Author's response to reviews

Title: Association between Leukocyte Telomere Shortening and Exposure to Traffic Pollution: a Cross-Sectional Study on Traffic Officers and Indoor Office Workers

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Author's response to reviews: see over
Dear Editor,

We are submitting a revised version of the manuscript MS: 1292946641254282 “Association between Leukocyte Telomere Shortening and Exposure to Traffic Pollution: a Cross-Sectional Study on Traffic Officers and Indoor Office Workers”.

We are very thankful for the reviewer’s comments that we found very helpful and constructive. We have addressed and fully resolved all of the reviewers comments, and revised our manuscript following the reviewer’s suggestions, as indicated in the itemized responses below.

We would also need that the first Authors of this manuscript be listed as “Mirjam Hoxha”, rather than “Mirjam Barbullushi” as in the original submission. Dr. Hoxha has recently obtained the Italian citizenship and is required according to the Italian customs to use her maiden name (Hoxha) in all her personal documents, including those pertaining her academic affiliation.

Thanks in advance for your consideration.

Best Regards,

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Point-by-point responses to the issues raised in the Editor’s letter

Referee 1

C1. The exposure assessment conducted in this study consists of two aspects: First, the authors select traffic officers who are thought to be highly exposed on a long-term basis due to their occupation. A reference group with low chronic occupational exposure to traffic is chosen from office workers. In addition, acute personal exposure on the day before the blood draw is measured. It would be helpful for the understanding of the time scale of the hypothesized effect to be more clear about the expected time frame of exposure and outcome and to comment on how well the acute personal exposure assessment relates to chronic traffic exposure. It would also be very convincing to see that the duration of high chronic traffic exposure, which is probably the more relevant exposure metric for this specific outcome than prior day quantitative exposure, is an independent predictor of LTL.

R1. All study participants had been employed for at least one year at the time of examination (see page 4, METHODS/Study Participants and Exposure Assessment, second sentence of the original submission). Due to the cross-sectional nature of the study design it is difficult to make inferences on the timeframe of exposure effects on LTL. Following Referee 1’s suggestion, we have now analyzed LTL data by duration of employment. However, we found no association between duration of employment and LTL, as shown in the Table below.

<table>
<thead>
<tr>
<th>Job Duration</th>
<th>n</th>
<th>Traffic Officers (unadjusted analysis)</th>
<th>Traffic Officers (adjusted analysis)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>mean* (95% CI)*</td>
<td>p-value</td>
</tr>
<tr>
<td>1-2 years</td>
<td>48</td>
<td>1.10 (1.04-1.18)</td>
<td>1.09 (1.01-1.17)</td>
</tr>
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<td>3-17 years</td>
<td>29</td>
<td>1.14 (1.04-1.24)</td>
<td>1.11 (1.03-1.21)</td>
</tr>
</tbody>
</table>

*Statistical analysis on telomere length was performed on log-transformed data to approximate normal distribution. Geometric means and 95% Confidence Intervals (CIs) are reported.

In the revised manuscript, we describe these results in the text (RESULTS, Telomere length and traffic exposure, page 9, lines 1-4)

C2. I would like to hear a more detailed discussion on the possible pathogenic component(s) of “traffic exposure” and how they relate to the measured markers of traffic exposure in this study. Also, could noise, which is also an important traffic emission, play a role in this observed association?

R2. In the revised manuscript, we discussed more in detail how “traffic exposure” may affect LTL. In particular, we indicated how traffic emissions are a mix of by-products of the combustion process including hundreds of pollutants in gaseous and particulate phases. Particles may stimulate directly the generation of Reactive Oxygen Species (ROS), or through transition metal and quinone structures that undergo redox cycling [Risom et al. Mutation Research 592 (2005) 119-137]. Other combustion products, including nitric oxide and benzene, have been shown to generate oxidative stress [(Lai et al. Occup Environ Med 2005; 62:216-222), (Møller et al. Cancer Letters 2008, 266:84-97), and (Avogbe et al. Carcinogenesis 2005, 26:613-620)]. In addition, exposure to traffic pollutants may indirectly produce oxidative stress by activation of inflammatory cells capable of generating ROS and reactive nitrogen species [Risom et al. Mutation Research 592 (2005) 119-137]. We speculate that ROS production and inflammation, which are known to increase the rate of telomere
attrition, may represent mechanism accounting for the association we found between LTL shortening and traffic exposure. We added this part of Discussion on page 10, lines 4-11. We agree with reviewer that noise from traffic could potentially affect telomere length by increasing oxidative stress [Kovacic P. et al. Med. Hypotheses 2008; 70(5):914-23] or other mechanisms. Unfortunately we have any measurement to evaluate this interesting hypothesis. In the revised Discussion, we indicate noise exposure as a potential determinant of LTL shortening on page 13, lines 7-11.

C3. Please elaborate a little bit on the hypothesized biologic link of traffic exposure – LTL - observed clinical and subclinical outcomes such as progressed atherosclerosis and cancer.

R3. In the revised Discussion, we elaborated on potential biological mechanisms relating shorter telomeres with age-related diseases, including atherosclerosis and cancer. (See DISCUSSION page 12, lines 1-10)

C4. As the authors state, LTL represent a marker of biological age and therefore reflect chronic influences on the organism. While I recognize that the authors have included the most important determinants such as age, sex and smoking habits in their analysis, it would be interesting to explore in more detail possible other differences between the two groups that are known to be related to oxidative stress and inflammation.

R4. All study participants were healthy subjects without any acute inflammation at the time of the study and no history of chronic inflammatory disease. LTL was not associated with white blood count and proportion of neutrophils and lymphocytes. Recent studies have demonstrated that LTL is inversely associated with leisure-time physical activity (Cherkas et al. Arch Intern Med, 2008; 168:154-8). However, if systematic differences in physical activities existed between the two groups in our study, one would expect lower activity among the referents (office workers) who have a more sedentary work. Therefore, potential confounding from physical activity would cause bias in the opposite direction from the one we observed. Unfortunately, because we did not collect information on work-related or leisure time physical activity, we cannot adjust our models for this relevant potential determinant of LTL.

We reported information on: i) absence of acute inflammation in the study participants description on page 4, lines 19-22; ii) differential blood count in the Results (page 8, lines 13-16); iii) potential role of physical exercise in the Discussion (page 13, lines 11-19).

C5. In the background section (page 3, 2nd paragraph), the authors refer to time-series and longitudinal studies, two of the cited papers however are based on a case-crossover study (Peters et al.) and a cross-sectional study (Hoffmann et al.).

R5. We are very thankful to the reviewer for having pointed out this error. We have modified it with “epidemiological studies”. (See page 3, lines 22)

C6. A few sentences in the results section are redundant because they contain information given in the methods section. These could be deleted.

R6. RESULTS, Characteristics of the study Population. We have deleted the first sentence.

Referee2

C1. Abstract

a. Conclusions. The sentence should state ‘…suggesting evidence of …’ rather
than ‘...potentially reflecting...’; The study has limitations due to the design (case-crossover) therefore it would be difficult to conclude beyond suggestive.
R1/a. We have changed ‘...potentially reflecting...’ to ‘...suggesting evidence of ...’

C2. Introduction
a. The first paragraph of the Discussion section seems more appropriate for the first section of the in Introduction. There are a few technical terms such as ‘senescence’ which may not be very familiar for the reader of EH, therefore need some clarifications (as done in the first paragraph of Discussion).
R2/a. We have deleted the second sentence of the INTRODUCTION and moved the first paragraph from the DISCUSSION to the first section of the INTRODUCTION.

C3. Methods
a. The last sentence of the first paragraph mentions that information on traffic conditions were obtained through the questionnaires for Traffic Officers. This seems as very valuable information. What kind of information was obtained (pattern of exposure, exposure intensity, length – months, years)? Please include this detail.
R3/a. All traffic officers have responded to a specific questionnaire item on the traffic conditions during their work shifts. This was a close-ended question with two possible choices: 1) low traffic; 2) high traffic. We specified this information in the Methods section, page 4, lines 17-19.

b. First sentence, 2nd paragraph: are benzene and toluene measures only ONCE? Please clarify.
R3/b. Airborne benzene and toluene were measured by passive samplers, worn near the breathing zone by each participant for the entire duration of their work shift. The sampling was done only once, though it covered an entire work-shift. At the end of the work-shift the passive samplers were taken back to the laboratory, where benzene and toluene absorbed on the samplers’ filters were analyzed. Information on methods for benzene and toluene exposure measurements is detailed on the last 3 lines on page 4 and the first 6 lines on page 5.

c. Age, smoking (pack-years) are analyzed as categorical. Please specify this in this section.
R3/c. In the description of the statistical analysis in the Methods we specified how age and pack-years were handled (page 6, lines 16-17).

C4. Results
a. There is repetition of information on covariates adjusted for in the last paragraph of the methods and first paragraph of the Results. I would suggest this information goes (or stays) in the Methods section.
R4/a. We have deleted the third sentence from Characteristics of the study population, RESULTS.

b. p-values are not informative as a statistic on the effect magnitude and precision. Therefore, it would be better to only present (where you can) the effects and their CIs. One example is a finding reported in the Results section of the abstract of the LTL mean of 1.10 (95%CI 1.04-1.16) for traffic Officers, and 1.27 (95%CI 1.20-1.35), p-value< 0.001. I think the reader can get all the information by just looking at the difference of effects, as well as the precise (not
including null), tight and non-overlapping CI. The p-value is unnecessary and not informative in this case (and others).

R4/b. We fully agree with the reviewer, that presentation of means and CIs would be appropriate for our data. However, we feel that results presented without p-values could be hard to interpret for readers without appropriate statistical or epidemiological expertise. Although usual Environmental Health readers might have this background, we believe that our data may be interesting for molecular biologists or physicians who are accustomed to the use of p-value. Therefore we request to keep the use of p-values in our manuscript, although we are open to drop them should the referee request so.

c. One of the limitations of this study is the cross-sectional nature of it. The authors recognize this in a brief statement in the discussion section. I think this needs more attention and a more elaborate discussion. In the Results section (page 7, 2nd paragraph) the authors report the association between reporting high traffic exposure and toluene and benzene levels. However, if information on exposure length and pattern exists (see comment 3a), then include some results on this.

R4/c. Our data are based on a cross-sectional study, which does not provide direct information on dynamic changes in LTL over time. In addition our study did not have measures of long-term exposure to traffic pollutants. Following the Referees’ suggestion, we have now analyzed LTL data by duration of employment. However, we found no association between duration of employment and LTL, as shown in the Table below.

<table>
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<td>3-17 years</td>
<td>29</td>
<td>1.14 (1.04-1.24) 0.67</td>
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Statistical analysis on telomere length was performed on log-transformed data to approximate normal distribution. Geometric means and 95% Confidence Intervals (CIs) are reported.

In the revised manuscript, we describe these results in the text (RESULTS, Telomere length and traffic exposure, page 9, lines 1-4)

d. age, smoking (pack-years) were treated as categorical variables in your models. How did you decide about these categories? Did you try to use them as continuous non-linear function (maybe quadratic)? I suggest that additional analyses with these variables as continuous non-linear terms are included.

R4/d. We understand that our statistical methods were unclear about how covariates were handled in multivariate models. In the revised manuscript, we specified that age and pack-years of smoking were fitted as continuous variables (METHODS, Statistical analysis, page 6, lines 19, 21-22, and footnote c to Table 3).

As a sensitivity analysis, we also fitted a set of models in which we used a quadratic term for age, in addition to the linear term. Result from this set of models had only marginal differences from the results reported in the paper. (See METHODS, Statistical analysis, page 6, lines 23-25).

e. Page8, second last sentence: ‘...as a comparison, the percent decrease in TLT...’ – comparison with what?
R4/e. The sentence was meant just to give a benchmark for the magnitude of effects of benzene and toluene exposures. In the revised manuscript we clarified this sentence as follows: “To get a perception of the magnitude of benzene and toluene effects, LTL decreases associated with the exposures can be compared to the percent decrease in LTL associated with age. Among the referent subjects, each year of age was associated with a 0.5% (95% CI 0.1%-0.9%) decrease in LTL” (See page 9, lines14-18).

f. last sentence (cont in page 9). Give effects and 95% CI instead.
R4/f. In the revised manuscript, we indicated the effects and 95% CIs: 6.1% (95% CI 1.5%-10.4%) for benzene, and 6.0% (95% CI 1.0%-10.7%) for toluene. (See page 9, lines 21-22).

C5. Discussion
a. First paragraph belongs to Introduction section.
R5/a. We have removed first paragraph from DISCUSSION to INTRODUCTION section (page 9, lines 4-9).

b. Page 10, 1st paragraph, 3rd sentence: 6.2%-6.4%. Specify which result belongs to what exposure.
R5/b. In the revised manuscript, we specified this information (6.2% and 6.4% respectively for toluene and benzene exposure).

c. Limitation: the authors need to discuss the limitation of this study due to lack of long term exposure measurements, the implications for the temporal relationship with shortening of telomere length.
R5/c. We are thankful to the Reviewer for pointing out this important omission. In the revised manuscript we elaborated on this issue on page 11, lines 5-9.

Minor Essential Revisions
1. The paper need editing for language and minor mistakes throughout
Reply: The revised manuscript was proofread and edited by a scientific writing specialist.

2. When reporting effects and their 95% confidence interval, keep that reporting consistent throughout the paper (for example there are times when ‘to’ is used, and other times when ‘-‘ is used). Also report the unites of the effects for the 95% CI.
Reply: We now use ‘-‘ throughout.