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Association between Fine Particulate Matter and Oxidative DNA Damage May Be Modified in Individuals with Hypertension

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Abstract

Objective—To investigate the association between PM_{2.5} and urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) in hypertensive and non-hypertensive individuals.

Methods—Twelve hypertensives and nine non-hypertensives were monitored during a 36-hour period using a repeated-measures panel study design. Personal exposure to PM_{2.5} was assessed using a real-time continuous monitor. Spot urine samples collected at 12-hour intervals were analyzed for 8-OHdG.

Results—Exposure to PM_{2.5} was associated with a decrease in 8-OHdG in hypertensives compared to an increase in non-hypertensives, after adjusting for age, gender, smoking status, and time of day.

Conclusions—Results suggest modification of the association between PM_{2.5} exposure and urinary 8-OHdG by hypertension status. Antioxidant activity present in antihypertensive medications may play a role or PM_{2.5} exposure may reduce the capacity to repair DNA damage in hypertensives. These results should be confirmed with further investigation.

Keywords

particulate matter; oxidative DNA damage; biomarkers; epidemiology; susceptibility

Introduction

The adverse health effects of fine particulate matter, or PM_{2.5} (particulate matter with an aerodynamic mass median diameter $\leq 2.5 \mu\text{m}$), have been widely noted in the epidemiology and toxicology literature. Short- and long-term exposure to PM_{2.5} has been associated with various health effects, including, but not limited to, decreased lung function and increased respiratory symptoms;^{1, 2} altered heart rate and heart rate variability;^{3, 4} pulmonary and systemic inflammation;^{5, 6} cardiopulmonary hospitalizations and mortality;^{7, 8} and lung cancer mortality.⁴

Epidemiologic studies have observed associations between personal PM_{2.5} exposure and various biomarkers of oxidative DNA damage in different cohorts with a wide range of exposures,⁹ e.g., university students,¹⁰ urban area bus drivers,¹¹ and boilermakers exposed to high levels of residual oil fly ash.¹² PM_{2.5} is a complex mixture of compounds, including transition metals, which are capable of producing reactive oxygen species (ROS) through the Fenton reaction,¹³ and polycyclic aromatic hydrocarbons, which are metabolically activated to o-quinones that can generate ROS through redox cycling.¹⁴ These ROS can cause oxidative damage to DNA, which can further lead to mutations that are associated with the initiation and

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progression of human cancers.¹⁵ Oxidative stress and associated oxidative damage also have been found to mediate vascular injury and inflammation in many cardiovascular diseases, including hypertension, hyperlipidemia, and diabetes.¹⁶

Increased susceptibility to various PM_{2.5} health effects has been observed in individuals with preexisting cardiovascular and respiratory diseases.¹⁷ However, the susceptibility of these individuals specifically to PM_{2.5}-induced oxidative DNA damage has not been examined previously. Given their diseased state, individuals with cardiovascular disease may be more sensitive to oxidative DNA damage associated with PM_{2.5} exposure.

In this study, we investigated the association between personal exposure to PM_{2.5} and oxidative DNA damage and repair, as indicated by urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), in non-hypertensive and hypertensive individuals. Previous studies have found urinary 8-OHdG to be a useful biomarker in assessing ROS-induced DNA damage in both the clinical and epidemiological setting.¹⁸⁻²³ The purpose of this study was to examine whether individuals with hypertension were more susceptible to PM_{2.5} effects on oxidative DNA damage.

Method

Study Population

The study was approved by the Institutional Review Board of the Harvard School of Public Health (Boston, MA). Written informed consent was obtained from each study participant. The study cohort consisted of an inner-city population living in close proximity to a bus terminal (one of the busiest Massachusetts Bay Transportation Authority bus stops in the Boston area). The study population was recruited from a local health clinic. A list of the adult clients living in four zip codes (02124, 02122, 02121, 02119) in Roxbury, MA and North Dorchester, MA was generated and cleaned to remove patients with duplicate, incomplete, or invalid contact information. Lists were separated by “healthy” and “compromised” health status by excluding and selecting, respectively, relevant International Classification of Diseases-9 (ICD-9) codes in the health center patient database (Diabetes [250]; Chronic Bronchitis [491, 491.9]; Asthma [493]; Emphysema [492, 492.8]; and Hypertension [401]). Twenty-seven healthy individuals and 40 compromised individuals were contacted, of which 22 agreed to participate in the study. The individuals were monitored during the period of March to August 2004. Of the 22 monitored individuals, one individual was excluded from the analysis because she withdrew from the study before the first 12-hour monitoring period was completed, leaving 21 individuals with relevant data for inclusion in this analysis. Six of the 21 individuals were “healthy,” while 15 individuals were “compromised,” with one or additional disease diagnoses as stated above. Eighteen of the 21 individuals were monitored for the entire 36-hour period; two individuals were monitored for a 24-hour period and one individual was monitored for a 12-hour period.

Each participant completed a modified American Thoracic Society questionnaire.²⁴ The self-administered questionnaires were used to obtain information on personal demographic information, smoking history, medical history, including cardiopulmonary symptoms and diseases, and medication use. In cases where the participant was not fluent in English, the questionnaire was administered by a translator in Spanish or Portuguese. Data from questionnaires were used to identify individuals with hypertension, inflammatory lung diseases (i.e., chronic bronchitis, asthma, and emphysema), and diabetes.

PM_{2.5} Sample Collection

The TSI Incorporated model AM510 SIDEPAK personal aerosol monitor (TSI Incorporated, Shoreview, MN), a light scattering laser photometer, with a 2.5- μ m cutoff impactor was used to assess personal exposure to PM_{2.5} over a 36-hour period. The air sample was drawn through

a 4-feet long Tygon tube into the impaction inlet at a flow rate of 1.7 L/min. PM_{2.5} concentrations were logged every minute, an average of a series of measurements taken every 10 seconds. The minute-to-minute PM_{2.5} concentrations were averaged over an approximately 12-hour interval which coincided with the time when spot urine samples were collected.

The SIDEPAK was factory-calibrated to the respirable fraction of the International Organization for Standardization (ISO) 12103-1, A1 Arizona test dust.²⁵ Arizona test dust is a polydispersed test aerosol commonly used because it is representative of a wide variety of ambient aerosols. Previous studies have shown that measurements from similar light scattering laser photometers overestimate PM_{2.5} concentrations by a factor of two to three when compared to measurements taken using a gravimetric method.²⁶⁻²⁸ To examine the possible overestimation of PM_{2.5} concentrations when using the SIDEPAK, comparisons were made with PM_{2.5} samples collected on a 37 mm polytetrafluoroethylene membrane filter (Gelman Laboratories, Ann Arbor, MI) using KTL cyclones (GK2.05SH, BGI Incorporated, Waltham, MA) with a 50% aerodynamic diameter cutpoint of 2.5 µm. The cyclone was collocated with the SIDEPAK and PM_{2.5} was collected over the entire monitoring period for all subjects, resulting in an approximately 36-hour average concentration. PM_{2.5} concentrations from the SIDEPAK and the cyclone gravimetric method were compared using analyses described previously.²⁹ Eighteen of the 21 collocated cyclone samples provided data useful to this analysis. For the 18 samples, the geometric mean of the PM_{2.5} concentrations obtained using the gravimetric method was 0.026 mg/m³ (GSD 2.032) while the corresponding geometric mean using the Sidepak method was 0.059 mg/m³ (GSD 2.360). Among the non-smokers, the geometric means for the gravimetric and Sidepak concentrations were 0.020 mg/m³ (GSD 1.410) and 0.042 mg/m³ (GSD 1.379), respectively. Among the smokers, the geometric means of the gravimetric and SidePak concentrations were 0.094 mg/m³ (GSD 1.922) and 0.317 mg/m³ (GSD 2.020), respectively. The gravimetric PM_{2.5} concentrations were regressed on SIDEPAK PM_{2.5} concentrations and an interaction term was included to study the effect of smoking status on the association between gravimetric and SIDEPAK PM_{2.5} concentrations. Comparisons indicated that the SIDEPAK overestimated PM_{2.5} concentrations and the degree of overestimation depended on the smoking status of the individual. To correct for the overestimation, a factor of 0.45 for smokers and 0.29 for nonsmokers was applied to the SIDEPAK PM_{2.5} concentrations.

Urine Collection and Analysis for 8-OHdG

Spot urine samples were collected at baseline and at approximately 12-hour intervals during the 36-hour monitoring period, resulting in the collection of up to four samples for each individual. The baseline and 24-hour spot urine samples were generally collected between 7:00 AM to 9:00 AM while the 12-hour and 36-hour spot urine samples were collected between 7:00 P.M. to 9:00 P.M. Spot urine samples were collected instead of 12-hour pooled urine samples due to concerns of subject compliance. A study by Miwa et al.³⁰ observed significant correlations between 8-OHdG levels in a morning spot urine and in a 24-hour pooled urine, and concluded that a morning spot urine sample can be used for the measurement of 8-OHdG instead of 24-hour sampling, which can be cumbersome and inconvenient for the subjects.

After samples were collected in sterile 120 mL urine collection cups, they were aliquoted into 15 mL polypropylene tubes. One 5 mL aliquot was sent to Path Lab Incorporated (Portsmouth, NH) for creatinine analysis. The remaining specimen was stored at -20°C until further analysis.

Urine analysis for 8-OHdG was performed by Genox Corporation (Baltimore, MD). Urinary 8-OHdG was determined using a competitive enzyme-linked immunosorbent assay.^{18,31} Briefly, 50 µL of urine samples and standards were added to precoated 8-OHdG protein conjugate microtiter plates, followed by 50 µL of the primary antibody, anti-8-OHdG monoclonal antibody solution, and incubated for 1 hour at 37°C. The plates were washed and

the enzyme-labeled horseradish peroxidase-conjugated secondary antibody (100 μ L) was applied for 1 hour at 37°C. After washing, 100 μ L of the chromatic substrate, (3,3',5,5') tetramethylbenzidine, was added to the plate and allowed to react at room temperature for 15 minutes. The intensity of color produced for each sample was measured at an optical density of 490 nm. Pooled urine samples from several healthy adults were used as the quality control samples. For each standard 96-well microplate, six to nine quality control samples were randomly placed among the unknown samples. The measured quality control values were averaged and compared with previously established values. Acceptable quality control values were defined as mean $\pm$ 2 SDs. The limit of detection for 8-OHdG was 0.64 ng/mL. Seven of 78 available samples (9%) had concentrations below the limit of detection and were given the value of 0.32 ng/mL ($\frac{1}{2}$ the limit of detection). For each sample, either a duplicate or triplicate measurement was performed. The mean, SD, and coefficient of variation (percent) were calculated. Any sample with a coefficient of variation equal to or greater than 20% was retested. Urinary 8-OHdG concentrations are dependent on kidney function. Creatinine levels are a standard indicator of kidney function; therefore, we adjusted the urinary 8-OHdG levels for urinary creatinine levels in all our analyses.

Statistical Analyses

Statistical analyses were performed using SAS version 9.1 (SAS Institute Incorporated, Cary, NC). Urinary creatinine-adjusted 8-OHdG concentrations and PM_{2.5} concentrations with the correction factors applied were used in all analyses. Baseline urinary 8-OHdG concentrations were excluded from the analysis as PM_{2.5} concentration data were not available prior to baseline urine sample collection. As multiple samples were collected from each individual, linear mixed models were used to account for the correlation of repeated measurements within individuals.

These models were used first to examine whether urinary 8-OHdG and PM_{2.5} concentrations differed by age, gender, smoking status, and hypertension status. The 12-hour-average PM_{2.5} concentrations were found to be positively skewed (skewness of 4.3); as PM_{2.5} concentrations were an outcome variable in these analyses, it was necessary to log-transform the PM_{2.5} data to improve skewness. Then, multivariate regression models were constructed to investigate the association between urinary 8-OHdG concentrations and average PM_{2.5} concentrations over the prior 12-hour interval. In these models, the PM_{2.5} concentration was a predictor, not an outcome; therefore the non-transformed PM_{2.5} concentrations were used. Only a small percentage of urinary 8-OHdG and PM_{2.5} exposure data were missing (2% and 7%, respectively) and the missing data generally appeared random; therefore, all analyses were conducted using the available data only. A compound symmetry correlation structure was used because it resulted in the best Akaike's Information Criterion compared with models with other covariance structures, including a first-order autoregressive structure.³² Restricted maximum likelihood was used to estimate the covariance parameters. To investigate effect modification of the association between urinary 8-OHdG and PM_{2.5} by hypertension status, an interaction term between PM_{2.5} and an indicator variable for hypertension was included in the model. Potential confounding by age, gender, and tobacco smoking (dichotomized as current smoker or non-current smoker) also was examined. Due to possible circadian variation of urinary 8-OHdG levels, all models also were adjusted for time of day (dichotomized as day or evening).³³ As concerns remain regarding residual confounding by tobacco smoking and circadian variation even after adjustment using a dichotomized variable in the statistical models, data also were reanalyzed after restricting to morning urinary 8-OHdG samples from nonsmokers. In addition, as study subjects were monitored over two seasons, from March to August, a sensitivity analysis adjusting for seasonal variation was also conducted.

Results

Study Population Demographics

The study population consisted of 21 individuals, five men and sixteen women, with a mean age of 45 years (range, 21-70) (Table 1). Twelve individuals had physician-diagnosed hypertension (≥ 140 mm Hg systolic pressure or ≥ 90 mm Hg diastolic pressure). Eleven of the twelve individuals indicated that they were on antihypertensive medications; one individual did not provide that information. The antihypertensive medications currently taken by the individuals included angiotensin-converting enzyme (ACE) inhibitors (lisinopril, enalapril), angiotensin II receptor blockers (losartan), calcium channel blockers (amlodipine), diuretics (hydrochlorothiazide, triamterene), and beta-adrenoceptor antagonists (atenolol, metoprolol).

Individuals with hypertension were significantly older than those without hypertension (mean age 54 years versus 33 years, respectively [$p = 0.001$]). In addition, a greater percentage of individuals with hypertension compared to those without hypertension were current smokers (42% versus 11%, respectively [$p = 0.14$]). Two of the individuals with hypertension also had diabetes and five also had inflammatory lung disease (asthma and/or chronic bronchitis). Three of the nine individuals without hypertension had asthma; none had diabetes or chronic bronchitis.

PM_{2.5} Exposure Assessment

The personal PM_{2.5} exposure data are summarized in Table 2. Fifty-five 12-hour-average PM_{2.5} measurements were collected from the 21 participants. The number of consecutive 12-hour intervals for which an individual had PM_{2.5} data ranged from 1 to 3. The coefficient of variance (COV) of the PM_{2.5} concentrations was calculated for each individual subject. The COVs varied widely, from a minimum value of 5% to 111% across the subjects, with a median value of 55%. These results suggest that though autocorrelation may be of concern for a small number of subjects (only 4 subjects had a COV of less than 25%), there appears to be considerable variation in the PM_{2.5} data within an individual.

The 12-hour-average PM_{2.5} concentrations were found to be positively skewed. Linear mixed models indicated that the log-transformed 12-hour-average PM_{2.5} concentrations were not significantly different by hypertension status ($p = 0.24$). Additional analyses indicated that PM_{2.5} exposures significantly differed by gender ($p = 0.04$) and marginally differed by smoking status ($p = 0.06$), but not by age ($p = 0.11$). Men and current smokers were found to have higher 12-hour-average PM_{2.5} concentrations compared to women and non-smokers, respectively.

Urinary 8-OHdG Analysis

A total of 78 spot urine samples were collected from the 21 individuals, with each individual providing 2 to 4 samples. The baseline urine samples ($n = 20$, one subject did not provide a baseline sample) were excluded from the analysis as PM_{2.5} concentration data were not available prior to baseline urine sample collection. The Shapiro-Wilk test indicated that urinary 8-OHdG concentrations were normally distributed ($p = 0.17$). The distribution of urinary 8-OHdG levels for the remaining samples ($n = 58$) is presented in Table 3. In crude analyses, urinary 8-OHdG concentrations were not significantly different in individuals with hypertension compared to those without hypertension ($p = 0.30$). Urinary 8-OHdG concentrations did not significantly differ by gender ($p = 0.39$), smoking status ($p = 0.79$), or age ($p = 0.60$). Examination of the urinary 8-OHdG concentrations by time of day indicated that levels were higher in the evening (7:00 PM - 9:00 PM) compared to the morning (7:00 AM - 9:00 AM) ($p = 0.01$).

Regression Analysis of Urinary 8-OHdG and PM_{2.5} Exposure

The 12-hour-average PM_{2.5} concentrations were positively skewed largely due to two outlying data (196 $\mu\text{g}/\text{m}^3$ and 262 $\mu\text{g}/\text{m}^3$). Excluding the outlying data reduced the positive skewness to 1.1. Regression models excluding the two outlying data points were conducted as sensitivity analyses.

Linear mixed regression models were constructed to investigate the association between urinary 8-OHdG and PM_{2.5} (Table 4). In models individually adjusting for age, gender, smoking status, and time of day, all but age were found to affect the PM_{2.5} effect estimate. However, as previous studies have indicated that age may influence urinary 8-OHdG levels,³⁰ all four variables were included in the adjusted model. In the adjusted model, an inverse association between urinary 8-OHdG and PM_{2.5} was observed. In the model excluding the two outlying data points, similar results were observed; however, the effect estimate was not statistically significant. An additional sensitivity analysis was conducted to consider possible influences of seasonal variation. In models adjusted for seasonal variation, similar results were once again observed, with only slight changes in the effect estimates.

Prior to performing statistical analyses to investigate effect modification of the association between urinary 8-OHdG levels and PM_{2.5} by hypertension status, crude scatter plots stratified by hypertension status were examined (Figure 1). The scatter plots suggested that the relationship between urinary 8-OHdG and PM_{2.5} differed by hypertension status. Regression models including an interaction term between PM_{2.5} and hypertension were used to formally investigate modification by hypertensive status. In the adjusted model, the interaction term between PM_{2.5} and hypertension status was marginally significant when using all available data ($p = 0.06$). The interaction term indicated that each 10 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} was associated with a 1.71 $\mu\text{g}/\text{g}$ creatinine (95% CI: -0.10, 3.52) greater increase in urinary 8-OHdG in individuals without hypertension compared to those with hypertension. Similar results were observed in models excluding the outlying data points (2.44 $\mu\text{g}/\text{g}$ creatinine [95% CI: 0.34, 4.54] greater increase in urinary 8-OHdG with each 10 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5}). Effect estimates for those with and without hypertension were calculated from the adjusted model with the interaction term for hypertension status. The effect estimate for PM_{2.5} was positive, though nonsignificant for individuals without hypertension, while an inverse association was observed in those with hypertension.

Model assumptions were tested by examining a plot of the residuals against the predicted values and the normality of the residuals. In models using all available data, as well as those excluding outlying data, model assumptions were found to be met. The regression residuals were normally distributed (Shapiro-Wilk test $p > 0.90$) and the plots of the residuals against the predicted values indicated good model fit.

Due to concerns regarding residual confounding by tobacco smoking and circadian variation, a restricted analysis using only morning urinary 8-OHdG samples from non-smoking individuals was conducted (Table 4). For this analysis, a total of 15 spot urine samples were available from 15 non-smoking individuals. The same trend in results was observed in this restricted analysis - a positive association between PM_{2.5} and urinary 8-OHdG levels in individuals without hypertension, while an inverse association was observed in individuals with hypertension. Both effect estimates were not statistically significant.

Discussion and Conclusions

Exposure to PM_{2.5} has been linked to the induction of oxidative DNA damage in toxicologic studies.³⁴ Particles may contain soluble transition metals, including iron, copper, chromium and vanadium, which can generate ROS through Fenton-type reactions. Polycyclic aromatic

hydrocarbons absorbed onto the surface of carbonaceous particles also may be metabolically activated to quinone radicals that may undergo redox-cycling to produce ROS. Oxidative DNA damage resulting from the ROS may be implicated in cancer risk and possibly serve as a marker for oxidative stress relevant for other health conditions, including cardiovascular diseases, caused by PM_{2.5} or other air pollutants.³⁴

Recent interest has focused on 8-OHdG, measured either in white blood cells or urine, as an indicator of oxidative DNA damage and repair.³⁵ 8-OHdG is formed from a hydroxyl radical attack at the C-8 position of deoxyguanosine in DNA.³⁶ The repair mechanism after 8-OHdG is incorporated into DNA involves base and nucleotide excision with DNA-specific nucleosides excreted into urine. Pilger and Rudiger³⁷ noted that the 8-OHdG is the most studied oxidative DNA lesion due to its significance as an endogenous mutagen and its likely role in the process of carcinogenesis.

In this study, the association between personal PM_{2.5} exposure and oxidative DNA damage, as indicated by urinary 8-OHdG concentrations, was examined in a cohort of inner-city adults living in close proximity to a bus terminal. In the analysis including all subjects, PM_{2.5} concentration was associated with a decrease in urinary 8-OHdG after adjusting for age, gender, smoking status, and time of day. However, results from the analysis including an interaction term between PM_{2.5} and hypertension status suggested that the relationship between PM_{2.5} exposure and urinary 8-OHdG concentrations is modified by hypertension status. PM_{2.5} concentration was associated with a decrease in urinary 8-OHdG in individuals with hypertension compared to an increase in those without hypertension after adjusting for age, gender, smoking status, and time of day.

The positive, though not statistically significant, association between PM_{2.5} exposure and urinary 8-OHdG observed in those without hypertension is consistent with results from previous epidemiologic studies. In a recent study, Abder-Rahman and Nusair³⁸ observed that urinary 8-OHdG concentrations in a variety of occupationally- and environmentally-exposed populations were higher compared to the control population, suggesting that environmental toxins can induce DNA damage. In a group of non-smoking bus drivers from the greater Copenhagen area, higher urinary 8-oxo-2'-deoxyguanosine (8-oxodG) levels, another biomarker of oxidative DNA damage, were observed in bus drivers from the urban area compared to those from rural areas, suggesting that exposure to ambient air pollution was associated with oxidative damage to DNA.¹¹ Sørensen et al.¹⁰ examined the association between personal PM_{2.5} exposures and biomarkers of oxidative DNA damage in students living in central Copenhagen, Denmark, and observed that each 10 µg/m³ increase in PM_{2.5} exposure was associated with an 11% increase in lymphocyte 8-oxodG levels (p = 0.007), though not with urinary 8-oxodG concentrations.

Oxidative stress may constitute a major pathogenic factor in the development of hypertension.³⁹ Experimental studies by de Champlain et al.³⁹ indicated that ROS, mainly through the production of superoxide anion, could cause important alterations in the cellular signal transduction systems, ultimately leading to vasoconstriction. Further, hypertension is also associated with an impairment of endogenous antioxidant mechanisms.⁴⁰ However, antihypertensive medications have been shown to have antioxidant activities, including calcium channel blockers (amlodipine), ACE inhibitors (enalapril and lisinopril), and angiotensin II receptor blockers (losartan).⁴¹⁻⁴⁴ In this study, eleven of the twelve hypertensives indicated that they were taking antihypertensive medication and eight of them were on treatments with some antioxidant capability. The antioxidant activity present in some antihypertensive medications may play a role in reducing the effects of PM_{2.5} on oxidative stress; however, it does not fully explain the inverse association observed between PM_{2.5} exposure and urinary 8-OHdG levels among individuals with hypertension. Further studies

may consider measuring antioxidant activity to examine the effects of hypertensive medication use on the relationship between PM_{2.5} and 8-OHdG levels.

Another possible hypothesis for the observed inverse association in hypertensive individuals may be that PM_{2.5} exposure does not decrease oxidative damage, but rather reduces capacity to repair DNA damage. DNA damage that is not repaired tends to promote mutagenesis. As mentioned earlier, urinary 8-OHdG is a specific DNA repair product in the urine and although it is often considered a marker of oxidative damage, perhaps it also reflects repair of that damage. Gackowski et al.⁴⁵ examined 8-OHdG levels in leukocytes and urine in patients with lung cancer and two control groups. They observed that the level of 8-OHdG in DNA isolated from leukocytes of cancer patients was significantly higher than that in DNA from the two control groups. However, urinary 8-OHdG levels were similar in the cancer patients and control group. The authors concluded that the higher rate of oxidative damage in the cellular DNA of lung cancer patients was a result of a deficiency of repair mechanisms in this group. Likewise, the inverse association between PM_{2.5} concentrations and urinary 8-OHdG levels observed in the hypertensive individuals in our study may suggest a reduced capacity for DNA repair with increased PM_{2.5} exposure in this potentially susceptible population.

We acknowledge limitations to our study. In this panel study, we investigated the association between PM_{2.5} concentrations and urinary 8-OHdG levels in 21 individuals, 12 hypertensives and 9 non-hypertensives, monitored repeatedly during a 36-hour period. While the small sample size is a limitation, the use of repeated measurements increased the power of the study, allowing us to examine this relationship.

We were unable to examine the modifying effect of PM_{2.5} on oxidative DNA damage in hypertensives untreated by medication compared to non-hypertensives. Both hypertensive and non-hypertensive individuals were recruited from a health center and selected using ICD-9 codes. Generally, individuals diagnosed with hypertension at the health center were prescribed antihypertensive medications for treatment. For ethical reasons, we could not request that hypertensive individuals refrain from taking their medications during the monitoring period. Due to the variety of medications taken by the hypertensive individuals (e.g., ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, diuretics, and beta-adrenoceptor antagonists) and the small sample size of our study, we could not examine the effects of the different types of medications in our analyses.

Another limitation of this study is possible confounding of the association between PM_{2.5} and urinary 8-OHdG by copollutants, though the use of personal PM_{2.5} measurements rather than ambient PM_{2.5} concentrations from a community monitor likely reduced this risk. One study by Sarnat et al.⁴⁶ observed that personal PM_{2.5} exposures were not highly correlated with personal measurements of copollutants, particularly ozone, nitrogen dioxide, and sulfur dioxide. It should be noted, however, that in another study by Sarnat et al.,⁴⁷ personal exposure to PM_{2.5} was found to be associated with personal exposures to ozone and nitrogen dioxide in the summer.

In this study, associations between total exposure to PM_{2.5} and urinary 8-OHdG were examined. We were unable to distinguish between health effects associated with PM_{2.5} from ambient and nonambient origins. Of particular concern in our study is PM_{2.5} exposure from tobacco smoking. Tobacco smoke has a particle size range of <0.1 µm to just over 1 µm in particle diameter,⁴⁸ thus, it has an impact on total PM_{2.5} exposure, as was observed in our study. A recent study by Ebelt et al.⁴⁹ examined cardiovascular and respiratory health effects after separating total personal particle exposure into that of ambient and nonambient origins. The strongest associations were observed with personal exposure to PM_{2.5} of ambient origin compared to ambient PM_{2.5} concentrations, total personal exposure to PM_{2.5}, or personal

exposure to PM_{2.5} of nonambient origin. We were unable to examine the associations between ambient PM_{2.5} and oxidative DNA damage since we did not collect the necessary information (time-activity data, measurements of PM infiltration) to estimate the ambient component of PM_{2.5} from our personal exposure samples.

Another limitation of this study is the possibility of confounding from age, gender, tobacco smoking, and circadian variation. Though these variables were controlled for in the multivariate regression models, the possibility of residual confounding remains, particularly for tobacco smoking and circadian variation as the biologically-relevant surrogate measures for these variables in assessing the relationship between PM_{2.5} and urinary 8-OHdG were difficult to determine and adjusted for in the models. Concerns of residual confounding led us to conduct an analysis restricting the data to morning urinary 8-OHdG samples from non-smoking individuals. In this restricted analysis, the same trend was observed, though the 95% CIs were wider due to reduced power.

In conclusion, this study shows that the association between personal exposure to PM_{2.5} and urinary 8-OHdG, a biomarker of oxidative DNA damage and repair, was modified by hypertension status. A positive, but not statistically significant, exposure-response relationship was observed between urinary 8-OHdG concentrations and PM_{2.5} exposure in individuals without hypertension. In contrast, a statistically significant decrease in urinary 8-OHdG levels in relation to PM_{2.5} exposure was observed in those with hypertension. Since it is highly unlikely that PM_{2.5} has healthful effects on hypertensive individuals, these counterintuitive results require confirmation in further studies and exploration of possible mechanisms.

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Abbreviations

The following abbreviations are used (in alphabetical order):

8-OHdG, 8-hydroxy-2'-deoxyguanosine; 8-oxodG, 8-oxo-2'-deoxyguanosine; 95% CI, 95% confidence interval; ACE, angiotensin-converting enzyme; COV, coefficient of variance; IQR, interquartile range; PM_{2.5}, particulate matter with an aerodynamic mass median diameter $\leq$ 2.5 μ m; ROS, reactive oxygen species; SD, standard deviation.

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Figure 1.

Crude scatterplots of 12-hour-average PM_{2.5} concentrations (on natural log scale) and urinary 8-OHdG concentrations in individuals with hypertension (A, including all data points; B, excluding two outlying data points) and without hypertension (C).

(A) Individuals with hypertension (n = 12; 30 data points)

(B) Individuals with hypertension, excluding two outlying data points (n = 12; 28 data points)

(C) Individuals without hypertension (n = 9; 25 data points)

Table 1

Study population characteristics

Characteristics		
Total number of participants		21
Gender	Males	5
	Females	16
Race/Ethnicity	Non-Hispanic white	1
	Non-Hispanic black	15
	Hispanic	5
Mean age, years (range)		45 (21-70)
Number (%) of current smokers		6 (29%)
Number (%) with hypertension		12 (57%)

Table 2

Summary of adjusted 12-hour-average PM2.5 concentrations ($\mu\text{g}/\text{m}^3$)^a

	Number of samples	Percentile				
		Minimum	25 th	50 th	75 th	Maximum
All individuals (<i>n</i> = 21)	55	3.7	10.8	18.4	30.1	261.9
Individuals with hypertension (<i>n</i> = 12)	30	5.3	11.3	21.8	46.8	261.9
Individuals without hypertension (<i>n</i> = 9)	25	3.7	10.4	15.8	27.8	46.2

^a Comparisons with gravimetric measurements indicated that SIDEPAK overestimated PM2.5 concentrations. A correction factor of 0.45 for smokers and 0.29 for nonsmokers applied.

Table 3

Summary of creatinine-adjusted urinary 8-OHdG concentrations (µg/g creatinine)

	Number of samples	Mean (SD)	Percentile				
			Minimum	25 th	50 th	75 th	Maximum
All individuals (n = 21)	58	10.7 (6.1)	0.6	6.3	9.5	15.2	26.3
Individuals with hypertension (n = 12)	32	9.6 (6.6)	0.6	4.4	8.6	15.4	26.3
Individuals without hypertension (n = 9)	26	12.0 (5.3)	1.2	9.1	10.3	15.1	22.8

Regression coefficients and 95% CIs for urinary 8-OHdG concentrations regressed on 12-hour-average PM_{2.5} concentrations^a

	Including all data points		Excluding outlying data points ^b	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Crude association	-0.40 (-0.79, -0.01)	p = 0.04	-0.33 (-1.35, 0.69)	p = 0.51
Adjusted association ^c	-0.51 (-0.95, -0.07)	p = 0.02	-0.69 (-1.74, 0.36)	p = 0.19
Effect modification by hypertension status ^c				
Hypertensives	-0.60 (-1.03, -0.17)	p = 0.01	-1.36 (-2.54, -0.19)	p = 0.02
Non-hypertensives	1.10 (-0.68, 2.88)	p = 0.22	1.08 (-0.71, 2.86)	p = 0.23

^a Coefficient is expressed as the change in urinary 8-OHdG concentration (µg/g creatinine) per 10 µg/m³ increase in PM_{2.5}.

^b Two outlying data points, both from the same hypertensive individual, were excluded in these analyses.

^c Models adjusted for age, gender, current smoking status, and time of day