ORIGINAL ARTICLE

Associations of multiple metals with kidney outcomes in lead workers

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► Additional appendices are published online only. To view these files please visit the journal online (http://dx.doi.org/10.1136/oemed-2012-100765).

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Accepted 27 May 2012

ABSTRACT

Objectives Environmental exposure to multiple metals is common. A number of metals cause nephrotoxicity with acute and/or chronic exposure. However, few epidemiologic studies have examined the impact of metal coexposure on kidney function. Therefore, the authors evaluated associations of antimony and thallium with kidney outcomes and assessed the impact of cadmium exposure on those associations in lead workers.

Methods Multiple linear regression was used to examine associations between ln-urine thallium, antimony and cadmium levels with serum creatinine- and cystatin-C-based glomerular filtration measures and ln-urine N-acetyl-β-D-glucosaminidase (NAG).

Results In 684 participants, median urine thallium and antimony were 0.39 and 0.36 μg/g creatinine, respectively. After adjustment for lead dose, urine creatinine and kidney risk factors, higher ln-urine thallium was associated with higher serum creatinine- and cystatin-C-based estimates of glomerular filtration rate; associations remained significant after adjustment for antimony and cadmium (regression coefficient for serum creatinine-based estimates of glomerular filtration rate =5.2 ml/min/1.73 m^2; 95% CI =2.4 to 8.0). Antimony associations with kidney outcomes were attenuated by thallium and cadmium adjustment; thallium and antimony associations with NAG were attenuated by cadmium.

Conclusions Urine thallium levels were significantly associated with both serum creatinine- and cystatin-C-based glomerular filtration measures in a direction opposite that expected with nephrotoxicity. Given similarities to associations recently observed with cadmium, these results suggest that interpretation of urine metal values, at exposure levels currently present in the environment, may be more complex than previously appreciated. These results also support multiple metal analysis approaches to decrease the potential for inaccurate risk conclusions.

What is already known about this subject

Environmental exposure to multiple metals is common and chronic kidney disease is increasingly prevalent. However, few epidemiologic studies have examined the impact of metal coexposure on kidney function measures such as glomerular filtration rate estimates.

What this study adds

This study reports additional associations between urine metal levels and glomerular filtration measures in a direction opposite to that expected in nephrotoxicity. Given similarities to associations recently observed with cadmium, these results suggest that interpretation of urine metal values, at exposure levels currently present in the environment, may be more complex than previously appreciated. This analysis also revealed attenuation of thallium and antimony associations with NAG, a commonly used kidney biomarker, following adjustment with cadmium.

INTRODUCTION

A number of metals are known or suspected occupational and/or environmental nephrotoxicants. Lead and cadmium are widely recognised in this regard.[1–3] Recent advances in analytical technology, allowing measurement of multiple metals in urine samples, have revealed that most US residents are exposed via the environment to a wide range of metals.[4] However, data on nephrotoxic effects of metals other than lead and cadmium are limited and the effect of kidney exposure to multiple metals is relatively unknown. Populations with coexposures might be at increased risk for adverse kidney effects. Antimony and thallium are metals that are nephrotoxic in acute and subacute exposures[5 6] and are also common environmental exposures as evidenced by biomonitoring in the US general population,[4] wherein urine antimony and thallium were detectable in 86.7% and 99.9%, respectively, of National Health and Nutrition Examination Survey (NHANES) 05–06 participants.[7] However, to the best of our knowledge, no epidemiologic studies have examined associations of urine levels of either metal with kidney outcomes that include glomerular filtration measures. Therefore, to address the kidney impact of multiple metal exposures, we performed a cross-sectional analysis examining associations between urine antimony and thallium levels and kidney outcomes in 684 current and former lead

workers in the Republic of Korea in whom cadmium associations with kidney function have been reported.[8 9]

MATERIALS AND METHODS

Study overview and design

We performed a cross-sectional analysis of data from current and former inorganic lead workers who completed the fourth evaluation in a prospective study. Evaluations were performed between 8 April 2004 and 24 September 2005. All participants provided written informed consent. Participation in the study was voluntary. The study protocol was approved by Institutional Review Boards at the SoonChunHyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health.

Study population

As previously described,[8 10 11] participants in the initial cohort of this study were recruited between 1997 and 1999 (phase I) for three annual evaluations via medical surveillance programmes at secondary lead smelters and plants that produced lead batteries, lead oxide, lead crystal or radiators. The population is 100% Korean. In 2004, recruitment for three additional annual evaluations (phase II) began; 498 (62%) of the 803 lead workers in the original cohort were re-enrolled (many had been laid-off in the economic crisis of the late 1990s and lost to follow-up) and 279 new participants were recruited. Inclusion criteria included occupational lead exposure and, for new participants, age ≥40 years, in order to enrich the study with participants with increased risk for adverse kidney outcomes. There were no medical exclusionary criteria. At the end of the second enrolment phase (24 September 2005), 778 current and former lead workers had completed the fourth (initial phase II) evaluation. In order to optimise study data for both cross-sectional and longitudinal analyses within funding constraints, a urine metals panel, including thallium, antimony and cadmium, was measured in fourth evaluation samples in the 712 workers who came to both the fourth and fifth evaluations. Workers from a primary smelter (n=28) who were enrolled in the second recruitment phase were excluded from this analysis due to their potentially wider range of occupational metal exposures, leaving 684 workers for the current cross-sectional analysis.

Data collection

As previously described,[8] data collection and biologic specimens included a standardised interviewer-administered questionnaire; blood pressure measured with the IntelliSense blood pressure monitor (Model HEM-907; Omron, Vernon Hills, Illinois, USA); height and weight measurements; a blood specimen (for serum creatinine, cystatin-C and blood lead); 4-h urine collection (for thallium, antimony, cadmium and creatinine levels); a spot urine sample (for N-acetyl-β-D-glucosaminidase (NAG) and creatinine), collected just before beginning the 4-h urine collection, and tibia lead assessed via x-ray fluorescence.

Metals exposure assessment

Urine specimens were analysed for metals in the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State (NYS) Department of Health's Wadsworth Center (Albany, New York, USA) which is the principal reference laboratory for the measurement of trace metals in urine in NYS. A multielement method based on inductively coupled plasma mass spectrometry (ICP-MS) was used.[12] As previously described,[8 12] the method has been validated against the National Institute of Standards and Technology Standard Reference Materials 2670a Toxic Elements in Urine, as well as secondary reference materials from a number of External Quality Assessment Schemes in which the laboratory participates successfully.

Urine specimens for trace metals analysis were collected and stored at −80°C in 5 ml Nalgene Cryogenic polypropylene tubes. Urine concentrations of thallium (m/z=205), antimony (m/z=121) and cadmium (m/z=114) were measured in standard mode using an Elan DRC II inductively coupled plasma mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA) equipped with dynamic reaction cell (DRC-ICP-MS) technology. As previously described,[8 12] 500 µl of urine was diluted 1+19 with 2% (v/v) HNO_3 (Veritas double distilled; GFS Chemicals, Powell, Ohio, USA); 0.005% Triton X-100 as a surfactant (Sigma-Aldrich, St. Louis, Missouri, USA); 1 mg/l gold and 10 µg/l gallium, rhodium, yttrium and iridium (Spex Certiprep, Inc., Metuchen, New Jersey, USA) as internal standards. Multielement calibration standards were prepared by serial dilution of National Institute of Standards and Technology-traceable stock solution (High Purity Standards, Charleston, South Carolina, USA) using a six-point calibration curve for each element. Base human urine pools were used to matrix match the calibration standards. Samples were prepared under conditions (Clean Room and Class IIB Biosafety Cabinet) certified as class 100 or better to minimise the potential for contamination.

Quality control (QC) during the course of the study included analysis of urine-based internal QC materials before, during and after every analytical run. The mean coefficients of variation (CV) of the internal QC samples from 18 days over the 5-month period in which the samples assayed were for antimony, 10% at 0.81 µg/l (n=74), 9.1% at 2.8 µg/l (n=74) and 5.5% at 4.5 µg/l (n=62) and for thallium, 8.5% at 0.25 µg/l (n=74), 8.9% at 0.70 µg/l (n=74) and 5.3% at 0.95 µg/l (n=62). One thallium value was below the method detection limit (MDL) of 0.02 µg/l; median (range) CV was 3.2% (0.1 to 17.9) from 60 (8.8%) duplicate analyses (eg, inter-assay CV). Of the antimony results, 313 (45.8%) were below the MDL of 0.2 µg/l; median (range) CV was 5.5% (0.1 to 73.4 (of 9 CVs that were >20%, eight were from samples that were below the MDL)) from 60 duplicate analyses. Details of cadmium QC and correction for potential polyatomic interference from molybdenum were as previously published.[8] None of the results for cadmium were below the MDL of 0.02 µg/l; median (range) CV was 2.6% (0.2 to 19.1) based on 60 duplicate analyses.

Blood lead was measured with a Hitachi 8100 Zeeman-background-corrected atomic absorption spectrophotometer[13] (Hitachi Ltd. Instruments, Tokyo, Japan) at the Institute of Industrial Medicine (now the Institute of Environmental & Occupational Medicine), a certified reference laboratory for lead in South Korea. Tibia lead levels were assessed via a 30 min measurement of the left mid-tibia diaphysis using ^{109}Cd in a back-scatter geometry to fluoresce the K-shell x-rays of lead. The lead x-rays were recorded with a radiation detector and then quantified and compared with calibration data to estimate the concentration of lead in bone.[14–16]

Kidney outcome assessment

Serum and urine creatinine were measured via a Dimension® clinical chemistry system using a Flex reagent cartridge in a modified kinetic Jaffe assay (model RxL; Dade Behring, Glasgow, Delaware, USA). Serum cystatin C was measured using an automated Dade Behring nephelometry assay on a Dimension Vista Lab System (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA). Urine NAG concentration was determined using a colorimetric assay (PPR Diagnostics Ltd, London, UK). For QC purposes, the original serum creatinine and

cystatin C results were ordered by concentration and 5% of each was selected sequentially for duplication. Median inter-day CV for serum creatinine and cystatin C and urinary NAG samples run in duplicate were all <10%.

Calculated creatinine-based kidney outcomes included calculated creatinine clearance,[17] measured creatinine clearance ((urinary creatinine in mg/dl × urine volume in ml)/serum creatinine in mg/dl/collection time in minutes) and the Modification of Diet in Renal Disease (MDRD) creatinine-based estimates of glomerular filtration rate (eGFR):

- 186.3 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × 0.742 (if women).[18 19]

Three cystatin-C-based equations were also used to estimate GFR[20]:

- Single variable CYSeGFRs =76.7 × serum cystatin C$^{-1.19}$
- Multivariable CYSeGFRm =127.7 × serum cystatin C$^{-1.17}$ × age$^{-0.13}$ × (0.91, if female)
- Combined cystatin C/creatinine eGFRc =177.6 × serum creatinine$^{-0.65}$ × serum cystatin C$^{-0.57}$ × age$^{-0.20}$ × (0.82, if female.)

Statistical analysis

The goals of this analysis were to (1) evaluate the respective associations between urine antimony and thallium levels and kidney outcomes in lead workers, while controlling for covariates, and (2) to evaluate the effects of adjustment with the other urine metals on those associations (eg, urine cadmium and antimony on models with thallium), also controlling for covariates. Statistical analysis was performed using statistical software of the StataCorp LP (College Station, Texas, USA).[21]

Initially, variable distributions were examined. Both urine antimony and thallium, with and without adjustment for urine creatinine (μg/g creatinine), were right skewed and thus ln-transformed to minimise influential outliers. The NAG distribution also exhibited a non-normal distribution and was ln transformed; the efficacy of this transformation was confirmed by examination of the final regression model residuals. In linear regression models, urine metals were adjusted for urine dilution in two ways: via the traditional approach in which the metal concentration is divided by urine creatinine and via a more recent approach in which the urine metal and urine creatinine are included as separate covariates in the model.[22] Antimony was initially analysed using all data reported from the laboratory; subsequently, it was determined that 45.8% of values were below the MDL. Analyses including antimony were then rerun with values below the MDL replaced by MDL/square root of 2.[23] Results of this sensitivity analysis were consistent; hence, results using actual values are reported. A sensitivity analysis using only antimony values above the MDL was also performed and discussed below.

Covariate selection utilised a priori variables (age, sex, body mass index (BMI) (weight in kilograms divided by the square of height in meters) and ln-urine creatinine) in modelling that initially included urine antimony or thallium with other biologically relevant variables in separate models. Variables were retained in the final model if they substantially changed either the urine antimony or thallium regression coefficient or the explanatory value (r^2) of the model for any of the kidney outcomes, were statistically significant or were relevant based on a priori knowledge or hypotheses inherent to this study (eg, blood and tibia lead; ln-urine cadmium). Additional covariates considered for inclusion using this approach were diabetes and hypertension (both based on participant report of physician diagnosis or medication use), regular analgesic use (based on questionnaire data on medication usage), self-reported work status (current vs former lead worker), study status (phase I vs II study participant), systolic and diastolic blood pressure (average of three measures), tobacco use (smoking status: never, former, current), smoking dose ((cigarettes per day × years of smoking) in quartiles for current smokers and dichotomised for former smokers, alcohol consumption (never, former, current)), education (less than middle school graduate, less than high school graduate, high school graduate, greater than high school graduate) and annual income (≤10, 10–20, 20–30, 30–40 and >40 million won). Blood lead, tibia lead and urine cadmium were added to final models after all other covariates were selected. Job duration (years) was also added to the final antimony models because urine antimony is considered a recent occupational exposure measure in study participants employed in lead battery manufacturing facilities, and job duration may therefore approximate duration of antimony exposure.

Associations between each study metal were also examined in models stratified at the median of the respective outcome measure in order to assess the potential for reverse causality (ie, whether associations were present only in participants with worse kidney function). Models were evaluated for linear regression assumptions and the presence of outlying points using augmented component-plus-residual plots and added variable plots.[24 25] Models were repeated without outliers when applicable. Models were also assessed for collinearity via examination of variance inflation factors.

RESULTS

Selected demographics, exposure and health outcome measures

Information on demographics, metal dose biomarkers, kidney outcomes and selected covariates is presented in table 1 for all 684 lead workers and, separately, by current and former lead worker employment status. Men and current lead workers comprised 78.2% (n=535) and 65.8% (n=450), respectively, of the population. Median urine thallium and antimony levels were 0.39 and 0.36 μg/g creatinine, respectively. Median antimony levels were substantially higher in current than former workers (0.77 and 0.1 μg/g creatinine, respectively), consistent with current occupational exposure. Mean values of the glomerular filtration measures were within normal limits.

Exposure biomarkers were generally correlated (table 2). Higher urine cadmium levels in former workers, but higher blood lead and urine antimony levels in current workers, were responsible for the lack of correlation between these metals. For example, antimony was positively correlated with cadmium in separated current and former worker groups (r=0.15, 0.16, respectively, p<0.05 for both) but not when all workers were considered together (−0.06, p=0.49). Urine thallium and cadmium were correlated with blood and tibia lead in current, but not former workers.

Kidney outcomes and urine creatinine were also correlated (online appendix table 1). Correlations were generally higher for measures based on the same biomarker (eg, serum creatinine-based measures). Urine creatinine was not correlated with any cystatin C variables.

Associations of urine metals with kidney outcomes

In all lead workers, after adjustment for age, sex, BMI, current versus former lead worker status, phase I versus II study entry, annual income, education, alcohol consumption, smoking dose, diastolic blood pressure, blood lead, tibia lead and, in antimony models only, lead job duration, higher urine concentrations of antimony and thallium (in separate models) were significantly associated with higher NAG, a direction consistent with nephrotoxicity (table 3, models 1 and 2, respectively).

Table 1 Selected demographic, exposure and health outcome measures in current and former lead workers

	All participants (n=684) N (%)	Current workers (n=450) N (%)	Former workers (n=234) N (%)
Characteristics			
Men	535 (78.2)	421 (93.6)	114 (48.7)
Diabetes	25 (3.7)	10 (2.2)	15 (6.4)
Hypertension	84 (12.3)	45 (10.0)	39 (16.7)
Current smokers	294 (43.0)	232 (51.6)	62 (26.5)

	All participants (n=684)			Current workers (n=450)		Former workers (n=234)	
	Median	Mean (SD)	Range	Median	Mean (SD)	Median	Mean (SD)
Age, years	46.5	47.6 (8.0)	24.1–71.3	44.7	45.2 (6.2)	54	52.0 (9.0)
BMI, kg/m^2	24.1	24.2 (2.9)	15.6–33.3	23.7	23.8 (2.7)	24.9	24.9 (3.1)
Systolic blood pressure, mm Hg	121.5	123.6 (15.7)	90.5–214.5	121.5	124.0 (15.4)	120.5	122.9 (16.3)
Diastolic blood pressure, mm Hg	74.5	75.1 (12.1)	46.0–148.0	74.5	75.9 (12.1)	73.0	73.6 (12.1)
Lead job duration, years	13.4	13.1 (7.2)	0.2–37.4	15.9	14.5 (6.5)	8.3	10.5 (7.7)
Exposure measures							
Antimony, μg/g creatinine	0.36	2.4 (7.5)	0.02–75.8	0.77	3.6 (9.0)	0.10	0.25 (0.48)
Thallium, μg/g creatinine	0.39	0.44 (0.23)	0.07–1.60	0.40	0.45 (0.23)	0.37	0.42 (0.24)
Cadmium, μg/g creatinine*	0.83	1.0 (0.62)	0.17–4.09	0.75	0.84 (0.43)	1.08	1.31 (0.79)
Blood lead, μg/dl	21.5	23.2 (14.3)	1.9–74.4	27.4	29.1 (13.0)	8.8	11.8 (8.8)
Tibia lead, μg/g bone mineral†	20.0	27.1 (29.3)	−12 to 231	20	27.0 (26.9)	19	27.4 (33.5)
Outcome measures							
Serum creatinine, mg/dl	0.87	0.87 (0.15)	0.42–1.53	0.89	0.88 (0.14)	0.81	0.83 (0.17)
MDRD eGFR, ml/min/1.73 m^2	95.8	97.7 (19.4)	23.6–189.7	97.9	100.2 (18.1)	90.7	92.9 (20.8)
Calculated creatinine clearance, ml/min	93.9	95.4 (22.2)	30.5–209.9	97.3	98.9 (20.1)	86.7	88.7 (24.4)
Measured creatinine clearance, ml/min	110.4	110.8 (30.9)	9.9–222.9	116.3	116.4 (30.8)	97.8	99.9 (28.3)
Serum cystatin C, mg/l	0.72	0.73 (0.12)	0.50–2.35	0.71	0.72 (0.11)	0.74	0.76 (0.13)
CYSeGFRm, ml/min/1.73 m^2	112.7	112.0 (17.8)	28.1–186.3	116.4	116.6 (15.5)	102.3	103.1 (18.6)
CYSeGFRs, ml/min/1.73 m^2	113.8	113.7 (16.9)	27.7–176.3	115.4	116.2 (15.4)	109.0	108.8 (18.4)
Combined eGFRc, ml/min/1.73 m^2	106.0	106.3 (17.7)	24.3–174.5	109.3	109.8 (15.7)	98.0	99.6 (19.3)
NAG μmol/h/g creatinine	318.1	386.3 (282.1)	46.1–3778.5	289.1	345.7 (230.3)	399.4	464.2 (348.9)

*Molybdenum corrected.
†n=678.
NAG, N-acetyl-β-D-glucosaminidase.

Ln-urine thallium was associated with seven of the eight serum creatinine and cystatin-C-based glomerular filtration measures but the direction of each of these associations was opposite to that expected with traditional nephrotoxicity (tables 3 and 4, model 2). Ln-urine antimony was only associated with serum creatinine-based glomerular filtration measures (serum creatinine and Modification of Diet in Renal Disease (MDRD) eGFR, a borderline significant association ($p<0.1$) with calculated creatinine clearance was also observed) (tables 3 and 4, model 1). As with ln-urine thallium, the directions were opposite

Table 2 Spearman correlation coefficients for metal dose variables in 684 current and former lead workers

	Antimony†	Thallium	Cadmium	Blood lead	Tibia
All workers					
Thallium, μg/g creatinine	0.23***				
Cadmium, μg/g creatinine	−0.06	0.21***			
Blood lead, μg/dl	0.51***	0.17***	−0.12**		
Tibia lead, μg/g bone	0.19***	0.16***	0.08*	0.47***	
Job duration, years	0.39***	0.11**	−0.11**	0.32***	0.41***
Current workers					
Thallium, μg/g creatinine	0.19***				
Cadmium, μg/g creatinine	0.15**	0.22***			
Blood lead, μg/dl	0.19***	0.19***	0.17***		
Tibia lead, μg/g bone	0.18***	0.22***	0.13**	0.57***	
Job duration, years	0.17***	0.06	0.004	−0.06	0.36***
Former workers					
Thallium, μg/g creatinine	0.32***				
Cadmium, μg/g creatinine	0.16*	0.30***			
Blood lead, μg/dl	0.44***	0.05	0.02		
Tibia lead, μg/g bone	0.28***	0.04	0.06	0.61***	
Job duration, years	0.43***	0.14*	0.02	0.60***	0.51***

†Antimony (μg/g creatinine).
*$p<0.05$; **$p<0.01$; ***$p<0.001$.

Table 3 Associations of urine metals with creatinine-based and NAG kidney outcomes in 684 lead workers

	β Coefficient (95 % CI)			
	Model 1	**Model 2**	**Model 3**	**Model 4**
Kidney outcome				
MDRD eGFR, ml/min/1.73 m^2				
Ln-urine antimony, μg/l	1.5 (0.5 to 2.5)**		1.0 (−0.03 to 1.9)	0.8 (−0.2 to 1.7)
Ln-urine thallium, μg/l		6.8 (4.1 to 9.6)***	6.4 (3.6 to 9.2)***	5.2 (2.4 to 8.0)***
Ln-urine cadmium, μg/l				6.7 (3.5 to 9.9)***
Ln-urine creatinine, mg/dl	−2.0 (−4.4 to 0.4)	−6.3 (−9.5 to −3.0)***	−6.8 (−10.1 to −3.5)***	−11.7 (−15.8 to −7.7)***
Calculated creatinine clearance, ml/min				
Ln-urine antimony, μg/l	0.8 (−0.1 to 1.6)		0.5 (−0.3 to 1.4)	0.4 (−0.4 to 1.3)
Ln-urine thallium, μg/l		4.0 (1.5 to 6.5)**	3.8 (1.3 to 6.2)**	3.0 (0.6 to 5.4)*
Ln-urine cadmium, μg/l				3.5 (0.7 to 6.3)*
Ln-urine creatinine, mg/dl	0.1 (−1.9 to 2.2)	−2.5 (−5.4 to 0.5)	−3.0 (−5.9 to −0.1)*	−5.5 (−9.0 to −1.9)**
Measured creatinine clearance, ml/min				
Ln-urine antimony, μg/l	−0.6 (−2.1 to 0.9)		−0.9 (−2.4 to 0.6)	−0.7 (−2.2 to 0.8)
Ln-urine thallium, μg/l		2.7 (−1.4 to 6.8)	3.1 (−1.0 to 7.3)	3.6 (−0.6 to 7.8)
Ln-urine cadmium, μg/l				−3.3 (−8.1 to 1.4)
Ln-urine creatinine, mg/dl	11.3 (7.8 to 14.8)***	8.2 (3.4 to 13.1)**	8.8 (3.8 to 13.7)**	11.3 (5.2 to 17.3)***
Serum creatinine, mg/dl				
Ln-urine antimony, μg/l	−0.010 (−0.018 to −0.003)**		−0.006 (−0.013 to 0.001)	−0.005 (−0.012 to 0.003)
Ln-urine thallium, μg/l		−0.053 (−0.073 to −0.033)***	−0.050 (−0.071 to −0.030)***	−0.042 (−0.063 to −0.021)***
Ln-urine cadmium, μg/l				−0.049 (−0.073 to −0.026)***
Ln-urine creatinine, mg/dl	0.013 (−0.005 to 0.031)	0.051 (0.027 to 0.075)***	0.054 (0.030 to 0.079)***	0.091 (0.061 to 0.121)***
Ln-NAG, μmol/h/g creatinine				
Ln-urine antimony, μg/l	0.033 (0.004 to 0.063)*		0.029 (−0.0004 to 0.058)	0.022 (−0.007 to 0.052)
Ln-urine thallium, μg/l		0.096 (0.015 to 0.178)*	0.083 (0.001 to 0.166)*	0.045 (−0.038 to 0.128)
Ln-urine cadmium, μg/l				0.225 (0.132 to 0.319)***
Ln-urine creatinine, mg/dl	−0.101 (−0.171 to −0.031)**	−0.154 (−0.251 to −0.057)**	−0.171 (−0.269 to −0.073)**	−0.337 (−0.456 to −0.218)**

Multiple linear regression models also adjusted for age, sex, BMI, employment status (current vs former lead worker), study status (phase I vs II study entry), annual income (10, 10–20, 20–30, 30–40 and >40 million won), education (less than middle school graduate, less than high school graduate, high school graduate, greater than high school), alcohol consumption (never, former, current), smoking dose ((cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status), diastolic blood pressure and blood and tibia lead. Model 1 also adjusted for lead job duration. *p<0.05; **p<0.01; ***p<0.001.
NAG, N-acetyl-β-D-glucosaminidase.

to that expected in nephrotoxicity. Neither thallium nor antimony was associated with measured creatinine clearance.

The direction of significant associations in fully adjusted models was the same in a priori models that adjusted only for age, sex, BMI and ln-urine creatinine (data not shown). Adjustment for blood and tibia lead attenuated the associations between ln-urine thallium and kidney outcomes (although they remained highly significant) but slightly increased the β coefficients of the associations between ln-urine antimony and the creatinine-based glomerular filtration measures.

Associations after adjustment for multiple metals

Associations with each urine metal were evaluated following additional adjustment for the other metals, for example, antimony further adjusted for thallium (tables 3 and 4, model 3) and for thallium and cadmium (tables 3 and 4, model 4). Previously significant associations with creatinine-based filtration outcomes were attenuated; however, thallium associations remained significant. With the exception of the combined creatinine- and cystatin-C-based eGFR, β coefficients for ln-urine thallium associations with cystatin-C-based filtration measures became stronger with additional adjustment. Results were consistent when the metal concentrations were entered as μg/g creatinine (online appendix table 2). The impact of cadmium adjustment on each metal is shown in online appendix table 3.

Adjustment for metals that were strongly associated with kidney outcomes also had a large impact on urine creatinine associations. An example is the increased significance of urine creatinine in models 2–4 for MDRD eGFR and CYSeGFRm compared with model 1 (tables 3 and 4). Associations between urine metals and kidney outcomes, fully adjusted as in model 4, but without urine creatinine, are shown in online appendix table 4. With the exception of measured creatinine clearance, thallium associations were attenuated but remained significant for cystatin-C-based outcomes. Cadmium associations with serum creatinine-based outcomes were attenuated but were strengthened for cystatin-C-based outcomes.

Sensitivity analyses

In order to determine if the observed thallium and cadmium attenuation of antimony associations was related to measurement uncertainty (45.8% of antimony values were less than MDL), sensitivity analyses, in which values below the MDL were replaced by MDL/square root of 2, were conducted in models of MDRD eGFR. Similar evidence of attenuation of associations in combined metal models was observed (data not shown). Models were also run only in those participants with antimony values greater than MDL; attenuation, although observed, was less than when concentrations less than MDL were included (data not shown).

To determine if the unexpected directions of the thallium and antimony associations were a result of different groups within our population, a priori models of MDRD eGFR stratified by worker status (current vs former) were examined. The directions of the associations in these analyses were consistent with results in the combined population (data not shown). Similarly, consistent directions were observed in a priori models of MDRD eGFR stratified by participant sex (data not shown). In models stratified by median kidney outcome (table 5), higher ln-urine thallium was associated only in the group of lead workers with

Table 4 Associations between urine metals and cystatin-C-based kidney outcomes in 684 lead workers

	β Coefficient (95 % CI)			
	Model 1	**Model 2**	**Model 3**	**Model 4**
Kidney outcome				
CYSeGFRm, ml/min/1.73 m^2				
Ln-urine antimony, μg/l	−0.2 (−1.0 to 0.7)		−0.5 (−1.3 to 0.3)	−0.4 (−1.3 to 0.4)
Ln-urine thallium, μg/l		4.9 (2.6 to 7.2)***	5.1 (2.8 to 7.4)***	5.4 (3.1 to 7.8)***
Ln-urine cadmium, μg/l				−1.9 (−4.6 to 0.7)
Ln-urine creatinine, mg/dl	−0.6 (−2.6 to 1.3)	−5.2 (−7.8 to −2.5)***	−4.9 (−7.6 to −2.2)***	−3.5 (−6.8 to −0.1)*
CYSeGFRs, ml/min/1.73 m^2				
Ln-urine antimony, μg/l	−0.1 (−1.0 to 0.7)		−0.5 (−1.3 to 0.4)	−0.4 (−1.3 to 0.4)
Ln-urine thallium, μg/l		5.3 (2.9 to 7.7)***	5.5 (3.1 to 7.9)***	5.8 (3.4 to 8.3)***
Ln-urine cadmium, μg/l				−2.0 (−4.7 to 0.8)
Ln-urine creatinine, mg/dl	−0.6 (−2.7 to 1.4)	−5.5 (−8.3 to −2.7)***	−5.2 (−8.1 to −2.4)***	−3.8 (−7.2 to −0.3)*
Combined eGFRc, ml/min/1.73 m^2				
Ln-urine antimony, μg/l	0.9 (−0.01 to 1.7)		0.4 (−0.5 to 1.2)	0.3 (−0.5 to 1.1)
Ln-urine thallium, μg/l		6.3 (3.9 to 8.6)***	6.1 (3.7 to 8.5)***	5.6 (3.1 to 8.0)***
Ln-urine cadmium, μg/l				3.1 (0.4 to 5.9)*
Ln-urine creatinine, mg/dl	−1.5 (−3.5 to 0.6)	−6.3 (−9.1 to −3.5)***	−6.5 (−9.3 to −3.7)***	−8.8 (−12.3 to −5.3)***
Serum cystatin C, mg/l				
Ln-urine antimony, μg/l	0.0004 (−0.004 to 0.005)		0.002 (−0.002 to 0.007)	0.002 (−0.003 to 0.006)
Ln-urine thallium, μg/l		−0.032 (−0.045 to −0.020)***	−0.033 (−0.046 to −0.021)***	−0.036 (−0.048 to −0.023)***
Ln-urine cadmium, μg/l				0.015 (0.001 to 0.030)*
Ln-urine creatinine, mg/dl	0.005 (−0.006 to 0.015)	0.034 (0.019 to 0.048)***	0.032 (0.018 to 0.047)***	0.021 (0.003 to 0.039)*

Multiple linear regression models also adjusted for age, sex, BMI, employment status (current vs former lead worker), study status (phase I vs II study entry), annual income (10, 10–20, 20–30, 30–40 and >40 million won), education (less than middle school graduate, less than high school graduate, high school graduate, greater than high school), alcohol consumption (never, former, current), smoking dose ((cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status), diastolic blood pressure and blood and tibia lead. Model 1 also adjusted for lead job duration. *p<0.05; **p<0.01; ***p<0.001.

worse kidney function (although in this occupational population, most kidney function values in this group were still in the normal range making any assessment for reverse causality difficult).

Similar results were observed in a priori models (data not shown).

In order to determine whether the thallium associations could be due to lead-related hyperfiltration, fully adjusted models were examined in 234 former lead workers whose blood lead levels were negatively associated with MDRD eGFR (β (95% CI) = −0.5 (−0.8 to −0.1)), a pattern consistent with traditional lead-related nephrotoxicity. However, urine thallium remained positively associated with MDRD eGFR in these workers as well (β (95% CI) = 7.0 (1.9 to 12.1)).

DISCUSSION

We compared associations of ln-urine antimony and thallium concentrations with a range of kidney outcomes, including eight serum creatinine- and cystatin-C-based glomerular filtration measures and NAG, a proximal tubular early biological effect marker, to determine the impact of coexposure to these metals on kidney function in lead-exposed workers. Two key findings were observed. First, contrary to expectation, higher ln-urine thallium levels were significantly associated with higher levels of both serum creatinine- and cystatin-C-based glomerular filtration measures (tables 3 and 4). These associations, which are in the opposite direction of those traditionally reported in nephrotoxicity, remained significant even after adjustment for ln-urine cadmium and antimony and lead dose. Measured creatinine clearance was an exception; no significant associations with ln-urine antimony or thallium were observed. In subgroup analyses stratified by median kidney outcome, ln-urine thallium associations were limited to participants in the lower kidney function group (table 5). Second, the analysis of multiple metals revealed evidence of attenuation. Antimony associations with kidney outcomes, only present with creatinine-based outcomes but in the same unexpected direction, became non-significant after thallium and cadmium adjustment (table 3). Similarly, associations of NAG with both antimony and thallium were attenuated by cadmium adjustment (table 3).

Thallium and antimony are widespread in the environment. Exposure sources in the general population include industrial releases, from smelting of other metals and coal combustion, and diet due to uptake of metals from soil.[4 26 27] Elevated levels of thallium have been reported in trout from Lake Michigan.[28] In contrast to cadmium, urine levels of antimony and thallium reflect more current exogenous exposure.[4] Thallium is concentrated in the kidneys; the half-life in rats is approximately 3 days; data in humans are extremely limited but the half-life appears to be as long as 30 days.[26] A renal excretion half-life of 4 days for antimony was reported in lead battery production workers although a longer half-life may be possible based on reports of elevated urine levels in treated patients and deposits in lungs of smelter workers.[27] In our population, median urine thallium levels were similar between current and former lead workers (0.40 and 0.37 μg/g creatinine, respectively) but urine antimony levels were higher in current workers (0.77 cf. 0.10 μg/g creatinine in former workers), likely from occupational exposure to antimony used as an alloy in lead storage batteries. In comparison, median and 95th percentile urine levels in adults in the 2003–2004 NHANES were 0.15 and 0.33 μg/g creatinine, respectively, for thallium, and 0.08 and 0.28 μg/g creatinine, respectively, for antimony.[4]

Nephrotoxicity is a side effect of pentavalent antimony use in the treatment of parasitic diseases, such as leishmaniasis.[29] Increased serum creatinine, blood urea nitrogen and proteinuria have been observed in animal models following 30 days of intramuscular injections with pentavalent antimony compounds.[30] Tubular necrosis and decreased creatinine clearance have also been reported.[29] Interestingly, consistent with our

Table 5 Associations between urine metals and eGFR in models stratified at median kidney outcome (n=684)†

Kidney function measure	β Coefficient (95 % CI)	β Coefficient (95 % CI)
MDRD eGFR, ml/min/1.73 m^2	<95.9 ml/min/1.73 m^2	≥95.9 ml/min/1.73 m^2
Ln-urine antimony, μg/l	−0.3 (−1.0 to 0.5)	1.0 (−0.02 to 2.1)
Ln-urine thallium, μg/l	2.4 (0.6 to 4.2)*	−0.1 (−3.8 to 3.7)
Ln-urine cadmium, μg/l	3.0 (0.7 to 5.4)*	5.2 (1.6 to 8.8)**
Calculated creatinine clearance, ml/min	<93.9 ml/min	≥93.9 ml/min
Ln-urine antimony, μg/l	0.4 (−0.3 to 1.1)	1.0 (−0.03 to 2.1)
Ln-urine thallium, μg/l	2.6 (0.8 to 4.4)**	0.02 (−3.5 to 3.5)
Ln-urine cadmium, μg/l	2.7 (0.5 to 5.0)*	5.0 (1.3 to 8.7)**
Serum creatinine, mg/dl	≥0.87 mg/dl	<0.87 mg/dl
Ln-urine antimony, μg/l	0.002 (−0.006 to 0.009)	−0.005 (−0.012 to 0.002)
Ln-urine thallium, μg/l	−0.024 (−0.044 to −0.004)*	−0.017 (−0.039 to 0.005)
Ln-urine cadmium, μg/l	−0.030 (−0.057 to −0.003)*	−0.040 (−0.061 to −0.019)***
CYSeGFRm, ml/min/1.73 m^2	<112.7 ml/min/1.73 m^2	≥112.7 ml/min/1.73 m^2
Ln-urine antimony, μg/l	−0.4 (−1.3 to 0.5)	−0.4 (−1.1 to 0.4)
Ln-urine thallium, μg/l	4.5 (2.6 to 6.5)***	1.0 (−1.9 to 3.9)
Ln-urine cadmium, μg/l	−2.9 (−5.3 to −0.5)*	0.3 (−2.5 to 3.2)

†Multiple linear regression models stratified by median outcome such that n=342 for each population model. Models also adjusted for age, sex, BMI, ln-urine creatinine, employment status (current vs former lead worker), smoking dose (cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status, alcohol consumption (never, former, current), education (less than middle school graduate, less than high school graduate, high school graduate, greater than high school), annual income (≤10, 10–20, 20–30, 30–40 and >40 million won), study status (phase I vs II study entry), diastolic blood pressure and blood and tibia lead. *p<0.05; **p<0.01; ***p<0.001.

results before adjustment for the other metals, decreased serum creatinine was observed in a dose-related manner in rats exposed to trivalent antimony salt via drinking water over a 90-day study period.[31] Route of administration may be relevant in animal data; nephrotoxicity from antimony potassium tartrate administered intraperitoneally but not via drinking water has been reported.[32] Tubular changes have also been observed in rabbits after 5 days of inhalational exposure to antimony trisulfide.[33] A recent analysis of NHANES data identified antimony as a potential risk factor for peripheral arterial disease[34] and for cardiovascular and cerebrovascular disease.[35]

Acute kidney injury has been reported in thallium poisoning.[36–38] Increased serum creatinine and proteinuria and decreased glomerular filtration rate have been observed with acute exposure in animal studies.[39–41] Pathologic changes have been reported throughout the renal tubule following acute and subacute thallium exposure in animal studies.[36 39 42–44] Decreased reabsorption in the proximal tubules, even without visible microscopic pathology, has also been observed.[40] We did not identify any comparable publications on associations between antimony or thallium and the kidney outcomes used in this analysis. Thallium was not associated with vascular outcomes in the NHANES analyses cited above for antimony.[34 35]

The data herein allow additional consideration of the previously published hypotheses[8 9] generated for associations between ln-urine cadmium- and serum creatinine-based filtration measures that were also in the opposite direction to that expected in nephrotoxicity. In the current analyses, we modelled traditional serum creatinine-based kidney outcomes and measured creatinine clearance in a 4 h urine collection. We also included the kidney proximal tubule biomarker, NAG, as well as kidney outcomes based on serum cystatin C, a cysteine protease inhibitor that is secreted by all nucleated cells,[45] thus avoiding confounding with serum creatinine related to its metabolism from muscle. Since creatinine and cystatin C are correlated measures of kidney function, associations present with both creatinine- and cystatin C-based eGFR suggest a kidney function, rather than an individual biomarker effect. Thus, associations between higher ln-urine thallium and higher levels of both serum creatinine- and cystatin-C-based glomerular filtration measures are consistent with a kidney function effect such as reverse causality or thallium-related hyperfiltration. Reverse causality implies that lower urine thallium levels reflect less kidney excretion of thallium due to chronic kidney disease. In contrast, thallium-related hyperfiltration implies an effect of the metal on kidney function. Hyperfiltration is a longitudinal process reported in humans with diabetes, hypertension and obesity[46] and lead-exposed rodents,[47] in which an initial increase in glomerular filtration rate is followed by a subsequent decline indicative of chronic kidney disease. We attempted to distinguish between these two hypotheses in models stratified by median outcome. The fact that thallium associations are confined to participants with filtration measures below the median is supportive of reverse causality. However, the two populations stratified by median outcome have a number of differences in terms of age, sex and lead worker status and, since this is an occupational population, few participants have kidney dysfunction to the extent necessary to result in reverse causality.

Antimony and cadmium associations were observed with serum creatinine- but not cystatin-C-based filtration measures and so neither hypothesis above is as relevant. As previously published, a statistical mechanism related to the use of creatinine in both exposure and outcome metrics is a consideration for these associations.[9] The striking change in urine creatinine associations when a statistically significant metal is in the model, as evidenced by models 1 and 2 (tables 3 and 4), lends support for this mechanism. However, given the differences in associations between metals, unique metal-specific mechanisms, such as metal–protein binding affecting excretion[48] must also be considered. Adding to the complexity of interpreting these findings is a recent publication in which lower levels of urinary bisphenol A were observed in National Health and Nutrition Examination Survey participants with lower MDRD eGFR levels, but not using a different creatinine-based eGFR measure.[49]

We were able to exclude several potential hypotheses. Thallium associations cannot be attributed to lead-related hyperfiltration in this analysis since we adjusted for lead dose;

moreover, urine thallium was positively associated with eGFR even in former workers in whom lead-related hyperfiltration was not apparent. We were also able to exclude metal collinearity by observing consistent association directions in simpler a priori models and evaluating variance inflation factors in fully adjusted models. As an additional check on our models, BMI, a traditional chronic kidney disease risk factor, was significantly and negatively associated (ie, direction consistent with nephrotoxicity) with MDRD eGFR.

Novel results were also observed in the multiple metals analysis. Although common in environmental exposure, mixtures are infrequently evaluated in research. Coexposures to metals with common target organs may have additive or multiplicative effects. A recent analysis of 1999–2006 NHANES data found that increased blood cadmium and lead levels were independently associated with increased prevalences of albuminuria and reduced eGFR.[50] Furthermore, odds of having both outcomes were fourfold higher in participants in the highest quartiles of both metals compared with those in the lowest. However, in the current analysis, attenuation was observed. In particular, NAG is routinely used in nephrotoxicant research. If we had limited our analysis to associations between antimony and thallium in separate models of NAG, we would have concluded that the results supported nephrotoxicity from both metals at these levels of exposure. Instead, our multiple metal analysis allowed us to identify cadmium as the primary toxicant associated with NAG. The antimony attenuation observed in our analysis may be related to measurement uncertainty, given the large proportion of samples below the MDL, or to population differences due to higher exposure in the lead battery workers.

In conclusion, we report two novel and important findings in these data. First, urine thallium levels were significantly associated with both serum creatinine- and cystatin-C-based glomerular filtration measures in the direction opposite to that traditionally reported with nephrotoxicity. Second, the analysis of multiple metals revealed attenuation of observed associations by other metals. These results suggest that interpretation of low-level urine metal values may be more complex than previously thought. Furthermore, single metal exposure analysis approaches might increase the potential for inaccurate risk conclusions. Additional research is needed to determine the mechanism(s) for associations between higher urine metal concentrations and higher glomerular filtration measures.

Contributors All authors included on our manuscript fulfil the OEM stated criteria of authorship and there is no one else who fulfils the criteria who has not been included as an author.

Funding This research was supported by National Institute of Environmental Health Sciences, grant 2 ES007198; National Institute for Occupational Safety and Health, grant T42 OH0008428, from the Education and Research Center for Occupational Safety and Health at the Johns Hopkins Bloomberg School of Public Health and Korea Research Foundation, grant 2000-00545. The funding sources had no involvement in study design; data collection, analysis and interpretation; manuscript writing or decisions to submit the work for publication.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The ethics approval was provided by SoonChunHyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health.

Provenance and peer review Not commissioned; externally peer reviewed.

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Associations of multiple metals with kidney outcomes in lead workers

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Occup Environ Med 2012 69: 727-735 originally published online July 26, 2012
doi: 10.1136/oemed-2012-100765