smoking at least one cigarette a day for more than one year at EGEA2. »

We have rewritten the lines 224 to 221 « Due to the familial dependence of the data, multivariate analyses (except mediation analyses) took into account dependence between observations. Linear regression models and logistic regression models with random effects on center and familial dependence were used to study the associations between outdoor air pollution with plasma FIOPs... »
levels, and between outdoor air pollution and persistent asthma, respectively. To control a potential effect of short-term exposure to O₃ in the associations between O₃ with plasma FlOPs levels and persistent asthma, further adjustment for the season of plasma collection (EGEA2) was conducted. Logistic regression models using generalized estimated equations (GEEs) on familial dependence were performed to study associations between plasma FlOPs levels and persistent asthma. »

Results:

- "At EGEA2, the mean age of the 204 adults was 39 years, 48 % were men, 24 % were current smoker, 79 % had persistent asthma": since persistent asthma is defined based on whether subjects in EGEA3 had current asthmatics or not, should not be EGEA3 instead of EGEA2?

All the 204 participants had current asthma at EGEA2. Participants with persistent asthma had current asthma at EGEA2 and at EGEA3 and those with remittent asthma had current asthma at EGEA2 only. We therefore described the 204 participants with current asthma at EGEA2, and compared them according to change in current asthma from EGEA2 to EGEA3.

We clarified this point by changing the title and adding information in Table 1.

- Line 253: "and were lower in non-smokers".

We reviewed this sentence as follows « Plasma FlOPs levels [...] were lower in never smokers than in smokers. »

- Overall, I prefer the terms "increase/decrease" rather than "positive/negative" because they can confuse the reader.

As suggested by the reviewer, we changed « positive/negative » by « increase/decrease » along the results section.

- Line 266: here the authors should describe the association between PM10 and persistent asthma.

- It is strange that the authors only present the results for O₃ and not for PM10 even they observed an association between PM10 and Flops and persistent asthma.

We agree with the reviewer and we rewrote the lines 259 to 267 as follows: «Among all pollutants studied, plasma FlOPs levels increased by 1 RFU/mL with PM10 and O₃ exposures (adjusted (a)β= 0.04, 95% CI 0.001–0.08, p=0.03 and aβ= 0.04, 95% CI 0.009–0.07, p=0.02 for an increase of 10 µg/m3 of O₃ and PM10, respectively, Table 2). The results were similar after excluding participants who lived at the same address for less than 1 year. The association
between PM10 and plasma FIOPs levels did not remain significant with back-extrapolated data (aβ= 0.03, 95% CI -0.01–0.07, p=0.22 for an increase of 10 µg/m3 of PM10, Table 2). The risk of persistent asthma decreased not significantly with exposure to NO2, NOx and PM2.5 (aOR ranging from 0.62 to 0.94), and increased not significantly with exposure to PM10, O3, O3-summer and road traffic, the greater effect being observed for O3 (aOR= 1.78, 95% CI 0.73–4.37, for an increase of 10 µg/m3 of O3, model 2, Supplementary Table 3). Results were similar after excluding participants who lived at the same address for less than 1 year (Table 2 and Supplementary Table 3).»

Discussion:
- Line 318: in the present study no association is observed with PM2.5. Authors should explain the reasons of these null findings although this pollutant has more capacity to induce oxidative stress than PM10, as the authors point out.

- Lines 322 and 324: authors need to justify why they explain these findings here (i.e. why PM2.5 has been associated with 8-isoprostane and not with FIOPs).

As suggested, we have rewritten the lines 318 to 324 and added possible explanations «Previously, we found that 8-isoprostane in exhaled breath condensate, a matrix close to the lungs, increased significantly with PM2.5 exposure in the same study [23]. 8-isoprostane is a biomarker of damages related to oxidative stress, and a specific product of lipid peroxidation. In the present analysis, plasma FIOPs levels increased with PM2.5 exposure but the association was not significant. Fine and ultrafine particulates are known to be more harmful by penetrating deeper into the lungs and inducing damages due to oxidative stress both at the airways and systemic compartment [24]. The discrepancies in the results could be partly explained by difference in the sample sizes, in the composition and concentration of the particulates, and by difference in underlying mechanism related to the studied biomarker. »

- Not necessary to repeat "for the first time" so many times.

"For the first time” is present once in the revised version.

- Lines 354-357: I would remove this sentence.

Done.

- It would be nice to describe difference between FIOPs and other oxidative stress markers such as telomere length, which has also been associated with persistent asthma in adults (Belsky et al Am J Respir Crit Care Med. 2014 Aug 15;190(4):384-91).

As suggested by the reviewer, we have referenced the results of this study (see line 344) as follows « Interestingly, leukocyte telomere length, which reflects oxidative-stress damages to
DNA (Clemente et al. 2019), was shorter in participants who had persistent asthma from childhood into adult as compared to those who had adolescent or adult-onset asthma (Belsky et al. 2014). »

- Table 3 heading needs to indicate that this analysis is for O3 exposure.

We added precisions in Table 3.

Supplementary material:

- The EGEA3 follow-up is not described in the respiratory symptoms section.

We have described the 204 participants at EGEA2 (see the response to the first comment on the result section). Therefore only respiratory symptoms at EGEA2 are described in the section.

- The flowchart is difficult to follow - I would try to simplify it by grouping some of the exclusion criteria (i.e. plasma Flops dosages with variation coefficient >20% and missing data on plasma Flops).

We have modified the flowchart accordingly.

Reviewer #2: This is a well written manuscript that describes associations observed between several modeled markers of air pollution, a biomarker of oxidative stress, and persistent asthma. The approach is interesting analyses provide some suggestive evidence, but a clear convincing signal is missing. Considering the small sample size, the authors are generally appropriately modest in the interpretation of their findings.

Major comment:

My major concern with this manuscript is the selective reporting of the (positive) results from statistical analysis in the text. More attention should be given to the results that are not in the expected direction (e.g. non-significant protective effects of PM on asthma). In the current manuscript these are included in the tables, but ignored in the text. It is important to also address these results to give the reader a balanced view of the results of this study. This also pertains to the abstract, where the conclusion section only focuses on O3.

We thank the reviewer for their helpful suggestions. We have detailed the associations between air pollution and persistent asthma, and have explained the negative associations between PM and persistent asthma. In the abstract, we have generalized the sentence of the conclusion.
Minor comments:

- In the discussion the authors state: "Plasma FlOPs levels might also increase with exposure to PM2.5". This statement should be further qualified as you have PM2.5 data in your own study.

We added explanations lines 318 to 324 (see answer below to Reviewer #1) «Previously, we found that 8-isoprostane in exhaled breath condensate, a matrix close to the lungs, increased significantly with PM2.5 exposure in the same study [23]. 8-isoprostane is a biomarker of damages related to oxidative stress, and a specific product of lipid peroxidation. In the present analysis, plasma FlOPs levels increased with PM2.5 exposure but the association was not significant. Fine and ultrafine particulates are known to be more harmful by penetrating deeper into the lungs and inducing damages due to oxidative stress both at the airways and systemic compartment [24]. The discrepancies in the results could be partly explained by difference in the sample sizes, in the composition and concentration of the particulates, and by difference in underlying mechanism related to the studied biomarker. »

- Is there a possibility to look at the relationship between the FIOPs data in this study and the 8-isoprostane biomarkers measured in EGEA before?

In the present analyses, no correlation was found between plasma FlOPs and exhaled 8-iso levels (correlation coefficient = 0.04, P=0.69) as previously reported in the paper by M. Andrianjafimasy conducted in the same study [11].

- How do you explain the protective effect on persistent asthma that is observed for PM2.5 and PM10?

The ORs for the association between not back-extrapolated PM10 data and persistent asthma were close to 1 and greater than 1 for back-extrapolated PM10 data. For PM2.5, the results were unexpected with ORs close to 0.60. We added possible explanations in the discussion section (lines 334-335) as follows « Most of the associations between pollution and persistent asthma were close to 1; the risk of persistent asthma decreased with PM2.5 and increased with O3 and O3-summer exposures. The unexpected result observed for PM2.5 may be partly due to the lack of back-extrapolated data leading to an inverse temporality between PM2.5 and persistent asthma, or to random effect or residual bias. »

- In table 2 and supp table 3 less individuals are included in the analyses for PM10 and PM2.5. What is the reason for this? Considering this affects a considerable proportion of your population, please state clearly in the manuscript text.

Back-extrapolated PM10 and PM2.5 data were available only in Paris and Grenoble, and back-extrapolated PM10 data were available only in Paris explaining the lower numbers in the tables. We have clarified this point by adding this information in the statistical methods section and in
the legend of Table 2 in the main text, and in the legend of Table 3 in the supplementary materials.

- As expected due to small effect and sample size, the estimates from the mediation analyses are quite imprecise. Please add some additional considerations in the discussion for the reader to make an interpretation of these findings.

As suggested by the reviewer, we added additional considerations in the discussion (line 368) «Due to the small sample size, the estimates from mediation analysis may be imprecise and the results should therefore be interpreted with caution.»

- Why are participants with remittent asthma used as reference in this analysis, rather than the asthma free controls that are part of the EGEA cohort. What is the potential consequence of this for the interpretation of the results from the statistical analyses?

Previously in the same study [11], no difference in plasma FlOPs levels was found between participants without or with asthma; and plasma FlOPs levels were associated with poor asthma control, asthma attacks and asthma treatments among participants with asthma. We therefore conducted the present analyses among participants with current asthma, and participants with remittent asthma were used as reference.

The consequence is that the interpretation of the results from the statistical analyses only concerns participants with asthma.