The association of expired nitric oxide with occupational particulate metal exposure

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Abstract

Toxicologic studies have shown that soluble transition metals in residual oil fly ash (ROFA) can induce pulmonary injury. In this study, we investigated the association between the fractional concentration of expired nitric oxide (FENO) and exposure to metal constituents of particulate matter with an aerodynamic mass median diameter less than 2.5 μm (PM2.5) in boilermakers exposed to ROFA and metal fume. Metals investigated included vanadium, chromium, manganese, nickel, copper, and lead. Subjects were monitored for 5 consecutive days during boiler repair overhauls in 1999 (n = 20) and 2000 (n = 14). In 1999, we found a significant inverse association between log-transformed FENO and PM2.5 metal concentrations. Log FENO changed by -0.03 (95% CI: -0.04, -0.01), -0.56 (95% CI: -0.88, -0.24), -0.09 (95% CI: -0.16, -0.02), and -0.04 (95% CI: -0.07, -0.02) per μg/m3 of PM2.5 vanadium, chromium, manganese, and nickel, respectively. In 2000, no significant associations were observed, most likely due to exposure misclassification resulting from the use of respirators. The inverse association between PM2.5 metal exposure and FENO in subjects with limited respirator usage suggests that soluble transition metals might be partially responsible for the adverse pulmonary responses seen in workers exposed to ROFA.

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1. Introduction

Epidemiologic studies have shown an association between particulate air pollution and an increased incidence of respiratory symptoms, increased hospitalizations for cardiopulmonary diseases, and excess morbidity and mortality (Dockery et al., 1992; Pope et al., 1995; Schwartz et al., 1993). The fine mode of particulate air pollution is of particular concern, as this fraction has been found to have high penetration and retention in the alveolar region of the lung (Hinds, 1999). Fine particulate matter, defined as particles with an aerodynamic mass median diameter greater than 100 nm and less than 2.5 μm (PM2.5), is composed primarily of sulfate, nitrate, organic and elemental carbon, ammonium ions, and various trace elements. Recent attention has focused on soluble transition metals as a biologically relevant component of PM2.5 (Costa and Dreher, 1997; Dreher et al., 1996).

Residual oil fly ash (ROFA) is an emission source air pollutant with a high content of bioavailable transition metals. Many metals, including vanadium, nickel, iron, zinc, and copper, are present in ROFA at significant concentrations (Huffman et al., 2000). The metals are three- to sixfold more concentrated in PM2.5 than in larger particles (Huffman et al., 2000). Toxicologic studies have shown that soluble transition metals in
ROFA play an important role in inducing acute pulmonary injury and airway hyperreactivity (Costa and Dreher, 1997; Dreher et al., 1997). Transition metals have variable oxidation states which allow them to support redox cycling and the generation of oxygen-based radical species (Gavett et al., 1997). Previous studies have documented pulmonary injury, including airway obstruction and inflammation, in individuals occupationally exposed to ROFA (Hauser et al., 1995a, b; Sjöberg, 1955; Williams, 1952; Woodin et al., 1998). While these studies showed that ROFA exposure adversely affected respiratory health, the relationship between specific transition metals and airway injury was unclear. Most studies found no exposure–response relationship between vanadium exposure and pulmonary responses. However, one study observed that an increased dose of vanadium to the lower airway was significantly associated with increased severity and frequency of respiratory symptoms in occupationally exposed subjects (Woodin et al., 2000).

Utilizing a sensitive biomarker for acute lower airway responses may clarify the association between transition metals and respiratory health effects in humans.

Expired nitric oxide (NO) is an effective, noninvasive marker for the early assessment of airway responses (Silkoff, 2000). The production of endogenous NO is catalyzed by the enzyme NO synthase (NOS) (Marletta, 1993). There are three types of NOS; neuronal NOS and endothelial NOS generally have constitutive activity, while inducible NOS is immunoactivated (Michel and Higgs, 1993). NO plays an important role in the airways; it serves as a neurotransmitter of bronchodilator nerves and is important in nonspecific host defense of the respiratory tract (Belvisi et al., 1992; Moncada and Higgs, 1993). Individuals with inflammatory lung diseases have elevated expired NO levels compared to healthy individuals due to increased expression of inducible NOS (Kharitonov and Barnes, 2000).

Some environmental studies have shown that expired NO levels were elevated on days with high particulate air pollution (Steerenberg et al., 2001; Van Amsterdam et al., 1999). Conversely, exposure to cigarette smoke was found to be associated with decreased expired NO concentrations, possibly resulting from reduced NOS activity (Kharitonov et al., 1995; Su et al., 1998; Yates et al., 2001). Hence, the direction of change in expired NO levels following environmental exposures may vary depending on the specific type of exposure.

In this study, we investigated the association between the fractional concentration of NO in mixed expired gas (FENO) and exposure to constituent metals of PM2.5 in a cohort of boilermakers exposed to ROFA and metal fume. The boilermakers were monitored during a 5-day work period using a repeated-measures study design. The acute airway responses to the following six metals were studied: vanadium, chromium, manganese, nickel, copper, and lead. The occupational PM2.5 metal exposure was hypothesized to be similar to cigarette smoke, as both ROFA and cigarette smoke contain relatively high concentrations of metals (Chiba and Masironi, 1992; Huffman et al., 2000). Therefore, the FENO levels in the boilermakers were hypothesized to decrease with PM2.5 metal exposure.

2. Materials and methods

2.1. Study population

The study was approved by the Institutional Review Board of the Harvard School of Public Health. Written informed consent was obtained from each subject prior to participation in the study. The study population consisted of 32 boilermakers working at a power plant during the overhaul of oil-fired boilers. Twenty subjects were studied in June 1999 and 14 subjects, including 2 from 1999, were studied in October 2000. A self-administered questionnaire was used to obtain information on medical history, including respiratory symptoms and diseases, smoking history, and occupational history.

The overhaul entailed removal and replacement of several large panels of the interior wall and the water-circulating tubing of the boiler. In addition, repair of the ash pit was performed. The various work tasks of the boilermakers included welding, burning, and grinding.

2.2. FENO collection

FENO samples were collected pre- and postworkshift each day during a 5-day sampling period. Baseline FENO samples were collected preworkshift on the first day of the workweek, after 1–2 days away from work. The offline collection and measurement of FENO was in accordance with the American Thoracic Society (ATS) recommendations (American Thoracic Society, 1999). Subjects were asked to refrain from smoking in the hour preceding NO sampling. Subjects wore nose clips and tidal breathed for 30 s through an apparatus containing two one-way valves with an NO-scrubbing filter attached to the intake limb. Subjects then inhaled to total lung capacity and expired their entire vital capacity into a Mylar balloon attached to the expiratory limb while maintaining an oropharyngeal pressure of 12.5 cm H2O. Three FENO samples were taken at each collection time. NO levels in the balloons were measured using a calibrated Sievers NOA 280 chemiluminescence analyzer (Boulder, CO, USA). The median NO concentration of the three samples was used in the statistical analysis because it was insensitive to any aberrant observations while providing a measure of central value.
2.3. Particulate sample collection

Subjects were randomly selected to wear personal exposure monitors (PEM) during their workshift. Workplace particulate samples were collected from 19 of the 20 subjects in 1999 and all 14 subjects in 2000. The number of workdays each subject wore the personal exposure monitor varied from 5 study days to 0. On average, each subject was monitored two to three times throughout the week. The Model 200 PEM (MSP Corp., MN, USA) with a 2.5-μm impactor cutsize was used in line with a Gilian GilAir5 pump (Sensidyne Inc., Clearwater, FL, USA) calibrated at a flow rate of 4 L/min. The air sample was collected on a 2-μm-pore-size polytetrafluoroethylene 37-mm filter membrane (Gelman Laboratories, Ann Arbor, MI, USA) placed within the PEM. The PEMs were placed on the lapels of the subjects, near their breathing zone. The duration of sample collection was recorded so that the time-weighted average exposure could be calculated.

2.4. Metals analysis of particulate samples

Samples were digested using a modified acid digestion procedure originally developed by Loring and Rantala (1992). The digestion protocol allowed for the determination of total metal concentration, including metal oxides. Briefly, after removal of the support ring, the filter was placed in a Teflon liner (Parr Instrument Co., Moline, IL, USA). Next, 5 mL of nitric acid (OPTIMA, Seastar Chemical Inc., Pittsburgh, PA, USA) and 400 μL of hydrofluoric acid (OPTIMA, Seastar Chemical Inc.) were added to the liner. The Teflon liner was placed in a Parr bomb (Parr Instrument Co.), and heated in a microwave oven for 3 min at 750 W. After cooling of the vessels, 10 mL of 1.5% boric acid (ULTREX, J.T. Baker, Phillipsburg, NJ, USA) was added to the liners to neutralize the excess hydrofluoric acid. The sample was reheated for an additional 3 min. After another cooling period, the digestate was transferred into a 50-mL polypropylene tube and diluted to 50 mL with ultrapure, deionized water.

Samples were analyzed using inductively coupled plasma–mass spectrometry (ICP-MS) (Perkin-Elmer/SCIEX ELAN Model 5000 and Model 6100, Perkin–Elmer Inc., Norwalk, CT, USA). The elements analyzed were vanadium, chromium, manganese, nickel, copper, and lead. Indium was used as the internal standard for vanadium, chromium, manganese, nickel, and copper. Bismuth was used as the internal standard for lead. The ICP-MS was calibrated using a multielement standard from SPEX CertiPrep (Metuchen, NJ, USA).

Quality assurance and quality control procedures were performed to ensure the accuracy of the metal analysis data. Method blanks were analyzed to determine the blank filter metal content and to monitor contamination. National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) standard reference material (SRM) 1648, urban particulate matter, was digested to evaluate the accuracy and extraction efficiency of the acid digestion procedure. NIST SRM 1643d, trace elements in water, was used as the ICP-MS calibration verification standard.

The limit of detection (LOD) was determined as three times the standard deviation of 10 replicate measurements of the filter blank samples. None of the metal concentrations were below the LOD. The mass of each metal was calculated after adjusting for the blank filter metal concentration and metal-extraction efficiency. The PM$_{2.5}$ metal concentrations were calculated by dividing the metal mass by the air volume sampled. The PM$_{2.5}$ metal contents were calculated by dividing the mass of each individual metal by the total PM$_{2.5}$ mass.

2.5. Statistical analysis

Statistical analyses were performed using SAS version 6.12 (SAS Institute Inc., Cary, NC, USA) and S-Plus2000 for Windows (MathSoft Inc., Cambridge, MA, USA). Wilcoxon rank-sum tests were performed to compare the baseline F$_E$NO in the two sampling years. The nonparametric rank sum test was used because the baseline F$_E$NO data were not normally distributed. The Spearman rank correlation coefficient was used to determine the correlation among the individual PM$_{2.5}$ metal concentrations. In addition, the correlation between F$_E$NO and the individual PM$_{2.5}$ metal concentrations was determined. The nonparametric Spearman coefficient was calculated because individual PM$_{2.5}$ metal concentrations and F$_E$NO data were not normally distributed.

Linear models were constructed to study the association between log-transformed F$_E$NO and PM$_{2.5}$ metal exposure. A linear model with independent and identically distributed errors was used because the repeated within-subject errors were found to be uncorrelated (Kleinbaum et al., 1998). While F$_E$NO data collection was complete, there were missing PM$_{2.5}$ metal concentration data. However, the PM$_{2.5}$ sampling data were missing at random, as subjects were randomly selected each day to wear exposure monitors. Therefore, all analyses were restricted to subjects who had both F$_E$NO and the corresponding PM$_{2.5}$ metal concentrations on a given day. Including baseline data, there were a total of 50 complete measurements in 1999 and 46 complete measurements in 2000. F$_E$NO values were log-transformed to improve normality. The models were adjusted for self-reported current cigarette smoking status (yes/no), age, and sampling year. In addition, an interaction term between sampling year and PM$_{2.5}$ metal exposure was included in the model. To determine the presence of influential points in the data, Cook’s distances were calculated.
calculated. A Cook’s distance is a diagnostic measure that assesses the impact of deleting a point. Cook’s distance greater than 0.5 may be considered influential (Cook and Weisberg, 1999). The level of significance for all analyses was 0.05.

3. Results

3.1. Description of study population

The demographic data are summarized in Table 1. The study population consisted of 32 men, 31 of whom were white (97%). Twenty subjects were sampled in 1999 and 12 additional subjects were sampled in 2000. Two subjects from 1999 were monitored in 2000 as well. The subjects ranged in age from 18 to 59 years, with 2 weeks to 40 years of boilermaking experience. Thirteen of the 32 subjects (41%) were current cigarette smokers. Six of the 32 subjects (19%) had chronic obstructive pulmonary disease (COPD). Five subjects were chronic bronchitics, as diagnosed by a physician or with symptoms as defined by the American Thoracic Society (1995). One subject had emphysema diagnosed by a physician.

The baseline measurements of FENO are shown in Table 1. Baseline measurements were taken on average after 2 days away from work in 1999 and 1 day away from work in 2000. Wilcoxon confidence intervals and corresponding medians are presented because of the positively skewed distribution of FENO. The median baseline FENO was 10.6 ppb (95% CI: 9.1, 12.7) in the 1999 subjects and 7.4 ppb (95% CI: 6.7, 8.0) in the 2000 subjects. The median baseline FENO in sampling year 2000 was significantly lower than the baseline FENO in 1999 ($P = 0.002$).

3.2. Exposure assessment

The occupational PM$_{2.5}$ metal concentrations for the 1999 and 2000 sampling period are shown in Table 2. The mean sampling time was 8.8 h (SD = 1.2) in 1999 and 10.9 h (SD = 1.3) in 2000. The average time monitored in the 2 sampling years was different due to the difference in workshift length. In 1999, the boiler-makers worked 10-h shifts, while in 2000 they generally worked 12-h shifts. The concentrations of PM$_{2.5}$ and its constituent metals were standardized to 8-h time-weighted averages (TWA) to account for this difference in workshift length.

Of the six metals, the subjects had the highest exposure to vanadium (median: 1.98 µg/m$^3$ in 1999, 4.53 µg/m$^3$ in 2000) and the lowest exposure to chromium (median: 0.18 µg/m$^3$ in 1999, 0.21 µg/m$^3$ in 2000). With the exception of chromium, all of the median PM$_{2.5}$ metal 8-h TWAs were significantly greater in 2000 than in 1999 ($P$ values $< 0.05$). The median total PM$_{2.5}$ 8-h TWAs were marginally different between the 1999 and 2000 samples ($P = 0.06$). The total PM$_{2.5}$ concentrations were moderately correlated
with each of the constituent metal concentrations (0.55 < r < 0.78, P < 0.001) in all samples from 1999 and 2000. The individual metal concentrations were also moderately to highly correlated with one another (0.51 < r < 0.95, P < 0.001) in all samples.

Table 3 summarizes the PM$_{2.5}$ metal content in the collected air samples. The largest metal component of PM$_{2.5}$ in the samples was vanadium, with a median PM$_{2.5}$ metal content of 3190 µg/g (95% CI: 2440, 4590) in 1999 and 5910 µg/g (95% CI: 4650, 7930) in 2000. The median PM$_{2.5}$ metal contents were not significantly different in the 2 sampling years, with the exception of vanadium and lead (P < 0.01). PM$_{2.5}$ in sampling year 2000 had a greater vanadium and lead component compared to PM$_{2.5}$ in 1999.

In both 1999 and 2000, 85% of the subjects stated in the questionnaires that they wore respirators while performing boiler maintenance and repair. Although quantitative data are not available, observations made by the field team indicated a much lower use of respirators during the 1999 sampling period. The subjects in 1999 rarely wore their respirators due to the high temperature and limited ventilation inside the power plant. Data from the National Weather Service, Boston Weather Forecast Office (Taunton, MA) indicated that the maximum temperature in Boston, MA was 92–97°F during the first half of the 1999 sampling period. In contrast, the maximum temperature in Boston during the 2000 sampling period ranged from 53 to 65°F. Respirator use was observed to be more common in 2000. The respirators typically used were the half-mask particulate respirators equipped with a high-efficiency particulate air filter, which has a particle filter efficiency of 99.97% for particles with an aerodynamic mass median diameter of 0.3 µm (NIOSH, 1996).

### 3.3. Association between F$_{ENO}$ and PM$_{2.5}$ metal exposure

Correlations between PM$_{2.5}$ metal concentrations and the postshift F$_{ENO}$ on the same day were weak, with r values ranging from −0.15 for copper to −0.05 for vanadium (P values > 0.35) in sampling year 1999. In 2000, the r values ranged from −0.26 to −0.13 (P > 0.10). Furthermore, the linear models did not indicate a significant association between postshift F$_{ENO}$ and the same day’s PM$_{2.5}$ metal concentrations in either year, after adjusting for preshift F$_{ENO}$. There was, however, a stronger lagged association between preworkshift F$_{ENO}$ and PM$_{2.5}$ metal concentrations from the previous workday in sampling year 1999. Correlations were statistically significant for all the six metals (−0.54 < r < −0.48, P values < 0.001) in 1999. In sampling year 2000, there was no significant correlation between PM$_{2.5}$ metal concentrations and preworkshift F$_{ENO}$ (0.002 < r < 0.21, P values > 0.15).

The regression coefficients and 95% confidence intervals for the association between preworkshift log-transformed F$_{ENO}$ and the previous workday’s PM$_{2.5}$ metal concentrations are shown in Table 4. In the 1999 subjects, the previous workday’s PM$_{2.5}$ vanadium, chromium, manganese, and nickel 8-h TWAs were individually associated with a significant decrease in log F$_{ENO}$, after adjusting for cigarette smoking status, age, and sampling year. Cigarette smoking status was significantly associated with a decrease that ranged from −0.20 to −0.25 in log F$_{ENO}$ in all the six metal models (P values < 0.005). The association between log F$_{ENO}$ and PM$_{2.5}$ metals was not found to be modified by COPD status. Each 1-µg/m$^3$ increase in PM$_{2.5}$ vanadium exposure was associated with a decrease of 0.03 (95% CI: −0.04, −0.01) in log F$_{ENO}$. Likewise, with each 1-µg/m$^3$ increase of PM$_{2.5}$ chromium, manganese, and nickel exposure, log F$_{ENO}$ changed by −0.56 (95% CI: −0.88, −0.24), −0.09 (95% CI: −0.16, −0.02), and −0.04 (95% CI: −0.07, −0.02), respectively. Copper and lead exposures were found to be marginally associated with log F$_{ENO}$ in 1999. To determine the presence of influential points in the data, Cook’s distances were calculated. None of the data points had Cook’s distances greater than 0.5; therefore there was little evidence that influential points were affecting the

### Table 3

<table>
<thead>
<tr>
<th>Sampling year 1999 (n = 30)$^b$</th>
<th>Sampling year 2000 (n = 33)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median$^c$</td>
</tr>
<tr>
<td>Vanadium (µg/g)</td>
<td>3190</td>
</tr>
<tr>
<td>Chromium (µg/g)</td>
<td>260</td>
</tr>
<tr>
<td>Manganese (µg/g)</td>
<td>1030</td>
</tr>
<tr>
<td>Nickel (µg/g)</td>
<td>2280</td>
</tr>
<tr>
<td>Copper (µg/g)</td>
<td>2130</td>
</tr>
<tr>
<td>Lead (µg/g)</td>
<td>350</td>
</tr>
</tbody>
</table>

$^a$Metal content was calculated by dividing the PM$_{2.5}$ metal mass by the total PM$_{2.5}$ mass.

$^b$n, number of samples.

$^c$Wilcoxon median.
association between logF\textsubscript{E}NO and PM\textsubscript{2.5} metal concentrations (Cook and Weisberg, 1999). For the subjects sampled in year 2000, no association between PM\textsubscript{2.5} metal concentrations on the previous workday and preworkshift logF\textsubscript{E}NO was observed. None of the PM\textsubscript{2.5} metal regression coefficients was found to be statistically significant.

Since the total PM\textsubscript{2.5} concentrations and the individual PM\textsubscript{2.5} metal concentrations were highly correlated, they could not be included in the same model. To adjust for total PM\textsubscript{2.5} exposure, analyses were rerun using PM\textsubscript{2.5} metal contents. The regression coefficients for the PM\textsubscript{2.5} metal contents are summarized in Table 5. As before, statistically significant associations were only observed in sampling year 1999 (2000 data not shown). The PM\textsubscript{2.5} metal contents of vanadium, chromium, and nickel were found to be significantly associated with logF\textsubscript{E}NO in 1999, after adjusting for cigarette smoking status, age, and sampling year. Each 1000-\mu g/g increase in PM\textsubscript{2.5} vanadium content was associated with a decrease of 0.02 (95% CI: −0.03, −0.01) in logF\textsubscript{E}NO. Likewise, with each 1000-\mu g/g increase of PM\textsubscript{2.5} chromium and nickel content, logF\textsubscript{E}NO changed by −0.29 (95% CI: −0.49, −0.08) and −0.02 (95% CI: −0.04, −0.003), respectively.

Calculation of Cook’s distances indicated that there was one potentially influential point in each of the metal content datasets, with the exception of vanadium. After the influential points were excluded, the magnitude of the regression coefficients increased three- to fourfold and the statistical significance improved for the chromium and nickel variables. The regression coefficients for the PM\textsubscript{2.5} metal contents after excluding the potentially influential points are summarized in Table 5. While there was no change in statistical significance for the copper and lead variables with the exclusion of the influential points, the association between logF\textsubscript{E}NO and PM\textsubscript{2.5} manganese content became significant. Each 1000-\mu g/g increase in PM\textsubscript{2.5} manganese content was associated with a decrease of 0.05 (95% CI: −0.09, −0.01) in logF\textsubscript{E}NO, after exclusion of an influential data point.

### Table 4

Total PM\textsubscript{2.5} and PM\textsubscript{2.5} metal concentration regression coefficients and 95% confidence intervals after adjusting for cigarette smoking status, age, and sampling year

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variable</th>
<th>Sampling year 1999</th>
<th>Regression coefficient(^a)</th>
<th>95% CI</th>
<th>Sampling year 2000</th>
<th>Regression coefficient(^a)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogF\textsubscript{E}NO</td>
<td>PM\textsubscript{2.5}</td>
<td>−0.23</td>
<td>(−0.38, −0.10)</td>
<td>0.02</td>
<td>(−0.15, 0.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanadium</td>
<td>−0.03</td>
<td>(−0.04, −0.01)</td>
<td>0.002</td>
<td>(−0.02, 0.02)</td>
<td>0.02</td>
<td>(−0.56, 0.59)</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>−0.56</td>
<td>(−0.88, −0.24)</td>
<td>−0.02</td>
<td>(−0.07, 0.04)</td>
<td>0.01</td>
<td>(−0.04, 0.05)</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>−0.09</td>
<td>(−0.16, −0.02)</td>
<td>0.001</td>
<td>(−0.04, 0.05)</td>
<td>−0.01</td>
<td>(−0.06, 0.03)</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>−0.04</td>
<td>(−0.07, −0.02)</td>
<td>−0.01</td>
<td>(−0.06, 0.03)</td>
<td>0.08</td>
<td>(−0.08, 0.24)</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>−0.02</td>
<td>(−0.05, 0.005)</td>
<td>0.001</td>
<td>(−0.04, 0.05)</td>
<td>−0.01</td>
<td>(−0.06, 0.03)</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>−0.14</td>
<td>(−0.29, 0.004)</td>
<td>−0.01</td>
<td>(−0.06, 0.03)</td>
<td>0.08</td>
<td>(−0.08, 0.24)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The coefficient is expressed as the change in logF\textsubscript{E}NO per 1-mg/m\(^3\) incremental change in PM\textsubscript{2.5} concentration or 1-\mu g/m\(^3\) incremental change in PM\textsubscript{2.5} metal concentration.

### Table 5

PM\textsubscript{2.5} metal content regression coefficients and 95% confidence intervals for sampling year 1999 after adjusting for cigarette smoking status, age, and sampling year\(^a\)

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variable</th>
<th>Including all data points</th>
<th>Regression coefficient(^c)</th>
<th>95% CI</th>
<th>Excluding influential data points(^b)</th>
<th>Regression coefficient(^c)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogF\textsubscript{E}NO</td>
<td>Vanadium</td>
<td>−0.02</td>
<td>(−0.03, −0.01)</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>−0.29</td>
<td>(−0.49, −0.08)</td>
<td>−1.11</td>
<td>(−1.55, −0.67)</td>
<td>−0.05</td>
<td>(−0.09, −0.01)</td>
<td></td>
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<tr>
<td>Manganese</td>
<td>−0.02</td>
<td>(−0.05, 0.004)</td>
<td>−0.07</td>
<td>(−0.11, −0.03)</td>
<td>−0.03</td>
<td>(−0.06, 0.01)</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>−0.02</td>
<td>(−0.04, −0.003)</td>
<td>−0.16</td>
<td>(−0.32, 0.01)</td>
<td>−0.02</td>
<td>(−0.06, 0.01)</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>−0.01</td>
<td>(−0.02, 0.004)</td>
<td>−0.02</td>
<td>(−0.06, 0.01)</td>
<td>0.00</td>
<td>(−0.02, 0.00)</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>−0.02</td>
<td>(−0.06, 0.01)</td>
<td>0.00</td>
<td>(−0.02, 0.00)</td>
<td>0.00</td>
<td>(−0.02, 0.00)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Metal content was calculated by dividing the PM\textsubscript{2.5} metal mass by the total PM\textsubscript{2.5} mass.

\(^b\) Influential data points were defined as having a Cook’s distance greater than 0.5 (Cook and Weisberg, 1999).

\(^c\) The coefficient is expressed as the change in logF\textsubscript{E}NO per 1000\-\mu g/g incremental change in PM\textsubscript{2.5} metal content.

NA, not applicable.
4. Discussion

While many previous studies have investigated the effect of particulate metals on respiratory health in animals, few studies have assessed the association between PM$_{2.5}$ metals and acute airway responses in humans. In our study, short-term occupational exposure to metal constituents of fine particulate matter was found to be associated with decreases in log F$_{E}$NO. However, these associations were seen only in subjects tested in 1999. In the group of boilermakers sampled in 2000, no exposure–response relationship was found between any of the PM$_{2.5}$ metals and log F$_{E}$NO.

A possible reason for detecting an association in 1999 but not in 2000 is exposure misclassification. The lack of an exposure–response relationship between PM$_{2.5}$ metal exposure and F$_{E}$NO in the 2000 subjects could be attributable to respirator use. During the sampling week in June 1999, the hot climate and limited ventilation prevented the boilermakers from wearing their respirators. In October 2000, however, the use of respirators was more tolerable due to the cooler climate. With a particle filter efficiency of greater than 99% for particles with an aerodynamic mass median diameter of 0.3 μm, the use of respirators would have significantly decreased the exposure to particulates. Since the subjects in 2000 were more likely to wear a respirator, the PM$_{2.5}$ measurements during this sampling year were less likely to represent the true exposure. The measurement error in PM$_{2.5}$ exposure might be responsible for the lack of an exposure–response relationship between PM$_{2.5}$ metals and F$_{E}$NO in 2000. It was not possible to adjust PM$_{2.5}$ exposure for respirator use because usage was inconsistent and the fit of the respirators was unknown due to factors such as the presence of facial hair.

A potential limitation of this study is the small sample size. Twenty subjects were studied in 1999 and 14 subjects, including 2 from 1999, were studied in 2000. Restricting the study population to healthy adult males reduced the variability in F$_{E}$NO measurements. In addition, the efficiency of the study was increased by using a repeated-measures study design. A total of 96 complete measurements were collected in 1999 and 2000. Retrospective power analysis of the linear model indicated that the sample size allowed detection of a 0.1-unit change in log F$_{E}$NO with 87% power using PM$_{2.5}$ vanadium metal concentration as the exposure of interest.

In this study, inverse exposure–response associations between the previous workday’s PM$_{2.5}$ metal 8-h TWA concentrations and the next day’s preshift log F$_{E}$NO were found during the 1999 sampling period. Statistically significant relationships were seen between F$_{E}$NO and the individual metals vanadium, chromium, nickel, and manganese. F$_{E}$NO decreased by 5% from baseline with the median PM$_{2.5}$ vanadium exposure of 1.98 μg/m$^3$. Likewise, with the median PM$_{2.5}$ chromium, manganese, and nickel exposure, F$_{E}$NO declined by 10%, 8%, and 6% from baseline, respectively. The analyses using PM$_{2.5}$ metal contents indicated that the individual components of vanadium, chromium, and nickel in total PM$_{2.5}$ were associated with decreases in F$_{E}$NO. Manganese metal content was found to be associated with F$_{E}$NO after excluding a potentially influential data point.

While the metal exposure levels in this occupational population were high compared to levels typically experienced by the general population, none of the air samples had metal concentrations exceeding the Occupational Safety and Health Administration (OSHA) permissible exposure limits (PEL). The median vanadium 8-h TWA was 1.98 μg/m$^3$ in 1999, compared to the OSHA PEL of 50 μg/m$^3$ for vanadium pentoxide respirable dust. The exposure levels of the five other metals were far below the OSHA PEL as well. In addition, none of the metal air concentrations, with the exception of one sample for nickel, exceeded the more stringent National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits or the American Conference of Governmental Industrial Hygienists threshold limit values.

Epidemiologic studies have found that particulate air pollution is associated with increased markers of airway inflammation (Steerenberg et al., 2001; Van Amsterdam et al., 1999). In particular, ambient PM$_{10}$ exposure is associated with increased F$_{E}$NO, presumably from increased immunostimulation of inducible NOS. Cigarette smoke also is known to induce acute inflammation in the airways. However, exposure to cigarette smoke, both actively and passively, is associated with decreased expired NO levels (Kharitonov et al., 1995; Yates et al., 2001). A possible hypothesis for the reduction in expired NO is that cigarette smoke negatively affects constitutive NOS activity. A study by Su and colleagues (1998) observed that exposure to cigarette smoke extract reduced the presence of endothelial NOS and endothelial NOS messenger RNA in the pulmonary artery endothelial cells from pigs.

After exposure to particulates from ROFA and various work tasks such as welding and burning, the boilermakers in this study also experienced decreased expired NO levels. The difference in the level of the PM$_{2.5}$ exposure may explain the contrasting results between the air pollution studies and the studies with cigarette smoke or ROFA and metal fume exposure. Typical urban air has a PM$_{2.5}$ concentration of approximately 10–30 μg/m$^3$, while the median PM$_{2.5}$ level from occupational particulate exposure in our study was 560 μg/m$^3$ in the sampling year 1999. Acute PM$_{2.5}$ exposure from cigarette smoking also can be up to 100-fold greater than PM$_{2.5}$ levels in urban air.
In addition, the composition of the PM$_{2.5}$ differs among the various exposures. PM$_{2.5}$ in urban air is composed of sulfates, ammonium compounds, hydrocarbons, elemental carbon, trace elements, and water (Hinds, 1999). While particulate air pollution contains trace amounts of transition metals, the levels are generally much lower than that in cigarette smoke or ROFA. One study showed that the vanadium and nickel metal contents were almost 30-fold greater in ROFA compared to ambient air (Pritchard et al., 1996). Work tasks such as welding and burning generate metal fume which may contain iron, manganese, chromium, or other transition metals depending on the composition of the base metal (Burgess, 1995). Cigarettes contain significant levels of various metals, including iron, nickel, and manganese (Chiba and Masironi, 1992).

The high metal content in cigarette smoke and ROFA may be responsible for reduced expired NO levels despite airway inflammation. Huang and colleagues observed that ROFA instilled intratraehally into isolated perfused rabbit lungs resulted in reduced production of NO, as determined by decreases in nitrite/nitrate accumulation (Huang et al., 2002). The vanadium component of ROFA specifically was found to be associated with inhibited NO production. Huang hypothesized that the reduction in NO production and accumulation following vanadium exposure might be related to decreased NOS activity. The reduction in expired NO levels experienced by our cohort of boilermakers also may have been attributable to decreases in NOS activity after exposure to metal-containing particulates. The significant reduction in F$_{E}$NO following exposure to ROFA and metal fume may be important, as we found that decreases in F$_{E}$NO were significantly correlated with decreases in FEV$_{1}$ in a previous study on the same cohort of workers (Kim et al., in press).

In conclusion, we found a statistically significant inverse exposure–response relationship between F$_{E}$NO and PM$_{2.5}$ vanadium, chromium, manganese, and nickel exposure in boilermakers. In this study, six metals were investigated—vanadium, chromium, manganese, nickel, copper, and lead. These metals were selected because they are present in moderate to high concentrations in ROFA and metal fume and are known to have significant health effects. Future studies should investigate the association between acute airway responses and other bioavailable transition metals such as iron and zinc.

Because of the crucial role of endogenous NO in the airways, reduction in NO levels might have potentially significant respiratory consequences. This study showed that the soluble metal component might contribute to the adverse respiratory health effects experienced by workers exposed to ROFA and metal fume.

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