Author's response to reviews

Title: Repeated measures of inflammation, blood pressure, and heart rate variability associated with traffic exposures in healthy adults

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Author's response to reviews: see over
Reviewer #1: Scott Weichenthal

1) It would be helpful to include overall exposure means in Table 3 along with the site specific values.

Overall exposure means were added to Table 3 per the request of the reviewer. In addition, we added p-values to compare between locations.

2) I am not sure I agree with including an indicator variables for location in the mixed models, this is essentially putting the exposure in the model twice and likely diluted the air pollutant effects. Perhaps models with and without these indicator variables could be shown? Alternatively, the authors could look at linear models within sites if they are worried about confounding by site specific factors and verify that within-site effects are similar to the overall effect.

Location was added in the model as the locations were very different (visually as well and noise levels) and we wanted to assess whether it was possible that the location might also be influencing the outcomes we measured. Therefore, we completed 2 different models: one with location and one without location in the model. As the point estimates decreased and the standard error increased when location was not included in the model, it suggests that factors in addition to air pollution might be responsible for the effects observed. Therefore, to look only at air pollution effects we presented the results of the model with location being controlled. Due to space concerns the results from the model without location were not included in the manuscript.

3) Did the authors consider heart rate during exercise as a covariate? This may be used to account for potential differences in exercise intensity, and thus dose, between visits.

As differences in heart rate were not observed after or during the exposures (Figure 1), and the walking pace did not differ by location (Additional file 1), they were not included in the model as we did not want to over-model our results given our sample size. The locations were also on fairly level ground, meaning that no additional exertion should have been found between locations.

4) The authors should explore the use of regional air quality data for NO₂ and O₃ since their portable monitor was not capable of measuring concentrations at the selected sites.

As suggested by the reviewer, we added regional ozone data, for the selected locations, to the model. As nitrogen dioxide varies spatially, particularly near traffic sources (Beckerman 2008, Roorda-Knap 1998), we did not think that regional concentrations would accurately predict the concentrations the subjects were exposed to at the traffic sources. Thus, regional NO₂ was not included in the study and our rationale has been described in the Discussion.

5) The authors should show the effect estimates after the 2 obese subjects were removed, the additional files are difficult to go through and most readers won’t bother.

Due to space limitations we chose to not include the table where obese subjects were removed, as part of the main manuscript but have included it as an Additional File (Additional file 4) that can be viewed if interested readers want to see the results. We have highlighted in the body of
the manuscript where differences were observed between the inclusion and removal of the obese subjects.

6) The findings of decreased blood pressure but increased LF:HF seems odd as increased sympathetic modulation would tend to increase blood pressure. This should be discussed.

An article recently published in Frontiers in Physiology (Billman 2014) discussed the meaning of the LF:HF, its relationship to the sympathetic nervous system, and other factors outside the SNS that could influence the LF:HF. A brief summary of key points in this article was added to the discussion and cited.

7) It would be interesting to examine potential non-linearity in the relationship between air pollution and HRV and Blood pressure. In our own work, we are seeing that decreases in HRV occur at lower exposure levels and flatten off as exposure increases. It may be worth examining this. Also, we recently observed that regional air pollution concentrations immediately preceding exposure days modifies the effects of traffic pollution on HRV. It might also be interesting to explore this possibility (Part Fibre Toxicol. 2014 Dec 9;11(1):70).

Although very interesting, as we only have personal exposure data for particle measurements, using regional air pollution concentrations would be less relevant and were not included in this study. In addition, it is not likely that we are able to look at potential for non-linearity between air pollution and cardiovascular measurements. One to five subjects were studied in each exposure session, thus lessening the variability of air pollution measurements (for example, the GWB location was visited only 9 times for the study, with many subjects being tested on the same day).

Reviewer #2: Ling Liu

1) The goal of this study is to evaluate the health effects of traffic. The authors used repeated measures ANOVA to compare health measures at three sites where traffic density and air pollution levels tend to be different (but the differences were not statistically different). However, the repeated measures were done on 3 different days (with a washout period of #2 weeks in between) when temperature and humidity etc. might be quite different. Temperature and humidity may affect cardiovascular and respiratory function measurements, and thus may confound the traffic effects. Therefore, repeated measures ANOVA may not be an appropriate statistical method to evaluate the traffic effects, since it does not adjust for potential confounders or effect modifiers. Mixed-effects regression models have been used in situations like this in other studies in which site was an indicator variable and temperature, humidity, day-of-the-week etc. were included as co-variates (Dales R, et al. 2013. Acute changes in lung function associated with proximity to a steel plant: a randomized study. Environ Int 55: 15-19). The authors may even be able to adjust for the distance each participant traveled to the study site, and the walking pace, by including them as a co-variate. But I do not recommend including pollutants as co-variates in the model, because they may be highly correlated with the site and so not independent.

The reviewer was concerned about the repeated-measures ANOVA analysis to compare health effects at the three locations (Figure 1). As presented in the original manuscript, we did 2 levels
of statistics as suggested by the reviewer. We used a repeated-measures ANOVA as our first step of analyses and also presented a mixed effect model (Table 5 and Additional file 3) that included temperature and humidity (referred to as “apparent temperature”), as well as location. We did not include other variables such as driving distance or walking pace in our model as these metrics were not different, per person, according to our analyses (Additional file 1). We were also concerned about “over modeling” our data.

2) Page 14: In Discussion, the authors state that “This present study is unique in its use of multiple locations and diverse outcomes”. The authors ran many models to calculate the associations of particle constituents with many health measures. With so many models done, some statistically significant associations may have occurred by chance - the multiple comparison fallacy. Suggest the authors discuss how they dealt with this issue. There are also statistical methods available to reduce the possibility of spurious relationships due to multiple comparisons (e.g. familywise error rate procedures).

We are well aware of the concern about the many analyses that were done in this study. We have added in a sentence in the Discussion about possibly spurious findings due to chance with the many statistical tests run in this work.

3) Page 18: In Conclusion, the language in the first sentence “We have shown that acute physiological and biological changes can occur in a healthy population following a 2 hr walking exposure to near-walkway traffic” seem a bit too strong, given that most of the physiological functional and protein biomarkers had no associations or moderate associations with the site, and some of the associations were counterintuitive (e.g. significantly reduced blood pressure). Also the influence of noise cannot be ruled out, although one may argue that noise is also part of the traffic pollution.

We agree that this sentence should be edited as it might be too strong to support our findings. Therefore, as suggested, we have rewritten this sentence to be more specific about the results of our study with the population of subjects we used without making too strong a statement generalizing our findings to the general population.

Minor issues:

4) Page 4, 1st paragraph, in the sentence “…as well as only requiring only a small amount of blood for the analysis…” Please remove one “only”.

The word “only” was removed from this sentence.

5) Page 7, in Exposure Assessment, air samplers were located at one monitoring station while participants walked along the road, correct? How far did participants walk away from the monitoring station at each site?

We added in additional information about the location of the samplers on Page 7 in the Methods section.

6) Page 7, 3rd paragraph, what is “A-weighted noise levels”?
A definition of A-weighted noise, from OSHA, has been added to the Methods section of the manuscript. There have been many concerns in recent studies about the effect of noise levels on health outcomes, particularly cardiovascular endpoints.

7) Page 10, 2nd paragraph, in the sentence “Repeated measures ANOVA was used to assess for statistical differences among baseline health values at the 3 exposure sites”, how was “baseline” defined? Was “baseline” the pre-exposure measures at each site?

The reviewer is correct: we referred to baseline measurements as the “pre-exposure” measures at each location. We addressed this concern by removing “baseline” and replacing with “pre-exposure” on Page 10 as well as in the remainder of the manuscript.

8) Page 12: Many results for three sites were not presented: spirometry, cortisol, CRP, SAA, DBP, LF HRV etc. These are important results relevant to the objective of this study. If space is an issue, suggest including the data in “Supplementary files”.

An additional file (Additional file 2) was added to the manuscript. This Figure shows the results of the remaining biological outcomes that were not presented in the body of the manuscript, as suggested by the reviewer. This figure shows trends in the data, but none of the results were statistically significant.

9) Noise is a big concern in a study like this one. Since noise level was measured only once at SF site, it could not be adjusted for in models. The authors determined cortisol levels in one summer to try to evaluate the stress levels at three study sites. The authors reported no significant difference in cortisol levels among three sites. Please present the results for three sites in the paper.

Cortisol measurements were not statistically different at the 3 locations, although they were elevated 24 hrs following exposures at the GWB location. The results from these measurements can now be found in Additional file 2.

10) Page 14, sentence “although wind direction could also play a role”. Please present wind data to back this up.

We realize that the mention of wind direction might be confusing and therefore removed any mention of wind from this study. We would like to note, however, that we did consider wind direction when we were selecting our locations. As the NJ/NY area usually has prevailing Northwesterly winds, the walkway at the GWB location was on the South side of the Bridge, and the walkway at the GSP location was located on the East side of the GSP. We did not consider including ambient wind measurements in this study (i.e. direction, velocity) as ambient measurements most likely would not have correlated at locations where cars were moving by, essentially creating their own “microenvironments”.

11) Page 16, 1st paragraph, “and remaining serum in IL-1b associated with PM10 [36], ambient air [39], and diesel [37]...” Do you mean “ambient air pollutants”?

Yes, and we replaced “ambient air” with “ambient air pollutants”.
12) **Figure Legend and the figure:** Is “baseline” the same-day pre-exposure values? Please clarify. Since the unit is % change, there is no need to have units such as ppb, mmHg, in the figure.

“Baseline” was replaced with “pre-exposure”. Additionally, the units were removed from the Figure.

13) **Table 3:** Were pollutant concentrations among three sites statistically significant?

Yes, there were differences found between locations in pollutants with the exception of ozone. P-values were added to the pollutant table to address this concern.

14) **Table 5:** Since the associations were expressed as % change per unit of pollutant, having other units (e.g. ppb, ng/ml, mmHg) in the table is confusing. Suggest removing these units.

The units were removed from the Table.

15) The expression “directly following exposures” is confusing in some sentences, such as the sentence “An approximate 5% decrease in DBP was also observed directly following all exposures…”, as if there were indirect observations. Suggest using “immediately” instead.

“Directly” was replaced with “immediately” in this sentence.

16) **Page 4,** a large part of the second paragraph talks about the methods and the merits of the study. Suggest moving it to “DISCUSSION”.

As per the reviewer’s request, we moved a large section of this introductory paragraph to the Discussion.

17) **Page 16,** a discussion on enhanced vagal tone indicated by decreased rMSSD. I wonder whether reduced blood pressure in more polluted site might also be attributable to enhanced vagal tone?

An article recently published in Frontiers in Physiology (Billman 2014) discussed the meaning of many of the HRV measurements and factors other than the sympathetic and parasympathetic nervous system that can contribute to changes in these metrics. A brief summary of key points in this article was added to the discussion and cited. JEM, separate comment Several other studies have also found decreases or no change in BP with air pollution exposures, and these papers were cited in the Discussion.

18) **Page 18,** 1st paragraph, about participants’ workload, suggest also mentioning that walking pace among three sites were not statistically significant (additional file #1).

We mentioned that the walking pace among the three sites were not statistically significant.