Smoking Status and Occupational Exposure Affects Oxidative DNA Injury in Boilermakers Exposed to Metal Fume and Residual Oil Fly Ash

Sutapa Mukherjee, Lyle J. Palmer, Jee Young Kim, David B. Aeschliman, Robert S. Houk, Mark A. Woodin, and David C. Christiani

Abstract

Epidemiologic studies demonstrate increased cancer incidence among workers exposed to polycyclic aromatic hydrocarbons (PAH) and metals, probably through cumulative oxidative DNA damage in response to carcinogens. Boilermakers are exposed to particulates of residual oil fly ash (ROFA) and metal fume that contain carcinogenic PAH and metals. We conducted a repeated-measures cohort study in boilermakers during the overhaul of an oil-fired boiler to determine a possible association between the level of 8-hydroxy-2-deoxyguanosine (8-OH-dG; an oxidative injury biomarker) and biomarkers of PAH (1-hydroxypyrene; 1-OHP) and metal exposure. Preshift and postshift urine samples were analyzed for 8-OH-dG, cotinine, 1-OHP, and metals. Generalized estimating equations were used to model the multivariate relationship of oxidative DNA damage in boilermakers is influenced through the Fenton reaction and lead to the formation of ROS. Transition metal oxides, nitrogen dioxide, and ozone from welding emissions (8, 9). ROFA itself is a complex chemical mixture that includes high concentrations of metals, such as vanadium and nickel as well as PAH, sulfates, silicates, and nitrogen-containing compounds (10). ROFA exposure in experimental models causes widespread lung injury (10–12) and this may be mediated through metal exposure, particularly vanadium. We have shown previously, using the PAH biomarker, 1-hydroxypyrene (1-OHP), that boilermakers exposed to particulates during the overhaul of an electricity-generating oil-fired boiler are exposed to PAH (13) from exposure to ROFA in and around the boiler.

The molecular and cellular mechanisms leading to tumor formation after exposure to PAH and metals are poorly understood (14). However, it has been shown that particle-induced inflammation in the lungs causes macrophages to release reactive oxygen species (ROS), and transition metals on the particle surface can generate ROS through the Fenton reaction and lead to the formation of oxidative DNA lesions (14, 15). The development of such oxidative DNA damage coupled with interference to DNA repair processes is likely to play an important role in the development of cancer (16).
The most widely studied biomarker of oxidative DNA damage is 8-hydroxy-2-deoxyguanosine (8-OH-dG). 8-OH-dG is a particular type of ROS-induced DNA base modification (i.e., the C8 hydroxylation of deoxyguanosine). 8-OH-dG is premutagenic because it can mispair with adenine instead of cytosine during DNA replication, and if this occurs, a G → T transversion mutation develops. The repair mechanism for DNA after 8-OH-dG is incorporated into DNA involves base and nucleotide excision with DNA-specific nucleosides excised into urine (17). Therefore, the measurement of urinary 8-OH-dG can be used as an overall estimate of repaired oxidative DNA damage within an individual (18).

The aim of this study was to investigate associations between urinary levels of PAH and metal exposure biomarkers and urinary levels of the oxidative injury biomarker, 8-OH-dG. We conducted a repeated-measures cohort study in boilermakers exposed occupationally to a complex chemical mixture of PAH (from ROFA) and metals (through welding and ROFA exposure) during the overhauling of an oil-fired boiler. The well-validated PAH exposure biomarker, 1-OHP, was chosen to determine PAH exposure (19) and six urinary metal exposure biomarkers were selected based on previous work that showed that these metals had the highest concentration in ROFA (11, 20). These were vanadium, chromium, manganese, nickel, copper, and lead. The present study is the first to evaluate the association between such exposure and oxidative injury biomarkers in a population of highly exposed workers.

Materials and Methods

Study Design. The study was a repeated-measures short-term prospective study of boilermakers working at a power plant during the overhaul of an oil-fired boiler.

Study Population. The study was approved by the Institutional Review Board of Harvard School of Public Health. The study population consisted of 20 boilermakers who were monitored during 5 consecutive days of work. Their hours of work were 7:00 a.m. to 5:00 p.m., with potential exposures to metal fume and ROFA that was present in and around the boiler.

Work Description. The work process has been described previously (13). Briefly, boilermaker construction workers at the power plant were responsible for the repair of sections of the interior wall of the boiler. This involved acetylene torch cutting, carbon stick, and electric arc welding. The occupational exposure to PAH occurred through contact with ROFA that coated the walls of the boiler and accumulated in an ash pit at the base of the boiler after high temperature combustion.

Data Collection. All participants completed a self-administered modified American Thoracic Society questionnaire with specific data collected on past medical history, respiratory symptoms, smoking, and work exposure history. Spot urine samples were collected from each subject preshift and postshift for 5 consecutive days of work leading to a maximum of 10 urine samples from each individual. All urine samples were frozen at −20°C until urinary 1-OHP, 8-OH-dG, and metal laboratory analysis was performed. Urinary creatinine and cotinine were measured for each sample of urine (ESA Laboratories, Inc., Chelmsford, MA). Urinary cotinine was determined using reverse-phase high-performance liquid chromatography (HPLC) with UV absorption detection, and urinary creatinine was measured using spectrophotometry.

Extraction Procedure and Reverse-Phase HPLC Analysis for 1-OHP. The analytical procedure to determine urinary 1-OHP levels by HPLC has been described previously (13, 21, 22). The calculated concentration of urinary 1-OHP was adjusted to the urinary concentration of creatinine (µg 1-OHP/g creatinine) to control for variation in urinary flow.

Urinary Metal Analysis. Calibration solutions were prepared from single-element stock solutions (1000 mg/l; Claritas PPT; SPEX CertiPrep, Metuchen, NJ) and diluted to the desired concentration using 5% high-purity aqueous nitric acid. A blank solution of 5% nitric acid was also prepared. A 1-ml aliquot of each urine sample was mixed with equal volumes of concentrated nitric acid and a stock solution containing 100-ppb scandium as an internal standard. The resultant mixture was diluted to 20 ml using distilled deionized water.

A Finnigan MAT Element 1 magnetic sector inductively coupled plasma-mass spectrometer (Bremen, Germany) was used for metal analysis (23–25). For the quantification experiments described here, the Element was operated in medium resolution mode (R = m/Δm = 4000) for all elements, which is sufficient to separate the main polyatomic ions at these m/z values (24, 25). The “shielded” load coil (CD-1 torch, Finnigan MAT) improved sensitivity by a factor of 3–20 (depending on m/z) while still maintaining the extremely low background and high precision of the double-focusing instrument (26). The instrument was also fitted with platinum-tipped sampler and skimmer cones to reduce the instrument background for nickel.

A Teflon spray chamber and microconcentric nebulizer (Model ES-2002; Elemental Scientific, Inc., Omaha, NE) were used. The sample was drawn up at ~100 µl/min by natural suction. The Teflon nebulizer was cleaned on-line by aspiration of 2% aqueous hydrofluoric acid for 15 s before the analysis of each urine sample. This process maintained a low background signal and minimized clogging or loss of uptake by the nebulizer.

Each urine sample was vortexed prior to analysis to insure sample homogeneity. Six metal analytes—vanadium, chromium, manganese, nickel, copper, and lead—were determined in each urine sample. The spectrometer measured 20 lines/peak using 50 ms/line for the following seven isotopes: 45Sc, 51V, 52Cr, 55Mn, 60Ni, 63Cu, and 209Pb. Ten spectra were measured and averaged for each urine sample. Each sample was aspirated for 45 s before data acquisition to allow for adequate signal equilibration. The peaks for each analyte were integrated and corrected for blank levels and isotopic abundance. The metal concentrations in the original samples were calculated after adjusting for the internal standard and dilution factor. The following percentage of urine samples had metal concentrations that were below the limit of detection: 3% of all samples for chromium, 17% for manganese, 7% for nickel, 1% for copper, and 9% for...
lead. The concentration of individual urinary metals was adjusted to the urinary concentration of creatinine (µg metal/g creatinine) to control for variation in urinary flow.

**Urinary 8-OH-dG Testing.** Urinary 8-OH-dG levels were determined using a competitive ELISA immunoassay (Japan Institute for the Control of Ageing, Fukuroi, Shizuoka, Japan) performed by Genox Corporation (Baltimore, MD) and has been described previously (27, 28). Briefly, 50-µl urine samples and standards were added to precoated 8-OH-dG protein conjugate microtitre plates followed by 50 µl of the primary antibody, anti-8-OH-dG monoclonal antibody solution. After incubation for 1 h at 37°C, the plates were washed and the enzyme-labeled secondary antibody (100 µl) was applied for 1 h at 37°C. After washing, 100 µl of the chromatic substrate, 3,3',5,5'-tetramethylbenzidine, were added to the plate and allowed to react at room temperature for 15 min. The intensity of color produced for each sample was measured at an absorbance 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 ± 2 SDs. The limit of detection for 8-OH-dG was 0.64 ng/ml. For each subject sample, either a duplicate or a triplicate measurement was performed. The mean, SD, and coefficient of variation (%) were calculated and any sample with coefficient of variation (%) ≥ 20% was retested. The concentration of urinary 8-OH-dG was adjusted to the urinary concentration of creatinine (µg 8-OH-dG/g creatinine) to control for variation in urinary flow.

**Statistical Analysis.** Statistical analysis investigated the association of urinary 8-OH-dG levels with the following potential explanatory variables: age, self-reported smoking status, urine level of metals (vanadium, chromium, manganese, nickel, copper, and lead), individual metal or 1-OHP × cotinine interaction, urinary 1-OHP, urinary cotinine level, day of urine collection, and assignment of daily exposure (i.e., preshift or postshift). Urinary 1-OHP, metal, and cotinine levels were skewed with a long right tail and therefore were loge transformed prior to analysis and were approximately normally distributed after loge transformation. Because there were some samples with zero values in the urinary metal raw data, a constant was added to all values prior to loge transformation. Urinary 8-OH-dG levels were normally distributed. The day of measurement (days 1–5) and self-reported smoking status were analyzed as categorical covariates. All other variables were analyzed as continuous.

Generalized estimating equations (GEE; 29) were used to investigate the bivariate and multivariate relationships and predictive value of the explanatory variables and covariates of interest with the 8-OH-dG levels at each time point and to appropriately adjust for the within-individual correlations present in these data. Both forward and backward stepwise procedures were used to select a set of independent predictors. The modeling included an investigation of interaction and polynomial terms.

Analysis and data management was carried out using STATA v5 (Stata Corporation, College Station, TX) and SPlus v2000 (Mathsoft, Inc., Cambridge, MA). Formal statistical significance was defined at the conventional 5% level.

**Results**

**Subject Characteristics.** Table 1 summarizes the subject characteristics of the boilermakers working at the power plant. The subjects were 20 male boilermakers with a broad age range of 18–59 years reflecting the usual work environment of experienced boilermakers (with up to 40 years in the trade) working with apprentice boilermakers (0–4 years as a boilermaker). In total, 181 urine samples from 20 male individuals underwent analysis for biomarkers. The percentage of current cigarette smokers was 50%. The mean number of urine samples obtained from each subject was 9.2 (range 8–10) because not all boilermakers were present on each day of the study or able to provide preshift and postshift urine samples for each day of the study. Sixty percent of subjects provided the complete set of 10 urine samples.

**Urinary Metal and 1-OHP Levels.** Geometric mean cross-week levels of 1-OHP and individual metal biomarkers (µg metal/g creatinine) by smoking status in boilermakers are shown in Table 2. The levels of all biomarkers increased across the workweek (day 1 preshift to day 5 postshift) in both smokers and nonsmokers. However, for several metals, the levels measured across the workweek varied by smoking status. For lead and vanadium, day 1 preshift levels in smokers and nonsmokers were similar; however, by the end of the workweek, nonsmokers had at least 2-fold higher levels than smokers, but this was not statistically significant (P > 0.10). The pattern for chromium was different with nonsmokers showing higher day 1 preshift levels than smokers (P > 0.10), yet the magnitude of increase by day 5 postshift was similar (0.08 g/g creatinine) for smokers and nonsmokers. For manganese and nickel, nonsmokers had ~2-fold higher day 1 preshift levels than smokers (P > 0.10), but by day 5 postshift, the levels in both smokers and nonsmokers were comparable. For copper, there was minimal

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
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<tbody>
<tr>
<td>No. subjects</td>
</tr>
<tr>
<td>Age (yr), mean ± SD</td>
</tr>
<tr>
<td>Age (yr), range</td>
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<tr>
<td>Current smokers (%)</td>
</tr>
<tr>
<td>Years employed as a boilermaker, mean</td>
</tr>
<tr>
<td>Years employed as a boilermaker, range</td>
</tr>
<tr>
<td>Total no. urine samples collected</td>
</tr>
<tr>
<td>Urine samples/subject, mean</td>
</tr>
<tr>
<td>Urine samples/subject, range</td>
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These analyses show that an increase in urinary log e-transformed predictors of the 8-OH-dG level (Table 3, models 1–5). That several urinary metals were significant positive PAH Biomarkers.

...increase in 8-OH-dG levels cross-shift for days 3–5 smokers are similar, although there may be a slight increase in 8-OH-dG levels onwards, the 8-OH-dG levels in smokers and non-smokers during the workweek. For 1-OHP, smokers had significantly higher difference in cross-week levels in smokers and non-smokers by preshift and postshift work day.

**Table 2. Mean levels of urinary metal and PAH biomarkers (µg/g creatinine) in smokers and nonsmokers by preshift and postshift work day**

<table>
<thead>
<tr>
<th>Urinary biomarker</th>
<th>Day 1 preshft</th>
<th>Day 5 postshift</th>
</tr>
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<tbody>
<tr>
<td>Vanadium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>0.77 (0.56 to 1.00)</td>
<td>1.01 (0.69 to 1.36)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>1.02 (0.70 to 1.37)</td>
<td>2.07 (0.87 to 3.77)</td>
</tr>
<tr>
<td>Chromium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>0.25 (0.08 to 0.44)</td>
<td>0.33 (0.16 to 0.44)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>0.50 (0.25 to 0.79)</td>
<td>0.58 (0.38 to 0.79)</td>
</tr>
<tr>
<td>Manganese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>0.28 (0.15 to 0.42)</td>
<td>0.98 (−0.15 to 2.78)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>0.46 (0.18 to 0.76)</td>
<td>0.91 (0.26 to 1.73)</td>
</tr>
<tr>
<td>Nickel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>1.04 (0.04 to 2.54)</td>
<td>8.44 (0.10 to 49.7)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>3.44 (0.85 to 8.30)</td>
<td>8.46 (2.00 to 25.33)</td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>2.92 (1.75 to 4.44)</td>
<td>4.03 (2.52 to 6.04)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>2.90 (1.85 to 4.23)</td>
<td>3.85 (2.89 to 5.00)</td>
</tr>
<tr>
<td>Lead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>1.75 (0.41 to 3.82)</td>
<td>2.06 (0.70 to 4.12)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>2.33 (1.03 to 4.22)</td>
<td>4.57 (1.47 to 10.47)</td>
</tr>
<tr>
<td>1-OHP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokersa</td>
<td>0.39 (0.22 to 0.69)</td>
<td>0.49 (0.27 to 0.92)</td>
</tr>
<tr>
<td>Nonsmokersa</td>
<td>0.16 (0.10 to 0.25)</td>
<td>0.33 (0.17 to 0.61)</td>
</tr>
</tbody>
</table>

*Geometric mean (95% CI).

**Association between Urinary 8-OH-dG and Cotinine Interactions.** Multivariate GEE analysis indicated that vanadium × cotinine, chromium × cotinine, nickel × cotinine, and copper × cotinine interaction terms were significant predictors of the 8-OH-dG level (Table 3, models 1, 2, 4, and 5). All models were also adjusted for age, each individual metal, and loge (cotinine level). The vanadium × cotinine and nickel × cotinine interaction terms were found to be significantly associated with increased 8-OH-dG levels (Table 3, models 1 and 4); with increasing exposure to tobacco smoke, these metals were associated with a reduction in 8-OH-dG levels. In contrast, chromium × cotinine and copper × cotinine interaction terms were found to be significantly associated with increased 8-OH-dG levels (Table 3, models 2 and 5); with increasing exposure to tobacco smoke, these metals were associated with an elevation in 8-OH-dG levels. The manganese × cotinine, lead × cotinine, and 1-OHP × cotinine difference in cross-week levels in smokers and nonsmokers. For 1-OHP, smokers had significantly higher levels compared with nonsmokers (0.39 to 0.16 µg/g creatinine, P < 0.03), but nonsmokers exhibited a greater overall increase compared with smokers (0.16 to 0.33 µg/g creatinine). Urinary metal and 1-OHP levels varied markedly between individuals, as reflected by the wide confidence intervals (CI) around each time point, which increased during the week.

**Urinary 8-OH-dG Levels.** The mean 8-OH-dG levels in boilermakers by smoking status are shown in Fig. 1. Nonsmokers at the start of the workweek (day 1 preshift) had higher 8-OH-dG levels after adjusting for loge (cotinine level). The vanadium × cotinine, chromium × cotinine, nickel × cotinine, and copper × cotinine interaction terms were significant predictors of the 8-OH-dG level (Table 3, models 1, 2, 4, and 5). All models were also adjusted for age, each individual metal, and loge (cotinine level). The vanadium × cotinine and nickel × cotinine interaction terms were found to be significantly associated with decreased 8-OH-dG levels (Table 3, models 1 and 4); with increasing exposure to tobacco smoke, these metals were associated with a reduction in 8-OH-dG levels. In contrast, chromium × cotinine and copper × cotinine interaction terms were found to be significantly associated with increased 8-OH-dG levels (Table 3, models 2 and 5); with increasing exposure to tobacco smoke, these metals were associated with an elevation in 8-OH-dG levels. The manganese × cotinine, lead × cotinine, and 1-OHP × cotinine increase in 8-OH-dG levels after adjusting for loge (cotinine level). The magnitude of the increase in 8-OH-dG levels after a 1 unit increase in these urinary log e-transformed metals ranged from 0.18 (95% CI 0.17–0.19) for nickel to 1.89 (95% CI 1.48–2.30) for chromium. Similar analyses showed that neither urinary log e-transformed lead nor 1-OHP was a significant predictor of the 8-OH-dG level (Table 3, models 6 and 7).

**Association between Urinary 8-OH-dG and Cotinine.** Statistically significant associations between the 8-OH-dG level and loge (cotinine level) were observed after adjusting for all individual metals (Table 3, models 1–6). These analyses indicated an inverse relationship between 8-OH-dG level and loge (cotinine level). After a 1 unit increase in the loge (cotinine level), there was a decrease in 8-OH-dG levels from −0.12 (95% CI −0.18 to −0.06) for the models adjusting for vanadium, chromium, and lead to −0.16 (95% CI −0.19 to −0.12) for the model adjusting for nickel. There was a similar inverse association between 8-OH-dG level and loge (cotinine level) after adjusting for the PAH biomarker, 1-OHP; however, this did not reach statistical significance (P = 0.06; Table 3, model 7). Models using dichotomous, smoking status (yes/no) as a covariate produced similar results (data not shown).

**Association between Urinary 8-OH-dG and Metal/1-OHP × Cotinine Interactions.** Multivariate GEE analysis indicated that vanadium × cotinine, chromium × cotinine, nickel × cotinine, and copper × cotinine interaction terms were significant predictors of the 8-OH-dG level (Table 3, models 1, 2, 4, and 5). All models were also adjusted for age, each individual metal, and loge (cotinine level). The vanadium × cotinine and nickel × cotinine interaction terms were found to be significantly associated with decreased 8-OH-dG levels (Table 3, models 1 and 4); with increasing exposure to tobacco smoke, these metals were associated with a reduction in 8-OH-dG levels. In contrast, chromium × cotinine and copper × cotinine interaction terms were found to be significantly associated with increased 8-OH-dG levels (Table 3, models 2 and 5); with increasing exposure to tobacco smoke, these metals were associated with an elevation in 8-OH-dG levels. The manganese × cotinine, lead × cotinine, and 1-OHP × cotinine increase in 8-OH-dG levels after adjusting for loge (cotinine level). The magnitude of the increase in 8-OH-dG levels...
interaction terms were not significantly associated with the 8-OH-dG level (Table 3, models 3, 6, and 7).

**Discussion**

**Boilermakers Are Occupationally Exposed to Metals and PAH.** This study has measured metal and PAH biomarkers in boilermakers exposed to ROFA and metal fume. We show that boilermakers are exposed to PAH and different metals during their work and that the levels of all biomarkers of exposure increase during the workweek. Several PAH and metals, particularly nickel and chromium, are known carcinogens, and workers exposed to PAH and metals have increased rates of cancer (1–7, 14, 30). In addition, there is evidence that short-term exposure to vanadium, copper, and manganese leads to widespread cellular damage (31), induction of inflammatory mediators (32, 33), and increased production of cell signaling molecules involved in oxidative injury to cells (10, 34). Therefore, occupational PAH and metal exposure likely leads to oxidative stress at a cellular and systemic level, which may lead to an increased risk of cancer in this occupational group.

**Levels of Urinary Metals and PAH Biomarkers across the Workweek Vary by Smoking Status.** In this study, the patterns of increase of exposure biomarkers across the workweek varied by smoking status. For example, for urinary lead and vanadium levels, smokers and nonsmokers had similar levels at the start of the workweek, but nonsmokers developed much higher levels than smokers by the end of the workweek (Table 2). This observation may suggest that nonsmokers had higher levels of occupational exposure than smokers; however, particulate matter (diameter ≤ 2.5 μm) levels from filter measurements from this same cohort did not demonstrate a correlation between particulate matter (diameter ≤ 2.5 μm) exposure and smoking status (Spearman’s rank correlation $r = -0.06$, $P = 0.70$; unpublished observations). Alternatively, smokers may have improved clearance mechanisms for lead and vanadium. This enhanced ability to metabolize metals may apply to other metals because nonsmokers had manganese, nickel, and chromium levels approximately twice those of smokers at the beginning of the workweek. For 1-OHP, smokers had higher levels compared with nonsmokers and this reflects the fact that cigarettes contain much PAH. We have shown previously that 1-OHP is a useful biomarker of PAH exposure in boilermakers exposed to ROFA, particularly in nonsmokers (13). Likely, the ability to metabolize metals and 1-OHP from occupational sources will be directly affected by smoking. This has major implications for workers who are exposed occupationally to metals and PAH and will modify levels of oxidative damage that occur in response to these agents.

**Oxidative DNA Injury Biomarkers in Boilermakers.** Our results demonstrate that nonsmoking boilermakers have higher urinary 8-OH-dG levels at the start of the workweek than boilermakers who are smokers. There are several explanations for this result that must be considered. Firstly, nonsmoking boilermakers may develop higher levels of oxidative DNA damage in response to occupational ROFA and metal fume exposure. Secondly, nonsmoking boilermakers may be exposed to significant causes of oxidative damage on the weekends (e.g., marathon running and exposure to environmental tobacco smoke in nightclubs) compared with smoking boilermakers. In this study, we did not ask specific questions on weekend activities; therefore, we are unable to confirm this explanation. Thirdly, they may have a greater capacity to repair damaged DNA compared with boilermakers who smoke. Another
Patients with silicosis found that those with increased impaired ability of systemic lupus erythematosus lymphocytes decreased 8-OH-dG levels were suggested to reflect an identified in patients suffering from systemic lupus these other factors. Able to minimize these effects but not totally control for morning; 40) and by other factors \( \text{with lower levels recorded in the } \) morning change seen in boilermakers who smoke in our study, we were unable to collect urine samples from boilermakers on their off-work days. In addition, it would have been helpful to measure biomarker levels in non-ROFA- and metal fume-exposed workers to determine what role occupational exposure has on biomarker levels in nonexposed individuals.

These results are in contrast to several studies, which show that smoking elevates the urinary excretion of 8-OH-dG by 16–50% (35–37). However, these studies were often of small size and did not use the repeated-measures study design but focused on single spot urine measurement or cross-week changes. These methods to measure urinary 8-OH-dG are unlikely to represent individual long-term excretion rates and do not allow for intraindividual variability of 8-OH-dG as in our study. In addition, a study by Pilger et al. (37) found that a significant increase in 8-OH-dG levels in smokers was observed in only two of six series after repeated sampling of the same individuals during a 6-month period.

Few epidemiologic studies have investigated oxidative injury biomarkers in an occupational setting, but most studies show an increase in 8-OH-dG levels (in leukocytes and urine) after exposure to toxic chemicals, including styrene (38), benzene (39), coal-tar pitch dust, and asphalt fume (18), rather than the initial decrease demonstrated in nonsmokers and the minimal cross-week change seen in boilermakers who smoke in our study. There are likely to be a few reasons to explain this difference. Firstly, it is likely that the exposures (metal and ROFA particulates) in our study have markedly different cellular and systemic effects compared with the above agents. Secondly, we used a repeated-measures study design with twice-daily urine sampling for an entire week. However, most studies measure urinary and blood biomarkers either at the beginning and end of a work shift or as a single measurement. These readings may be affected by the known circadian variation in 8-OH-dG levels (with lower levels recorded in the morning; 40) and by other factors [e.g., age, diet (41), and smoking status]. With repeated sampling, we were able to minimize these effects but not totally control for these other factors.

Decreased 8-OH-dG Levels Associated with Disease. Markedly reduced urinary 8-OH-dG levels have been identified in patients suffering from systemic lupus erythematosus compared with healthy controls and patients with rheumatoid arthritis (42). In that study, decreased 8-OH-dG levels were suggested to reflect an impaired ability of systemic lupus erythematosus lymphocytes to repair damaged DNA. Another study of patients with silicosis found that those with increased 8-OH-dG in DNA showed significantly lower urinary excretion of 8-OH-dG compared with quartz-exposed healthy workers (17). This result was suggested to reflect a shift in the balance between oxidative DNA damage and repair mechanisms in patients with silicosis. In our study, we did not measure leukocyte DNA levels of 8-OH-dG, but taken together with the results from these two studies and the urinary 8-OH-dG levels in our study, there may be reduced ability to eliminate ROFA- and metal fume-induced 8-OH-dG, particularly in smoking boilermakers.

Determinants of the 8-OH-dG Level. Finally, we demonstrate that interactions between workplace cotinine and metals (nickel, vanadium, chromium, and copper) are also significant determinants of the 8-OH-dG level and that the direction of the interaction effect varies depending on the metal. These differential effects (Table 3) depending on the metal and the interaction with cigarette smoking is intriguing and unexpected. However, it is known that various metals can cause differential effects at the cellular level (14). The transition metal constituents of ROFA, including vanadium, chromium, manganese, nickel, and copper have been shown to be capable of inducing oxidative stress at a cellular level (43). Some of these metals can catalyze Fenton-type reactions and lead to the generation of ROS (44).

In this study, 1-OHP × cotinine, manganese × cotinine, and lead × cotinine interactions were not significantly associated with 8-OH-dG levels. For the metal biomarkers, this suggests that there are differential effects of each metal and some metals are more significant overall in conjunction with smoking than others. The lack of association of the 1-OHP level with 8-OH-dG levels may be explained by the fact that oxidative stress may not be a major or significant component of PAH carcinogenesis or there may be a time lag between exposure (as measured by the levels of the urinary biomarker 1-OHP) and effect (as measured by the 8-OH-dG level). Future studies focusing on longitudinal changes in biological markers of oxidative injury and exposure may provide further information on the mechanisms involved in metal and PAH exposure causing injury and disease.

Conclusions

In summary, we have demonstrated for the first time that biomarkers of PAH and metal exposure and oxidative DNA damage in boilermakers differ by smoking status. We show that nonsmokers have higher levels of 8-OH-dG in urine at the start of the workweek, but this rapidly declines to similar levels of smokers and there may be an overall down-regulation in the ability of boilermakers to repair damaged DNA compared with other occupational groups. This finding is clinically relevant because this may increase their susceptibility to cancer. In addition, we show that there are differential effects of the metal × cotinine interaction depending on the metal. This suggests that some metals (e.g., vanadium) may have a greater impact on cellular functions than others. Finally, a major implication of this work is that the overall oxidative damage due to occupational exposures to metals, PAH, and other toxic agents will be affected.
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by the level of exposure, including type of metal, smoking status of the worker, and other acquired susceptibility (e.g., diet) and genetic factors.

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References
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