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ORIGINAL ARTICLE

# Uranium associations with kidney outcomes vary by urine concentration adjustment method

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Uranium is a ubiquitous metal that is nephrotoxic at high doses. Few epidemiologic studies have examined the kidney filtration impact of chronic environmental exposure. In 684 lead workers environmentally exposed to uranium, multiple linear regression was used to examine associations of uranium measured in a 4-h urine collection with measured creatinine clearance, serum creatinine- and cystatin-C-based estimated glomerular filtration rates, and *N*-acetyl-$\beta$-D-glucosaminidase (NAG). Three methods were utilized, in separate models, to adjust uranium levels for urine concentration—$\mu$g uranium/g creatinine; $\mu$g uranium/l and urine creatinine as separate covariates; and $\mu$g uranium/4 h. Median urine uranium levels were 0.07 $\mu$g/g creatinine and 0.02 $\mu$g/4 h and were highly correlated ($r_s = 0.95$). After adjustment, higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG in models that used urine creatinine to adjust for urine concentration but not in models that used total uranium excreted ($\mu$g/4 h). These results suggest that, in some instances, associations between urine toxicants and kidney outcomes may be statistical, due to the use of urine creatinine in both exposure and outcome metrics, rather than nephrotoxic. These findings support consideration of non-creatinine-based methods of adjustment for urine concentration in nephrotoxicant research.

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## INTRODUCTION

Identification of environmental nephrotoxicants is increasingly important as the world-wide prevalence of chronic kidney disease grows.[1] Uranium, a naturally occurring radioactive element, is an important consideration in this regard. The US National Health and Nutrition Examination Survey (NHANES), a population sample considered representative of the non-military, non-institutionalized US general population, has consistently detected uranium in the urine of a majority of study participants.[2] Exposure in the general population occurs mainly through ingestion of natural uranium in drinking water and in food grown in contaminated soil. Exposure to uranium via contaminated ground water is a global concern as elevated levels have been detected in a wide range of geographic areas including the United States, Canada, Scandinavian countries, central Australia, India and South Korea.[3–5] The main industrial uses of uranium have been as a power source for nuclear reactors[3] and as depleted uranium used in the manufacture of munitions.

Uranium is nephrotoxic at acute, high-dose exposures in humans and animals.[5,6] However, despite widespread environmental exposure, few data on associations between chronic, low-level uranium exposure and kidney outcomes are available. Limited evidence indicates elevated levels of kidney early biological effect markers in populations chronically exposed to relatively low levels of uranium.[5–7] However, associations with glomerular filtration measures (GFR) have been inconsistent. Furthermore, the nephrotoxic effects of exposure to multiple metals are relatively unknown; populations co-exposed to uranium, lead and/or other nephrotoxic metals may be at increased risk for kidney damage. Thus, the data to date indicate considerable potential for nephrotoxicity associated with environmental uranium exposure and support the need for additional epidemiologic research. To address this knowledge gap, we performed a cross-sectional analysis of associations between urine uranium levels and kidney outcomes in 684 current and former lead workers in the Republic of Korea in whom cadmium, antimony and thallium associations with kidney outcomes have previously been analyzed.[8–10] Kidney outcomes examined include these traditional kidney filtration rate measures: serum creatinine- and cystatin-C-based estimated glomerular filtration rates (eGFR) and measured creatinine clearance. *N*-acetyl-$\beta$-glucosaminidase (NAG), an enzyme located in the lysosomes of the proximal tubule cells, was examined as an early biologic effect marker for the proximal tubule.

## MATERIALS AND METHODS

### Study Overview and Design

We performed a cross-sectional analysis of data from current and former inorganic lead workers who were voluntary participants in a longitudinal

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study established to look at the health effects of occupational lead exposure. Data used for this analysis were obtained in the fourth evaluation conducted between April 2004 and September 2005. All participants provided written, informed consent and 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,[9,11,12] participants were recruited via medical surveillance programs in 1997–1999 for the first enrollment phase of the cohort study and 2004–2005 for the second phase of the study. The population is 100% Korean. Inclusion criteria were occupational exposure to lead and, for phase II enrollees, age $\geq$40 years in order to enrich the study with participants with increased risk for adverse kidney outcomes. There were no medical exclusionary criteria. In order to optimize study data for both cross-sectional and longitudinal analyses, a urine metals panel, including uranium, thallium and cadmium, was measured in the fourth evaluation of the 712 workers who participated in both the fourth and fifth evaluations. Workers from a primary smelter ($n=28$) were excluded from this analysis due to their potentially wider range of occupational metal exposures. Thus, cross-sectional analysis of data in the remaining 684 workers was the focus of the current analysis.

## Data Collection

As previously described,[9] data collection and biological specimens included a standardized, interviewer-administered questionnaire; blood pressure obtained with the IntelliSense blood pressure monitor (Model HEM-907; Omron; Vernon Hills, IL, USA); height and weight; a blood sample (for serum creatinine, cystatin C and blood lead); a 4-h urine collection (for uranium, thallium, cadmium and creatinine levels); a spot urine sample collected just before beginning the 4-h urine collection (for NAG and creatinine); and tibia lead *via* X-ray fluorescence. Four-hour urine collections were obtained over the course of the day with start times from 0700 to 2000 hours; 66% were started between 0800 and 1159 hours and an additional 28% were started between 1200 and 1559 hours.

## Metals Exposure Assessment

Urine specimens were analyzed for metals in the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health's Wadsworth Center (Albany, NY, USA). A multi-element method based dynamic reaction cell-inductively coupled plasma-mass spectrometry was used[13] as previously described (Weaver, et al.[10] The method detection limit (MDL) for uranium was calculated according to International Union of Pure and Applied Chemistry guidelines.[14] The MDL was calculated as three times the SD measured in a urine matrix blank or low-level sample for a minimum of ten independent analytical runs and is typically 0.003 $\mu$g/l for urinary uranium. At the time of analysis for this study, the uranium MDL for urine was 0.001 $\mu$g/l, we have found the MDL to vary between 0.001–0.005 $\mu$g/l over the past 6 years. The limit of quantitation for uranium in urine, defined as ten times the SD measured in a urine matrix blank or low-level sample as described above is $\sim$0.009 $\mu$g/l, but may vary from 0.003–0.015 $\mu$g/l. Urine-based internal quality control (IQC) materials were analyzed before, during and after every analytical run. For uranium, the mean coefficient of variation (CV) of the IQC samples over the 5-month period in which the samples were assayed was 19% at 0.03 $\mu$g/l ($n=74$), 13% at 0.06 $\mu$g/l ($n=75$), 10% at 0.09 $\mu$g/l ($n=63$). Method accuracy was assessed via analysis of National Institute of Science and Technology Standard Reference Material 2670a toxic elements in urine (freeze-dried) for uranium content. Found values were $0.099\pm0.006$ $\mu$g/l ($n=22$) (certified value $0.102\pm0.002$ $\mu$g/l) in the low level and $4.88\pm0.07$ $\mu$g/l ($n=22$) (certified value $4.997\pm0.071$ $\mu$g/l) in the high level. The laboratory also successfully participates in the external QC program operated by the Institut National de Santé Publique du Québec, Le Center de Toxicologie du Québec's (CTQ) Intercomparison Program that includes uranium in urine. The laboratory routinely achieved a performance of $\pm$5% from the assigned target value on urine uranium challenges from CTQ.

Blood lead was measured with a Hitachi 8100 Zeeman background-corrected atomic absorption spectrophotometer[15] (Hitachi Instruments, Tokyo, Japan). 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 to calibration data to estimate the concentration of lead in bone.[16–18]

## Kidney Outcome Assessment

Serum and urine creatinine were measured with a Dimension clinical chemistry system using a Flex reagent cartridge in a modified kinetic Jaffe assay (model RxL; Dade Behring, Glasgow, DE, USA). Serum cystatin C was measured via an automated Dade Behring nephelometry assay on a Dimension Vista Lab System (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The PPR NAG test kit was used to determine urine NAG concentrations (PPR Diagnostics Ltd, London, UK). Median inter-day CV for serum creatinine and cystatin C and urinary NAG samples run in duplicate were all $<$10%.

Calculated kidney outcomes included measured creatinine clearance using the 4 h collection:

- (urine creatinine in mg/dl $\times$ urine volume in ml)/serum creatinine in mg/dl)/collection time in minutes)
  the Modification of Diet in Renal Disease (MDRD) creatinine-based eGFR:[19,20]
- $186.3\times$ (serum creatinine)$^{-1.154}\times$ (age)$^{-0.203}\times 0.742$ (if female)
  and a multi-variable cystatin-C-based equation:[21]
- $127.7\times$ serum cystatin C$^{-1.17}\times$ age$^{-0.13}\times 0.91$ if female

## Statistical Analysis

The goals of the analysis were to: (1) evaluate associations between urine uranium levels and kidney outcomes in current and former lead workers, while controlling for a range of covariates including other metals (blood and tibia lead and urine thallium and cadmium); and, (2) evaluate consistency of those associations in models that differed by method of adjustment for urine concentration. Statistical analysis was completed using statistical software from StataCorp LP (College Station, TX, USA).[22] As previously described[8] skewed variable distributions (urine uranium, thallium, cadmium, creatinine and NAG) were *ln*-transformed to minimize influential outliers and comply with statistical assumptions.

In separate multiple linear regression models, uranium measured in the 4-h urine collection was evaluated using three different methods to adjust for urine concentration: the traditional approach in which the metal concentration is adjusted for urine concentration by dividing by urine creatinine ($\mu$g uranium/g creatinine); a more recent approach in which the urine metal ($\mu$g/l) and creatinine (g/l) are included as separate covariates in the regression model;[23] and a non-creatinine-based approach using the total amount of uranium excreted during the 4 h urine-collection (urine uranium ($\mu$g/l) $\times$ total urine volume (L) $\times$ 4/actual collection time (h) resulting in $\mu$g uranium/4 h).

Initial regression models included *a priori* variables (age, gender, and BMI (weight in kilograms divided by the square of height in meters) and urine uranium (as *ln*-transformed $\mu$g/g creatinine, or as *ln*-transformed $\mu$g/l and *ln*-urine creatinine as separate covariates, or as *ln*-transformed $\mu$g/4 h). Additional covariates considered for inclusion 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); lead job duration (years); study status (phase I *vs* phase II enrollee); systolic and diastolic blood pressure (average of three measures); tobacco use (smoking status: never, former, current); smoking dose ((cigarettes per day $\times$ years of smoking) in quartiles for current smokers and dichotomized for former smokers); alcohol consumption (never, former and current); education ($<$middle school graduate, $<$high school graduate, high school graduate, $>$high school); and annual income ($\leq$10, 10–20, 20–30, 30–40, and $>$40 million won). Variables were retained in the final model if they substantially changed the uranium regression coefficient or the explanatory value ($r^2$) of the model for any of the kidney outcomes, were statistically significant ($P\leq0.05$), or were relevant based on *a priori* knowledge or hypotheses inherent to this study (e.g., *ln*-urine cadmium). Blood and tibia lead and urine thallium and cadmium were added to the final models after all other covariates were selected.

Models were evaluated for linear regression assumptions and the presence of outlying points using augmented component-plus-residual plots, added-variable plots and residual *vs* predicted plots[24,25] and repeated without outliers when applicable. Our goal in removing outliers was to avoid having results driven by the 1–2% of the population who

have extreme values for outcomes (abnormal kidney function) and/or exposure metrics. As previously published,[12] a standard set of outlier data has been identified and removed from the majority of analyses done in this population to date. These data are from workers with known kidney disease. Other outliers related to the metals in the current analyses were also removed. Models were also assessed for collinearity through examination of variance inflation factors, all of which were below 4.3.

## RESULTS

Information on demographics, uranium and other metal dose biomarkers, kidney outcomes, and selected covariates is presented in Table 1. Males comprised 78.2% ($n = 535$) of the population. Median urine uranium levels were 0.07 $\mu$g/g creatinine and 0.02 $\mu$g excreted in 4 h. Median levels of urine thallium and cadmium were 0.39 and 0.83 $\mu$g/g creatinine and 0.09 and 0.19 $\mu$g/4 h, respectively.

Urine creatinine-adjusted urine metals were correlated with their respective total metal excreted concentrations ($r_s \geq 0.8$; $P < 0.001$ for all; Table 2). Other metal correlations are shown in Table 2. As previously published,[8] the three GFR measures (MDRD eGFR, cystatin-C-based eGFR and measured creatinine clearance) were significantly correlated ($P < 0.001$); NAG was not correlated with MDRD eGFR but was negatively correlated ($P < 0.001$) with the two other glomerular filtration measures. Urine creatinine, measured in the 4 h urine specimen, was correlated with measured creatinine clearance, spot urine creatinine (used for NAG adjustment), and NAG ($r_s = 0.33$, 0.48 and $-0.12$, respectively; $P < 0.01$ for all), but not with eGFR as determined by either the serum creatinine- or cystatin-C-based equations.

**Table 1.** Selected demographic, exposure and health outcome measures in 684 lead workers.[a]

| *Characteristic* | N *(%)* | |
|---|---|---|
| Male | 535 (78.2) | |
| Current workers | 450 (65.8) | |
| Diabetes (yes) | 25 (3.7) | |
| Hypertension (yes) | 84 (12.3) | |
| Current smokers | 294 (43.0) | |
| | Median | Mean (SD) |
| Age, years | 46.5 | 47.6 (8.0) |
| BMI, kg/m$^2$ | 24.1 | 24.2 (2.9) |
| Systolic blood pressure, mm Hg | 121.5 | 123.6 (15.7) |
| Diastolic blood pressure, mm Hg | 74.5 | 75.1 (12.1) |
| Blood lead, $\mu$g/dl | 21.5 | 23.2 (14.3) |
| Tibia lead, $\mu$g Pb/g bone mineral[b] | 20.0 | 27.1 (29.3) |
| Uranium, $\mu$g/g creatinine | 0.07 | 0.13 (0.18) |
| Uranium, $\mu$g/4 h | 0.02 | 0.03 (0.04) |
| Thallium, $\mu$g/g creatinine | 0.39 | 0.44 (0.23) |
| Thallium, $\mu$g/4 h | 0.09 | 0.10 (0.06) |
| Cadmium,$\mu$g/g creatinine | 0.83 | 1.0 (0.6) |
| Cadmium, $\mu$g/4 h | 0.19 | 0.21 (0.10) |
| MDRD eGFR, ml/min/1.73 m$^2$ | 95.8 | 97.7 (19.4) |
| Cystatin-C-based eGFR, ml/min/ 1.73 m$^2$ | 112.7 | 112.0 (17.8) |
| Measured creat. clearance, ml/min | 110.4 | 110.8 (30.9) |
| NAG $\mu$mol/h/g creatinine | 318.1 | 386.3 (282.1) |

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; NAG, *N*-acetyl-$\beta$-D-glucosaminidase.
[a]modified from Shelley et al.[8]
[b]$n = 678$.

### Associations of Urine Uranium with Kidney Outcomes

In the two models that used urine creatinine to adjust for urine concentration, higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG after adjustment for age, sex, BMI, current *vs* former lead worker status, phase I *vs* II study entry, income, education, alcohol consumption, smoking dose, diastolic blood pressure, blood lead, tibia lead, *ln*-urine thallium and cadmium (Table 3; Models 1 and 2). In Model 1, *ln*-urine creatinine was entered as a separate covariate, and in Model 2, uranium and the other urine metals were divided by urine creatinine and the resulting variable, expressed in $\mu$g/g creatinine, was *ln*-transformed and entered so that *ln*-urine creatinine was not entered as a separate covariate. However, these associations were no longer significant when the uranium concentration metric was entered as $\mu$g excreted in the 4-h urine collection ($\mu$g/4 h) (Table 3; Model 3). Attenuation was much greater for measured creatinine clearance than NAG. Uranium was not associated with either creatinine- or cystatin-C-based eGFR in any of the three models (Table 3) nor with serum creatinine or cystatin C (data not shown). Results were consistent in *a priori* models that adjusted for age, sex and BMI and, in models with *ln*-urine uranium entered as $\mu$g/l, *ln*-urine creatinine. Specifically, significant associations with measured creatinine clearance and NAG were observed in the *a priori* models that used urine creatinine-based methods to adjust for urine concentration but not in those that used total uranium excreted ($\mu$g/4 h) (data not shown).

We also removed *ln*-urine creatinine from fully adjusted models of measured creatinine clearance and NAG (Model 1; Table 3). The *ln*-uranium association with NAG was no longer significant ($\beta$ (95% CI) = 0.02 ($-0.02$, 0.05)). However, the *ln*-uranium association with measured creatinine clearance was attenuated but remained significant ($\beta$ (95% CI) = $-2.0$ ($-3.7$, $-0.3$)). When *ln*-urine creatinine was added to fully adjusted models of measured creatinine clearance and NAG in which *ln*-uranium was entered as $\mu$g/4 h (Model 3; Table 3), the *ln*-uranium associations remained nonsignificant in both models (data not shown).

**Table 2.** Spearman correlation coefficients among urine metals and blood and tibia lead in 684 lead workers.

| | *Uranium* $\mu g/g$ *creatinine* | *Uranium* $\mu g/4\,h$ | *Thallium* $\mu g/g$ *creatinine* | *Thallium* $\mu g/4\,h$ | *Cadmium* $\mu g/g$ *creatinine* | *Cadmium* $\mu g/4\,h$ | *Blood lead* $\mu g/dl$ |
|---|---|---|---|---|---|---|---|
| Uranium, $\mu$g/4 h | 0.95# | | | | | | |
| Thallium, $\mu$g/g creat. | 0.08* | 0.06 | | | | | |
| Thallium, $\mu$g/4 h | 0.01 | 0.14# | 0.80# | | | | |
| Cadmium, $\mu$g/g creat. | 0.04 | $-0.09$* | 0.21# | $-0.09$* | | | |
| Cadmium, $\mu$g/4 h | $-0.02$ | 0.004 | 0.18# | 0.20# | 0.81# | | |
| Blood lead, $\mu$g/dl | $-0.21$# | $-0.15$# | 0.16# | 0.24# | $-0.12$** | $-0.03$ | |
| Tibia lead, $\mu$g/g bone | $-0.03$ | $-0.04$ | 0.15# | 0.08* | 0.08* | 0.04 | 0.48# |

*$P$-value $< 0.05$.
**$P$-value $< 0.01$.
#$P$-value $< 0.001$.

**Table 3.** Associations of urine uranium and kidney outcomes in 684 lead workers.[a]

| | | *Model 1* | | *Model 2* | | *Model 3* | |
|---|---|---|---|---|---|---|---|
| | | Ln-*uranium* μg/*l* | | Ln-*uranium* μg/*g creatinine* | | Ln-*uranium*, μg/*4 h* | |
| *Kidney outcome* | N[b] | β *coeff (95% CI)* | $r^2$ | β *coeff (95% CI)* | $r^2$ | β *coeff (95% CI)* | $r^2$ |
| MDRD eGFR, ml/min/1.73 $m^2$ | 678 | 0.02 (−1.0, 1.1) | 0.19 | 0.01 (−1.0, 1.0) | 0.19 | −0.2 (−1.2, 0.9) | 0.15 |
| Cystatin-C-based eGFR, ml/min/1.73 $m^2$ | 677 | −0.3 (−1.2, 0.5) | 0.33 | −0.3 (−1.2, 0.6) | 0.33 | −0.3 (−1.1, 0.6) | 0.33 |
| Measured creatinine clearance, ml/min | 668 | −2.5 (−4.2, −0.8)** | 0.30 | −3.0 (−4.7, −1.2)*** | 0.26 | −0.1 (−1.7, 1.5) | 0.40 |
| *Ln*-NAG, μmol/h/g creatinine | 669 | 0.034 (−0.0002, 0.07)* | 0.21 | 0.035 (0.002, 0.069)* | 0.21 | 0.025 (−0.010, 0.060) | 0.18 |

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; NAG, *N*-acetyl-β-D-glucosaminidase.
[a]Models adjusted for age, gender, BMI, employment status (current *vs* former lead worker), enrollee status (phase I *vs* II study entry), annual income (10, 10–20, 20–30, 30–40 and >40 million won), education (<middle school graduate, <high school graduate, high school graduate, >high school), alcohol consumption (never, former and current), smoking dose ((cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status), diastolic blood pressure, blood lead, tibia lead, *ln*-urine thallium, *ln*-urine cadmium and, in model 1 only, *ln*-urine creatinine. In models 2 and 3, urine cadmium and thallium were adjusted for urine concentration using the same approach as uranium (e.g., μg/g creatinine and μg/4 h, respectively).
[b]*N* for models 1–3 after removal of outliers for each outcome.
**P*-value ≤ 0.05.
***P*-value < 0.01.
****P*-value < 0.001.

## DISCUSSION

We examined associations of urine uranium concentrations with four kidney outcome measures (three filtration measures (measured creatinine clearance and serum creatinine- and cystatin-C-based glomerular filtration rates) and NAG, a proximal tubule early biological effect marker) to determine the impact of environmental exposure to uranium on kidney function in lead-exposed workers. We employed three different methods of adjustment for urine concentration and compared consistency of results across the three respective linear regression models. Distinct differences in the associations were observed by method of adjustment for urine concentration. *Ln*-urine uranium was significantly associated with lower measured creatinine clearance and higher NAG in the two models that used urine creatinine to adjust for urine concentration, one in which urine creatinine was included as a separate covariate in the regression model and the other using the more traditional creatinine-adjusted metal (i.e., urine uranium concentration expressed in μg/g creatinine) as a single variable. However, associations were no longer significant when the third urine concentration adjustment method (total uranium excreted (μg/4 h)) was used, although attenuation was much greater for measured creatinine clearance than for NAG.

Uranium is a ubiquitous heavy metal naturally found in rocks and soil. It is mined and used as an energy source and as depleted uranium in military munitions. Environmental exposure to natural uranium occurs mainly through ingestion of ground water or food.[7,26] Urine is the primary route of excretion for absorbed uranium.[7,26] An estimated 66% of uranium that enters the human bloodstream is rapidly excreted within 24 h and nearly 90% within a month. The remainder is primarily distributed to bone, and to a lesser extent, liver and kidneys.[27] The half-life in bone may range from months to years. Therefore, urine uranium concentration is considered to be a recent exogenous dose measure with a small contribution from cumulative endogenous exposure.[26] Median and 95$^{th}$ percentile urine uranium levels in our population were 0.070 and 0.531 μg/g creatinine compared with 0.005 and 0.026 μg/g creatinine, respectively, in 2005–2006 NHANES participants.[28]

Nephrotoxicity has been reported in animal studies using a variety of uranium compounds. Morphological abnormalities and dysfunction of the proximal tubule and glomerulus have been reported in acute and subacute studies with proximal tubule damage reported from chronic exposure.[5–7,29] Decreased creatinine clearance and increased proteinuria have been reported in acute high dose human case reports, such as in industrial accidents.[6] Epidemiological studies have reported associations between uranium and adverse proximal tubule effects. Beta 2-microglobulin is the proximal tubule biomarker that has been most consistently associated with uranium exposure in humans.[5]

Epidemiological studies examining associations between uranium exposure and glomerular filtration measures are scarce and the results are inconsistent (Table 4). Findings range from no associations;[30–34] to associations consistent with nephrotoxicity (increased serum creatinine);[35] to associations contrary to that expected with nephrotoxicity (i.e., lower serum creatinine and/or higher creatinine clearance with higher exposure).[36–41] In the studies in Table 4 in which NAG was included, no significant associations were observed.

In the data herein, increased *ln*-urine uranium was associated with decreased measured creatinine clearance and increased NAG, but only if urine concentration adjustment involved the use of urine creatinine and not if total uranium excreted (μg/4 h) was used as the exposure metric. This inconsistency is all the more perplexing as creatinine adjusted urine uranium (μg/g creatinine) was highly correlated with total uranium excreted (μg/4 h); thus, consistent results among these exposure metrics would be expected. This suggests that the significant associations observed may be statistical aberrations due to the use of urine creatinine in both exposure and outcome metrics. Urine uranium concentration (μg/l) is directly divided by the 4 h urine creatinine concentration (g/l) when entered as μg/g creatinine; the equivalent effect is achieved when both variables are entered separately into the model. The same urine creatinine value was also used to calculate measured creatinine clearance: ((urine creatinine (expressed as mg/dl for this equation) × urine volume in mL)/serum creatinine in mg/dl)/collection time in minutes. Futhermore, NAG was directly divided by the spot urine creatinine, which was correlated with the 4 h urine creatinine, as noted above. Thus, inverse and positive associations of urine uranium concentration with measured creatinine clearance and NAG, respectively, are potentially consistent with the use of urine creatinine in these variables. Supporting this hypothesis is the fact that the associations of excreted uranium (μg/4 h) with measured creatinine clearance and NAG remain non-significant when urine creatinine is added to those models. However, removing urine creatinine from the model of measured creatinine clearance in which it is added as a covariate for urine concentration adjustment reduces the association but does not completely attenuate it.

Similarities in renal handling of urine proteins used as kidney outcome markers and protein bound metals were recently implicated as causal factors for observed associations between

**Table 4.** Published associations between uranium exposure and glomerular filtration measures.

| *Reference/location Study population* | *Study design* | *Exposure assessment* | *Filtration measures and findings* |
|---|---|---|---|
| Thun et al. ,[36] Colorado Current and former uranium mill workers ($n=39$) with at least 1 year of work and cement plant worker controls ($n=36$) | $\chi^2$-Test used to examine differences between exposed and control participants for dichotomous variables. Matched and unmatched Student's *t*-test (matched on race, sex, age) used for continuous variables | 27 Mill workers exposed to "yellowcake", a powder containing salts and oxides of natural uranium including uranium diuranate; 12 workers in the crushing area exposed to less soluble uranium compounds<br>Mean blood lead ($\mu$mol/l) in exposed was 0.45 *vs* 0.78 in controls ($P<0.001$); mean blood cadmium (nmol/l) in exposed was 31.2 *vs* 24.7 in controls ($P>0.05$). Uranium biomonitoring is not obtained | Serum creatinine; 8-h urine collection for creatinine clearance (standardized to body surface area)<br>Serum creatinine was lower ($P=0.02$) and measured creatinine clearance was higher ($P=0.04$; matched analysis) in the exposed group; these differences are in the opposite direction from that expected in nephrotoxicity. The authors note that, in the control group, creatinine clearance was correlated with blood lead ($r=0.32$, $P=0.057$). |
| Kurrtio et al.,[31] Southern Finland<br>General population (ages 15–82) currently drinking well water in an area of known high uranium water concentrations ($n=325$)<br>Persons with diabetes excluded ($n=4$) | Urine uranium in $\mu$g/mmol creatinine used in generalized linear regression models that adjusted for age, sex and BMI<br>Adjustment for duration of uranium exposure, education, occupation, analgesic use or smoking (never, ex-smoker or current smoker) in linear models did not affect uranium exposure associations with kidney functions and were not used as covariates. | Oral exposure to natural uranium via drinking water. Wells used as main source of drinking water; mean = 13 years (range: 1–34 years). Uranium in drinking water: median = 28 $\mu$g/l (interquartile range (IQR) 6–135, maximum 1920 $\mu$g/l)<br>Overnight urine samples for uranium measurement (median collection time = 11 h, range 7–17 h). Uranium in urine: median 13 ng/mmol creatinine (IQR: 2–75 ng/mmol creatinine)<br>Median daily uranium intake of 39 $\mu$g (IQR: 8–224 $\mu$g) | No association of measured creatinine clearance (standardized to body surface area) with any of the four uranium measures: urine uranium (measured on the same overnight urine samples used for creatinine clearance); uranium in drinking water; daily or cumulative intake from drinking water (daily intake $\times$ duration of water consumption).<br>Serum creatinine obtained but not modeled. |
| Pinney et al.,[35] Fernald, Ohio<br>Residents who lived within 5 miles of a uranium processing plant for at least 2 years from 1/1/52–12/18/84 ($n=8464$) | Linear regression adjusted for age and sex | Oral exposure to natural uranium via drinking water and to various uranium types from the plant resulting in inhalation via airborne emissions and oral exposures via contaminated food and drinking water<br>Exposure variables: (1) residence proximity to plant ($\leq$2 miles *vs* >2 miles); (2) water source (well/cistern or municipal); (3) in the direction of groundwater run-off | Increased serum creatinine in residents living within 2 miles of the plant compared with residents living greater than 2 miles from plant |
| Kurrtio et al.,[32] Southern Finland<br>Subset of Kurrtio et al.[31] ages 18–81 who still used the same well for drinking water ($n=193$) | Log-transformed urine uranium variables used in linear regression models that adjusted for age (in models of serum cystatin C) and analgesic use (in models of measured creatinine clearance) | Oral exposure to natural uranium via drinking water. Wells used as main source of drinking water; mean = 16 years (range: 5–40 years). Uranium in drinking water: median = 25 $\mu$g/l (IQR: 5–148 $\mu$g/l; maximum, 1500 $\mu$g/l)<br>Overnight urine samples for uranium measurement as in Kurrtio et al.[31] (median collection time = 8 h, range 5–12 h). Uranium concentrations in urine stated to be 44% greater on average than in Kurrtio et al.[31] mean/median values not provided<br>Uranium dose biomarkers also included hair and toenail uranium concentrations | No association of urine uranium ($\mu$g/l) with serum cystatin C or measured creatinine clearance (standardized to body surface area and measured in overnight urine collection). Authors state results similar with creatinine correction of urinary uranium as well as for other uranium exposure indicators: uranium in drinking water, hair, toenails and daily and cumulative uranium intake (data were not shown).<br>Serum creatinine obtained but not modeled. No association with NAG. |
| Hooper et al.,[30] McDiarmid et al.[33,34,37–41]<br>Multiple follow-ups of Gulf War Veteran cohort exposed to depleted uranium (DU) munitions by "friendly-fire" in 1991<br>*N* for high group ranged from 10–14 and for low group from 18–53 | Differences in outcome measures between high low urine uranium exposure groups examined using Mann–Whitney *U*-test. Primarily unadjusted due to small sample size.<br>Authors note *P*-values <0.2 reflecting lack of power due to small sample size | DU exposures include: acute inhalation exposure to DU particles; acute dermal exposure to DU particles in wounds, and chronic endogenous exposure to embedded DU shrapnel<br>24-H urine collection for uranium and creatinine ($\mu$g/g creatinine)<br>Participants divided into high and low uranium exposure groups based on urine uranium level cutpoint of 0.10 $\mu$g/g creatinine. With and without shrapnel dichotomy used in 1999 | Serum creatinine: lower in high exposure group*: 2001, 2004, 2006, 2009, 2011 ($P=0.14$; 0.03; 0.11; 0.11; 0.02)<br>No difference: 1999, 2000, 2007<br>Calculated creatinine clearance: not significantly different: 2007; 2009—mean ~ 20% higher in high exposure group* although $P=0.44$<br>MDRD GFR: higher in high exposure group*: 2009—mean ~ 20% higher ($P=0.06$); 2011 ($P=0.2$)<br>*Inconsistent with traditional nephrotoxicity pattern<br>No difference in NAG: 2006, 2007, 2009, 2011 |

the two.[42] In addition to glomerular filtration, creatinine is secreted in the proximal tubule of the kidney; organic cation and anion transporters are involved in this process.[43–45] Uranium is a proximal tubular toxicant, however, its transport mechanisms are not well defined. Recent data suggest that a sodium-dependent phosphate co-transporter may be involved.[46] Thus, currently available information does not allow a determination of the possible role of kidney processing in our results.

To our knowledge, there are no published studies that have compared results among creatinine-based methods of adjustment for urine concentration and total uranium excreted ($\mu$g/4 h). However, Kurrtio et al.[32] found no association between creatinine unadjusted urine uranium ($\mu$g/l) and NAG, serum cystatin C or measured creatinine clearance, noting that results were similar when using creatinine-corrected urinary uranium ($\mu$g/g creatinine) and also when using uranium exposure measures that did not

require adjustment for urine concentration (e.g., uranium in hair and toenails). Thus, their results are not consistent with our findings. Other evidence also indicates that the statistical hypothesis, if applicable, does not affect all urine biomarkers uniformly. Urine creatinine-adjusted thallium, cadmium and antimony were not associated with measured creatinine clearance in previous analyses of the lead workers herein although associations with other creatinine-based kidney outcomes were observed.[8,9] Furthermore, the most common study design in nephrotoxicant research is analysis of associations between urine toxicants and kidney early biological effect markers, also measured in urine. Most adjust for urine concentration using urine creatinine and such publications routinely report some null associations. In addition, when urine creatinine was removed from the two significant models in Table 3, Model 1, uranium remained associated, although attenuated, in the measured creatinine clearance model. Our group has published three recent articles in the lead worker population in which the directions of urine metal associations have been unexpected.[8–10] Similar results have been observed with urine cadmium[47] and other urine exposure measures in recent NHANES analyses.[48] Overall, these recent results suggest that the use of urine biomarkers is more complex than has been previously appreciated.

In conclusion, we found significant associations between urine uranium and measured creatinine clearance and NAG in models that used urine creatinine to adjust for urine concentration, but not in those using the total uranium excreted metric ($\mu$g/4 h), an approach to adjust urine biomarkers for urine concentration that is less commonly employed in occupational and environmental studies due to the need for a timed urine collection. Considered in isolation, the results obtained with urine creatinine adjustment suggest uranium nephrotoxicity. However, when reviewed in conjunction with results from the excreted uranium ($\mu$g/4 h) models, alternative interpretations must be considered, although the more limited attenuation observed with NAG could still be interpreted as supportive of nephrotoxicity. Our results suggest that some associations between urine toxicants and kidney outcomes may be statistical, due to the use of urine creatinine in both exposure and outcome metrics, rather than nephrotoxic. These findings support consideration of non-creatinine-based methods of adjustment for urine concentration as well as non-urine based exposure measures (e.g., blood levels) in nephrotoxicant research. Additional studies that collect urine samples over a range of times, such as spot, overnight and 24-h, and examine the utility of various urine concentration adjustments, for example, urine creatinine, specific gravity and osmolality, as recently published by Akerstrom et al[49] are essential in this regard.

## ABBREVIATIONS

BMI, body mass index; Cd, cadmium; Cr, creatinine; CV, coefficient of variation; DU, depleted uranium; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; ICP-MS, inductively coupled plasma–mass spectrometer; IQC, internal quality control; *ln*-, natural logarithm; MDL, method detection limit; MDRD, Modification of Diet in Renal Disease; NAG, *N*-acetyl-$\beta$-D-glucosaminidase; NHANES, National Health and Nutrition Examination Survey; NIST, National Institute of Standards and Technology; NU, natural uranium

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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