Short-and medium-term associations of particle number concentration with cardiovascular markers in a Puerto Rican cohort

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1. Introduction

Particulate matter pollution in ambient air is a significant contributor to the global burden of disease (Lim et al., 2012). A large proportion of this burden is the association between airborne particulate matter and cardiovascular morbidity and mortality (Brook et al., 2010). Multiple mechanisms may explain these associations, including pulmonary inflammation leading to the release of inflammatory cytokines and acute-phase proteins, resulting in endothelial dysfunction, atherosclerosis, plaque rupture and thrombosis (Brook et al., 2010; Knol et al., 2009). C-reactive protein (CRP) is an acute-phase protein and a marker of systemic inflammation from cellular injury that has been studied as a subclinical indicator of this mechanistic pathway (Brook et al., 2010; Li et al., 2012). Particulate matter has been associated with increased CRP in short timeframes in studies of various cohorts including the young, elderly and those with co-morbid conditions (Brook et al., 2010). A second mechanism for the biological effects of PM involves disruption of the autonomic nervous system, vasoconstriction and heart function abnormalities (Brook et al., 2010; Knol et al., 2009). Supporting evidence includes associations between particulate matter and blood pressure, which is a traditional predictor of future adverse cardiac events. (Brook et al., 2010; Giorgini et al., 2016).

While many studies have examined cardiovascular effects of particulate matter less than 2.5 µm in diameter (PM$_{2.5}$), there is evidence of potentially greater impacts from smaller particles including those that are classified as ultrafine particles (UFP, aerodynamic diameter < 0.1 µm) (Knol et al., 2009). Total particle number concentration (PNC) in urban areas is dominated by UFP, and many studies have determined
that PNC is a reliable proxy for UFP (Kumar et al., 2014; Morawska et al., 1998; Pant and Harrison, 2013). We use the terms PNC or UFP as appropriate in this paper, depending upon the specific measurements made in particular studies, as well as refer to them jointly as PNC/UFP.

We have identified several studies that evaluated short-term associations of CRP with PNC/UFP (Delfino et al., 2009; Fuller et al., 2015; Hertel et al., 2010; Karotki et al., 2015; Wang et al., 2016). The majority identified non-significant increases in CRP with higher UFP up to 28 days prior. A recent review of studies of short-term ambient UFP and blood pressure reported mixed results for associations with systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Magalhaes et al., 2018). Several studies reported higher effect estimates linking UFP and blood pressure, but only a few reached statistical significance. A small number reported an inverse association between UFP and DBP (Magalhaes et al., 2018). It is important to note that the review did not include any studies that examined UFP for more than 7 days.

Another key question to explore is whether populations with pre-existing burdens of chronic disease may be especially susceptible to UFP exposures. Cardiovascular disease patients in New York State were found to experience higher associations between UFP and CRP and blood pressure (Rich et al., 2012). In a German cohort, patients with type 2 diabetes or impaired glucose tolerance with specific genotypes showed a greater response in CRP and inflammatory markers with exposure to PNC, compared to those without these conditions (Rückerl et al., 2014).

Puerto Ricans may be susceptible to air pollution due to a high prevalence of chronic diseases. Puerto Ricans make up the second largest Hispanic subgroup in the U.S., after Mexican Americans, and are at increased cardiovascular risk compared to the American population at large, as well as other Hispanic groups (Tucker et al., 2010). Puerto Rican women had the highest prevalence of metabolic syndrome in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) (Ilieis et al., 2014). In the Multi-ethnic Study of Atherosclerosis (MESA), Puerto Ricans had the highest measures of left ventricular mass index (LVMI) and ankle-brachial pressure index (ABI) (Allison et al., 2008). As higher cardiovascular risk may confer susceptibility to air pollution, it is important to evaluate among this population.

In the present study, we explore air pollution and cardiovascular health using data from the Boston Puerto Rican Health Study (BPRHS); a longitudinal cohort study of Puerto Rican adults designed to examine physical and social predictors of cardiovascular health (Tucker et al., 2010). In particular, the BPRHS was designed to gather data on behaviors and exposures, including environmental particulate matter, that may explain health disparities between Puerto Ricans, other Hispanic groups and non-Hispanic whites. The cohort was recruited from the Boston metropolitan area beginning in 2004. The rich and detailed information available include cardiovascular health indicators and several other risk factors. The purpose of this paper is to evaluate the association between changes in short-term ambient PNC and cardiovascular function markers. Specifically, we evaluate the impact of selected lags and moving averages on CRP and blood pressure. In addition, this is the first analysis, to our knowledge, that examines the impact of ambient PNC for up to 28 days on blood pressure (Magalhaes et al., 2018).

2. Materials and methods

2.1. Study design and population

The methods for recruitment and data collection are detailed elsewhere (Tucker et al., 2010) and presented briefly here. Participants were recruited from the Boston metropolitan area (primarily the cities of Boston, Chelsea and Lawrence) and were between the ages of 45 and 75 years at enrollment. Recruitment was conducted via door-to-door solicitation in census tracts identified in the 2000 Census as having 25 or more Puerto Rican adults. In addition, participants were identified through referrals, calls to the study office, and community events. Participants were enrolled in the study on an ongoing basis from 2004 to 2009 (baseline). Based on entry into the study, follow up took place after approximately 2 years (2006–2011) and after approximately 5 years (2011–2015). Baseline questionnaires and clinical measurements were completed in English or Spanish at the participant’s home by trained bilingual staff. Demographic information on age, household income, education, employment history and family structure were collected at the baseline visit. Data on health outcomes and measures were taken at baseline and at the year 2 and 5 follow up visits. The study was approved by the Institutional Review Boards at Tufts Medical Center, Northeastern University and University of Massachusetts-Lowell. All participants provided written informed consent.

2.2. Biomarker measurement

We utilized data on CRP, systolic and diastolic blood pressure. These measurements were made at baseline and at up to two subsequent visits, resulting in repeated measures for the majority of participants (Tucker et al., 2010). Blood pressure measurements were taken while the participant was seated quietly, using an electronic sphygmomanometer (DinamapTM Model 8260, Critikon, Tampa, FL). SBP and DBP were measured three times during each visit and the second and third measurements were averaged. Standing height, weight, waist and hip circumference were measured in duplicate for the calculation of body mass index (BMI) and waist-hip ratio. A certified phlebotomist drew venous blood samples from each participant, in the home. CRP was analyzed in blood serum using the Immulite 1000 High Sensitive CRP Kit (LKCRP1) on the Immulite 1000 (Seimens Medical Solutions Diagnostics, Los Angeles, CA).

2.3. Air pollution measurement

We characterized ambient PNC using a fixed monitor located within 10.5 km of all participants. The monitor was positioned on a rooftop (six floors above street level) at the Countway Library of Medicine of Harvard Medical School on Huntington Avenue in Boston. Measurements at this site have been found to be a good estimate of temporal variation in PNC at other locations in Boston (Fuller et al., 2012). In addition, PNC at this height has been shown to be representative of ground level concentrations (Wu et al., 2014). Continuous hourly measurements of PNC were collected from January 1, 2004 through December 31, 2013 at the site using a butanol-based condensation particle counter (Model 3022A; TSI Inc., Shoreview, MN). Hourly values taken during each 24-h period were averaged to calculate daily mean concentrations (Chung et al., 2015; Fuller et al., 2015). We then constructed several PNC metrics during selected time periods: current day, 1-day, and 2-day lags and moving averages (MAs) of 3, 7 and 28 days. We selected time periods for evaluation based on past published observations of associations between PNC and CRP for a current-day lag up to a maximum of 28 days (Fuller et al., 2015; Hertel et al., 2010). Data on temperature was collected from a station at Logan International Airport in Boston. We averaged hourly measures for a 24 h period to calculate daily means.

2.4. Statistical analysis

The relationship between changes in ambient PNC and biomarkers was evaluated using mixed effects models with individual-specific random intercepts. Age and sex were included in the models a priori, given the strong association of each with the outcomes. Other covariates were included based on their strength of association with the outcome (p-value < 0.05) in univariate models and/or change in effect estimates of 10% or more. We considered for inclusion the following participant-level covariates: BMI, waist-hip ratio, household income, education, diabetes (self-reported yes or no), hypertension (according
to medication or measurement), alcohol intake within the past year (none, moderate, heavy), physical activity score, marital status, and smoking. Medications were categorized as: anti-lipid agents (i.e. statins), beta blockers, anti-diabetic agents, hypotensive agents and anti-inflammatory agents for ear, nose and throat issues. Detailed information on individual covariates can be found in prior work (Tucker et al., 2010).

We also considered the effect of season, temperature (daily mean or warm/cool) and day of the week (weekday vs weekend). We built separate models for CRP, SBP and DBP. We identified the most parsimonious model with the lowest Akaike Information Criteria (AIC) score. A generalized form of the model is given below based on a log-transformed outcome. Let $\log X_i$ be the natural log-transformed outcome value at time $t$, for individual $i$, we assume:

$$
\log X_i = \beta_{00} + \beta_{01} + \beta_{02} \text{Covariate} + \beta_{03} \text{EXP} + \epsilon_i
$$

where $\beta_{00}$ is the overall intercept; $\beta_{01}$ is the vector of regression coefficients associated with the set of covariates that includes age and sex, as well as other potential confounders; $\beta_{02}$ is the parameter of interest and corresponds to the overall association between PNC and outcome; $\epsilon_i$ is individual-specific random effects assumed to be normally distributed; and finally $\epsilon_i$ is the normal independent residual error.

Basic descriptive statistics were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and mixed effects models in R v 3.3.1 using the nlme package (Pinheiro et al., 2016). We evaluated our results as percent change in CRP per interquartile range (IQR) increase in PNC and an absolute change in SBP or DBP per IQR increase in PNC. We used 95th confidence intervals as the measure of precision for our estimates.

We conducted several sensitivity analyses for the purpose of analyzing the validity of measurements for both PNC metrics and cardiovascular biomarkers and the appropriateness of our underlying assumptions. We included all valid CRP values in our analyses. However, past studies have excluded values greater than 10 mg/L, because they may indicate active infection (Ridker, 2003). For this reason, we conducted a sensitivity analysis excluding CRP values greater than 10 mg/L. We also ran models restricting analysis to participants with complete data on all covariates to examine any non-random missingness of data. Because traffic volume and type varies by day of the week, we examined the relationship between PNC and outcomes on weekdays versus weekend days. We evaluated effect modification based on key covariates including sex, age, smoking, education, BMI diabetes status, anti-lipid medications (i.e. statins) and hypertension (Hertel et al., 2010; Lane et al., 2016).

3. Results

Of the 1504 participants recruited into the study, 1499 had appropriate baseline data for our analyses, with follow-up data collected between 2004 and 2013 to coincide with available PNC data. Demographic information on the cohort at baseline is given in Table 1. Due to death, re-location, or loss to follow-up there were 1258 participants in the cohort at the year 2 visit and 891 at the year 5 visit available for our analyses. The majority of the cohort (70%) were women and the mean age was 57 years at baseline. Approximately half (46%) had less than a 9th grade education. The median income was low; however, it is important to note that almost 80% were not working, many being retired, which suggest few had occupational exposures. Mean BMI was 31, in the obese range, and the majority were long-time residents of the Boston area. Large proportions of the cohort were diagnosed with diabetes (40%) and/or hypertension (68%).

Mean values for SBP, DBP and CRP (Table 2) at baseline were 135 mmHg (SD: 19), 81 mmHg (SD: 11) and 6.3 mg/L (SD: 8.7), respectively, which indicates that many were at elevated risk for cardiovascular disease (Ridker, 2003). The intra-class correlation was used to calculate within and between person differences in repeated measures. Mean PNC measured at the fixed site was 26,000 particles/cm$^3$ (SD: 8000) over the entire study period 2004–2013 (Table 3). There was minimal variation in mean PNC across years. The IQR range for the 28-day moving average for participants was 10,000 particles/cm$^3$. Missing data on ambient PNC were 0–15% for 9 of the 11 years of data, 21% in 2005 and 44% in 2011. We evaluated the impact of missing data by running models with and without the two years with greater than 20% missing data. Results differed minimally so we chose to include all years of data in our final analyses.

Interquartile range increases in PNC at all lag periods were associated with statistically significant increases in CRP (Table 4). CRP models were adjusted for age, sex, waist-hip-ratio, diabetes, physical activity, alcohol intake, hypotensive medication and anti-inflammatory medications. Changes in CRP were comparable among the lags and
moving averages per IQR; however, we observed the greatest magnitude of association (7.1% [95% CI: 2.0%, 12.2%]) for a 3-day moving average of PNC. We did not identify an association between PNC and DBP. Models for DBP were adjusted for age, sex, diabetes, alcohol intake, hypertension, temperature (warm vs cool), beta blockers and anti-lipid medications (statins). Similarly, there was no association between PNC and SBP in models adjusting for age, sex, education, alcohol intake, hypertension and temperature (warm vs cool). In that case the effect estimate for an IQR increase in current day PNC was an absolute change in SBP of $-0.20\text{mmHg}$ (95% CI: $-0.69, 0.29$). However, associations were higher and statistically significant when temperature was excluded from the model. Specifically, an IQR increase in current day PNC increased SBP by $1.00\text{mmHg}$ (95% CI: $0.25, 1.74$) (See Supplemental table).

We used stratification to examine effect modification by several factors and found higher effect estimates and a statistically significant association between PNC and CRP for women, but not men. (Fig. 1). Among women, the effect of an IQR increase in current-day PNC was a change in CRP of 13.5% (95% CI: 7.8%, 19.2%). The associations for other lags and moving averages were similar (results not shown). A significant association with CRP was found in current and former smokers (10.0% [95% CI: 3.7%, 16.2%]), but not in non-smokers (3.0% [95% CI: -3.6%, 9.7]). We also noted a significant association with CRP among younger participants, under 65y, (8.3% [95% CI: 3.2%, 13.4%]) but not participants 65y and older (2.3% [95% CI: -8.5%, 13.1%]). Slightly stronger and significant associations were seen for CRP in those without, vs. with, diabetes and for those with, vs. without, hypertension. We did not find effect modification for blood pressure outcomes, however, effect estimates for SBP were slightly higher among current and former smokers (Fig. 2). Sensitivity analyses did not reveal any difference in associations comparing weekday to weekend days, restriction to CRP values below 10 mg/L or running a complete case scenario.

### 4. Discussion

Short- and medium-term increases in ambient PNC were associated with a higher subclinical marker of cardiovascular risk, specifically CRP in this Boston-area Puerto Rican cohort. Markers of inflammation have been evaluated in other studies and several have identified positive effects of PNC on these indicators (Fuller et al., 2015; Hertel et al., 2010; Ruckerl et al., 2006), while others did not (Karottki et al., 2015; Wang et al., 2016). The mechanism for this effect begins with pulmonary inflammation followed by oxidative stress, endothelial dysfunction, which increases production of acute phase proteins (Li et al., 2012).

Hertel et al. (2010) identified a positive association between PNC and CRP for single day lags and for moving averages of up to 28 days. The highest effect observed was an increase of 7.1% (95% CI: 1.9%, 12.6%) in CRP corresponding to an IQR increase in 21-day PNC. Further, moving averages from 2 to 28 days in PNC showed small, but steadily increasing effect sizes as periods lengthened. A different cohort of adult men with coronary heart disease, reported increases in CRP corresponding with ambient PNC for a current-day lag and peaked on the 2-day lag. The highest odds ratio was 2.3 (95% CI: 1.3, 3.8) (Ruckerl et al., 2006). Higher effect estimates were identified in a separate Boston area study population, the Community Assessment of Freeway Exposure and Health (CAFEH), with positive, non-significant increases in CRP of 74% (95% CI: -6.6%, 223.0%) for a 5000 particles/cm$^3$ change in fixed site PNC (Fuller et al., 2015).

We did not identify statistically significant associations between PNC and blood pressure in our cohort. Previous studies have reported similar results (Delfino et al., 2010; Hoffmann et al., 2012; Liu et al., 2014). No association was found between 24-h PNC and blood pressure among a cohort of participants over 65y with coronary artery disease, although associations for shorter time frames were noted (Delfino et al., 2010). We also failed to find any difference when stratifying by factors such as hypertension and anti-lipid medications. Similar lack of association was observed in a cohort of diabetes patients, and in another of
healthy college students (Hoffmann et al., 2012; Liu et al., 2014). By contrast, other studies have identified associations between PNC and SBP, DBP or both endpoints (Chung et al., 2015; Gong et al., 2014; Rich et al., 2012; Wang et al., 2016). In a review by Magalhaes et al. (2018) a 10,000/cm³ increase in UFP was associated with increases in SBP of 0.09 to 4.7 mmHg and DBP ranging from 0.3 mmHg to 2.4 mmHg in separate studies. Inverse associations of UFP with DBP have also been reported (Ibald-Mulli et al., 2004; Rich et al., 2012). Our results for SBP changed a great deal and models showed significant associations with PNC when temperature was excluded from models. Temperature and PNC are significantly correlated in our data (r = -0.58), which makes it difficult to tease apart which may be the causal agent or marker.

Our analysis expands the evidence for a possible role of ambient PNC contributing to increases in systemic inflammation. Importantly, the Puerto Rican population we studied is particularly vulnerable, based on co-morbidities, and has not been included in previous studies of PNC. The magnitudes of our effect estimates were similar to those observed in other populations, after controlling for potential confounders. Past work has shown that CRP is an important predictor of future cardiac events and that a standard deviation difference increases risk by a similar amount as that of an equal change in cholesterol or blood pressure (Kaptoge et al., 2010; Ridker, 2013). Although the associations we identified were small in absolute terms, the impact of a 7% increase in CRP across the Puerto Rican population could greatly impact population health.

The association between air pollution and CRP was only seen in women in the Puerto Rican cohort. Hertel et al. (2010) did not identify differences in effect between men and women. However, a qualitative review of air pollution and respiratory effects concluded that the majority of studies noted greater effects of air pollution in women.
(Clougherty, 2010). This result may be due to sex-linked biological differences in lung-function or toxicity of particulate chemicals based on hormonal status. It is also possible that sex can drive differences in exposure patterns or psychosocial stressors that can increase effects of air pollution (Clougherty, 2010). For example, it is possible that there is a greater degree of exposure misclassification among men, due to factors such as job exposure, resulting in a lack of association in this group. Associations were only statistically significant among smokers, those with hypertension, without diabetes and those younger than 65y. We saw opposite effects of age compared to Hertel et al. (2010) but similar effect modification with respect to diabetes. Ruckerl et al. (2016) identified the greatest impacts of UFP exposure on those who were genetically susceptible. It is important to identify differences in effects between and among groups in order to identify who is most susceptible and identify the level of effects for different segments of the population.

Our analysis has limitations. Our results apply to PNC of outdoor origin, as different impacts may be identified with particles generated indoors or captured using personal monitoring (Olsen et al., 2014). The measurements for ambient PNC were collected from a single fixed site, which will result in some exposure misclassification, because PNC varies greatly with geographic location. However, fixed site monitoring has been found to be a reasonable measure of temporal changes in PNC as evidenced by prior studies (Cryys et al., 2008; Fuller et al., 2012). Specifically, a prior study from our group compared PNC measured at a fixed site to sites placed at or near participant homes. The fixed site was found to closely estimate temporal changes in PNC for similar time periods to those explored here (Fuller et al., 2012). This is likely because PNC levels vary in similar short-term temporal patterns across the metropolitan area. A strength of evaluating effects based on temporal contrast is that it may minimize concerns with pollutants and confounders that have high spatial variation. Longer averaging times may have larger misclassification in the range explored in our analysis. However, we expect misclassification to alter the estimation of the effect, but not mask or reduce a true association. We have evaluated the impact of many possible confounders of our association of interest, however, residual confounding by factors that we were not able to consider (e.g., noise, psychosocial stress) may exist.

5. Conclusions

We identified associations of ambient PNC levels with CRP in a cohort of low SES, Puerto Rican adults in the Boston area. The association with CRP was seen only among women, current and former smokers, those without diabetes, those younger than 65y, and those with hypertension. The effects were similar in size to those identified in other studies. We did not find associations with blood pressure except in models excluding temperature. Disentanglement of the role of temperature and PNC on cardiovascular markers is a topic to be explored in future studies. Our study broadens the knowledge of the field by adding evidence of health effects of PNC on cardiovascular risk indicators in a population that is understudied and may be particularly vulnerable. More research is needed to understand the relationship between pre-existing vulnerability and ambient PNC. Special attention should be paid to exploring potential sex or gender-linked differences in associations between ambient particulate matter and cardiovascular outcomes.

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Author contributions

CHF conceptualized the analysis, built and ran the statistical models, and did the majority of writing and editing of the manuscript. MSO and JAS provided guidance on the analysis, interpretation of results, and editing of the manuscript. HHC gave technical assistance with regards to statistical models and provided feedback on the manuscript. KLT is the principal investigator of the project, provided access to the data and editing of the manuscript. DB is the director of this sub-study, provided guidance on the analysis, interpretation of results and editing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2018.06.042.

References


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