Author’s response to reviews

Title: Ozone exposure is associated with acute changes in inflammation, fibrinolysis, and endothelial cell function in coronary artery disease patients

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Response to Reviewers

Reviewer #1:

1. In general, it is very difficult to understand the statistical conclusions. For certain correlative measures (ozone monitoring stations), the authors indicate p-values, but that is never again seen. Are the arterial and circulatory changes significant or not?
P values as well as 95% confidence intervals were added to the text in the Results section to highlight the significance and distribution of our findings in our results.

2. For the lag data presented in most figures, there does not seem to be much change from day to day. I would encourage the authors test a lag -1 (negative 1) or -5 day comparison to show some temporal congruity with exposures.

We investigated the potential use of "future" ozone as a negative control for unmeasured confounding. The correlation between the initial concentrations and the 5 day future lag for all days between the first and last subject visit, however, were above 50%, leading us to doubt the utility of this analysis. For comparison, the correlation between initial concentrations and the 5 day future lag in PM2.5 are 0.16; this low correlation in PM2.5 makes it more amenable to our particular modeling strategy.

3. The literature review seems a bit misbalanced and insular. It is odd that HRV is such a point of discussion rather than the findings you actually include in the main text. There are many studies on ozone and inflammatory markers in cohort and panel studies. There are far more pertinent studies of arterial function and ozone in rodents and in humans to cite.

Sections of the Introduction (lines 87-107) were rewritten to include more background information on studies that found positive associations between ozone and markers of inflammation and fibrinolysis, which is consistent with our results. In addition, more emphasis in the Discussion was made to include past studies that have found associations between ozone and systemic inflammatory markers (lines 377-389).

4. Using regression to eliminate the possibility that PM2.5 was a confounder was a good idea. I would also encourage testing for ambient temperature.

Thank you for the suggestion. We have included temperature in all our models (lines 252-254).

Reviewer #2:

Line 110: You wrote "...their ambient environment...", but environmental ozone is measured during hospital visits, it is not referred to subjects ambient environment. If you use "their" maybe it could be misunderstood.

This sentence was removed (lines 120-121). Details about using data from central monitoring stations were added to line 124 for further clarification.

Lines 113-114: "The results provide...": it is not the right place to describe results.
This sentence (lines 124-125) was altered to suggest that the results may help provide biological plausibility in support of the concept that ozone induces adverse cardiovascular effects in susceptible populations.

Line 118: It should be better to precise where the Duke University is located (i.e. North Carolina US).

A sentence was added about Duke University being located in central North Carolina, US (lines 131-132). An additional sentence was added about the location of EPA Human Studies Facility in relation to Duke University (8 miles southwest, line 160).

Line 122: The phase of recruitment should be better explained. For example, how many subjects received invitation? What kind of advertisement did you use? This is mentioned only in results, lines 248-249.

Information about recruitment from the source population of CATHGEN was included in lines 135-140.

More in general, could you justify the reasons for all the selecting conditions?

Since each participant (all of whom had coronary artery disease or they would not have been in the source CATHGEN population) was visiting our clinical facility for up to ten times over a ten week period, we needed to recruit only those whose disease would remain stable during that ten week period and try to ensure that their response to air pollution would not be impacted by other things. For example, smokers are excluded because smoking can also change many of the end points we are studying. Further, daily changes in hypertension, medication use, and/or recent clinical event could potentially be an effect modifier for the air-pollution associated changes in end points. Lastly, we did not want to complicate the study by having some participants with only coronary artery disease and others having additional diseases, which is why we excluded those with pulmonary disease.

Lines 141-143: could you please provide some more details about the season in which the visits on participants were done? They were distributed during the two-years period (May 2012 - April 2014) or concentrated in some season? Since you studied exposure to ozone, seasonality is an important point to consider.

Figure 1 shows data about the patient visits and mean daily ozone concentrations (ppm) between May 30, 2012-April 29, 2014. Specifically, Figure 1A shows data points that represent the days the patients visited the Human Studies Facility for their clinical measurements. Text was added (lines 290-291) to summarize how the clinical measurements were separated over the course of the 2 year study across various seasons.

Line 214: The acronym QT seems not defined in the list of abbreviations, where QTc refers to QT.
QT is not an acronym – it is the measurement from the beginning of the QRS complex to the end of the T wave on an ECG. When the “c” is added at the end of QTc, the length of the QT interval is corrected for heart rate. An additional explanation of this measurement and how it is measured has been added to the manuscript (line 232-234).

Lines 221-224: The choice of the measurement station has to be better explained. Why did you use a 44 km distant station while the one located only 18 km far has been used only for missing data?

The participants lived closer to the monitoring station that was used for the analysis (Millbrook) rather than the station that was closer to our research facility (Durham Armory). As stated in lines 280-281, the ozone concentrations were highly correlated at these stations (Spearman correlation coefficient = 0.92; p < 0.0001).

Lines 246-247: You established to exclude two subjects because they completed less than three study sessions, was it an a priori condition?

Yes, we chose to exclude these two subjects prior to conducting any analysis.

Lines 255-257: The total of 117 exposure days analyzed doesn't fit with the sum of all the visits you described in detail before. Did some subjects had a visit in the same day?

Yes, there were days in which several subjects had clinical measurements taken. Figure 1A shows data points that represent the days the patients visited the Human Studies Facility for their clinical measurements, and you can observe the days in which overlaps occurred. A reference to this Figure was added in line 276.