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Temporal variability of urinary cadmium in spot urine samples and first morning voids

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Abstract

Cadmium is a carcinogenic heavy metal. Urinary levels of cadmium are considered to be an indicator of long-term body burden, as cadmium accumulates in the kidneys and has a half-life of at least 10 years. However, the temporal stability of the biomarker in urine samples from a non-occupationally exposed population has not been rigorously established. We used repeated measurements of urinary cadmium (U-Cd) in spot urine samples and first morning voids from two separate cohorts, to assess the temporal stability of the samples. Urine samples from two cohorts including individuals of both sexes were measured for cadmium and creatinine. The first cohort

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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(Home Observation of Perinatal Exposure (HOPE)) consisted of 21 never-smokers, who provided four first morning urine samples 2–5 days apart, and one additional sample roughly 1 month later. The second cohort (World Trade Center-Health Program (WTC-HP)) consisted of 78 individuals, including 52 never-smokers, 22 former smokers and 4 current smokers, who provided 2 spot urine samples 6 months apart, on average. Intra-class correlation was computed for groups of replicates from each individual to assess temporal variability. The median creatinine-adjusted U-Cd level (0.19 and 0.21 $\mu\text{g/g}$ in the HOPE and WTC-HP, respectively) was similar to levels recorded in the United States by the National Health and Nutrition Examination Survey. The intra-class correlation (ICC) was high (0.76 and 0.78 for HOPE and WTC-HP, respectively) and similar between cohorts, irrespective of whether samples were collected days or months apart. Both single spot or first morning urine cadmium samples show good to excellent reproducibility in low-exposure populations.

Keywords

analytical methods; biomonitoring; metals

INTRODUCTION

Cadmium is a toxic metal that has been implicated in the occurrence of kidney disease, bone disease, and cardiovascular and lung cancer, and has been suggested to be associated with a number of other health outcomes.^{1,2} Being able to reliably quantify exposure is essential for epidemiologic studies of cadmium. Cadmium has been measured in human urine worldwide and is typically adjusted by the creatinine measured in the same samples. Urinary cadmium (U-Cd) is considered a biomarker for long-term cadmium body burden in people, because it accumulates in the renal tubular cells where it is transported by metallothionein.³ Occupational studies have revealed a curvilinear relationship between U-Cd and cadmium in kidney tissue, indicating that the overall body burden could be measured noninvasively using U-Cd.^{4,5} Research on U-Cd concentration has indicated that levels are (1) increased with age, reflecting chronic dietary exposure, (2) higher in smokers and ex-smokers than in non-smokers, as a result of cadmium present in the tobacco,^{6–11} and (3) higher in women than in men, most likely reflecting increased absorption due to lower iron levels.^{12–16} In addition, U-Cd concentrations are often higher in occupationally exposed populations. For example, the creatinine-adjusted U-Cd (U-Cd_{cr}) concentration in battery workers may exceed 10 $\mu\text{g Cd/g creatinine}$,⁶ levels much higher than that observed in general Western population samples (<2 $\mu\text{g Cd/g creatinine}$).⁷

Although U-Cd concentration serves as an important biomarker for disease states and a powerful tool for cadmium epidemiology, studies of intra-individual short-term stability of U-Cd_{cr} are equivocal. A handful of studies have examined temporal variability in U-Cd_{cr} and report ICC coefficients ranging from 0.50 to 0.89 (refs 10, 17–21). ICCs are highest for first morning void samples or 24 h samples measured within a few days of each other;²⁰ however, for samples collected months to up to a year apart, ICCs are reported from 0.50 to 0.75, regardless of the type of sample collected.^{10,17,21} It is not clear why these studies are generating different results. One plausible explanation could be analytic measurement error,

which may be especially important at these lower concentrations of U-Cd, where molybdenum oxide interference may be a factor.²² Additional studies are needed to quantify the intra-individual variability of U-Cd_{cr}, with a primary focus on precise measurement in both spot urine samples vs first morning voids, which are most commonly available in biobanks of large cohort studies.

In this study, we report ICCs in repeated measures of U-Cd_{cr} in two geographically distinct populations in the United States. We also examine factors that can potentially account for the intraclass variability.

METHODS

Samples were provided from two cohorts: the Home Observation of Perinatal Exposure (HOPE) Cohort in Utah and a cohort of responders to the 9–11 World Trade Center (WTC) disaster monitored at the Stony Brook University World Trade Center-Health Program (WTC-HP). The overall study design is shown in Supplementary Figure 1.

HOPE Cohort

We obtained urine samples and demographic data from 21 never-smoking adults, 9 males and 12 females, who were trying to conceive and enrolled in the HOPE study at the University of Utah. Males and females provided daily first morning void urine samples during the fertile window for up to two menstrual cycles of the female partner. We selected four samples 2–5 days apart from the first cycle and one from the second cycle for U-Cd analysis. For each participant there was no more than one non-first morning void urine sample among the five analyzed samples. Demographic data such as self-reported age, sex, and measured height and weight were also obtained from all participants. Body mass index (BMI) was calculated as weight (kg)/ height² (m²). The study was approved by the institutional review board of the University of Utah (IRB authorization 50740).

WTC-HP Cohort

We obtained 2 fasting spot urine samples from 78 adults without known diagnosis of kidney disease, diabetes, or cancer, enrolled at the WTC-HP on Long Island (NY).²³ Participants were asked to fast for 12 h before visiting the clinic by avoiding intake of all solid and liquid foods or beverages, with the exception of water. All samples were obtained between 0700 and 1300 h. Self-reported basic demographic information including age, sex, parity (women only), height, weight, ethnicity, smoking status, alcohol and smoking history, and second-hand smoking history were collected via a self-administered questionnaire. Height and weight were used to calculate BMI.

Each participant self-reported the frequency at which each of 65 foods or beverages, belonging to 16 food types, were consumed during the 3 days preceding the urine collection (Supplementary Table 1).

The study was approved by the institutional review board of Stony Brook University (IRB authorization 2013–2068).

Cadmium and Creatinine Measurement

Urine samples were collected in metal-free polypropylene cups (BD 354013, Franklin Lakes, NJ, USA) or polypropylene UROLEX cups (4 oz, Medical Action Industries, Mechanicsville, VA, USA) and then aliquoted in 15 ml polypropylene metal-free centrifuge tubes (VWR 89049-172, Radnor, PA, USA) or sterile, self-standing graduated polypropylene storage tubes (50 ml; 6256, Globe Scientific, Paramus, NJ, USA) for archiving. All samples were refrigerated for a maximum of 72 h after collection and then frozen at approximately -8°C or lower. Samples were never in contact with any metallic equipment and suppliers guaranteed that cadmium was not used in the manufacturing of collection and storage materials. However, the non-metal-free UROLEX collection cups and storage tubes used in the Utah cohort were screened for background Cd contamination. Representative cups of the type used for sample collection were filled with either deionized water ($18\text{ M}\Omega\text{ cm}^{-1}$, Pure Water Solutions, Raleigh, NC, USA) or diluted (1%) nitric acid (Optima purity, Thermo-Fisher Chemical, Waltham, MA, USA) for extraction of trace metal contaminants, which was then analyzed by inductively coupled plasma mass spectrometry (ICP-MS). In all cases, background Cd was not detected. The same products (brand and part number) have been used throughout the study, although multiple lot numbers were used for sample collection. Only trace-metal analysis grade pipette tips (VWR) were used for sample aliquoting and handling. U-Cd was measured by ICP-MS by the Analytical Sciences Laboratory at RTI International (RTP, NC, USA). Urine samples were blinded to analysts and prepared for analysis by digestion on a graphite heating block digestion unit. Briefly, sample volumes of 0.50 ml were digested with a mixture of 0.10 ml of high-purity nitric acid and 0.050 ml of hydrochloric acid (Ultrex purity, J.T. Baker, Center Valley, PA, USA), and 0.10 ml of 30% hydrogen peroxide (non-tin-stabilized, EMD-Millipore, Chicago, IL, USA) at a temperature of 90°C for 30 min, spiked with an internal standard mixture of Bi, Pr, Sc, and Y (High Purity Standards, Charleston, SC, USA) at a nominal concentration of 10 ng/ml and diluted to a final volume of 5 ml with deionized water, and Pr was used as the analytical internal standard. National Institute of Standards and Technology Standard Reference Material 2668 (Toxic Metals in Frozen Human Urine) were prepared and analyzed alongside study samples as a quality control (QC). Additional details on the metal quantification method are available in the Supplementary Material.

For both the HOPE and WTC-HP cohorts, Cd limit of detection (LOD) ranged between 0.014 and $0.020\text{ }\mu\text{g/l}$ and the limit of quantification (LOQ) was $0.125\text{ }\mu\text{g/l}$. U-Cd was above the LOD in all samples, but in 39% and 21% of the samples from the HOPE and the WTC-HP cohort, respectively, U-Cd was measured below the LOQ. Values below the LOQ were still used in the analysis.

To help correct for differences in urine concentration, urinary creatinine concentrations were quantified using a Cayman Chemicals Creatinine Assay Kit No. 500701 (Cayman Chemicals, Ann Arbor, MI, USA) with UV-VIS measurement at 500 nm employing a Beckman Coulter DU800 UV/VIS Spectrometer (Beckman Instruments, Brea, CA, USA).

At least 5% of samples were randomly selected from all batches and retested for both Cd and creatinine values to verify the accuracy of analytical results. A percent change below 20% was considered acceptable. One batch of creatinine tests demonstrated a re-test percent

change consistently above 20% and therefore the analysis was repeated for the entire batch. At least 5% of samples in the repeated measurements' batches were again repeated to confirm accuracy.

We applied three different criteria for retaining samples in the analysis based on “normal” creatinine levels: an inclusive criterion in which no samples were excluded, a “relaxed” criterion in which we removed samples with urinary creatinine levels outside of 2.5–97.5% percentile ranges measured in a 45,000 person cohort (129–2690 mg/l in women and 204–3520 mg/l in men),²⁴ and a “stringent” criterion in which we excluded samples with creatinine outside of 300–3000 mg/l, regardless of sex.²⁵

Statistical Analysis

U-Cd_{cr} was log-transformed to account for non-normal distribution. Mann–Whitney *U*-test was used to compare distributions across cohorts or different groups within the same cohort. Multiple linear regression models were used to assess the correlation between log-transformed levels of the participant's mean U-Cd_{cr} (the dependent variable) and demographic or experimental characteristics (age, sex, smoking status, second-hand smoke exposure, BMI, number of pregnancies, time of sampling, and visit or sample number) as the independent variables. β -Parameters were then back-transformed to express the percent changes of the U-Cd_{cr} with respect to one-unit increments of each independent variable (U-Cd_{cr} percent change = $((e^{\beta}) - 1) * 100$). The second-hand smoke exposure variable is defined as the number of years of self-reported exposure during adult age (>18 years) at either home or work, whichever was higher. Similar models were run using U-Cd as the dependent variable and creatinine as one of the predictor variables, and results were consistent and therefore are not reported here.

ICC was calculated according to Shrout and Fleiss,²⁶ with ICC defined as in ref. 1, where σ_b^2 and σ_w^2 are the between-individual variance and the within-individual variance, respectively.

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2} \quad (1)$$

In order to determine short, medium, and long-term U-Cd_{cr} variability, the ICC calculations were done on three sets of data: (i) HOPE cohort, visits 1–4 (includes all values from the first 4 visits, average time span of 6 days), (ii) HOPE cohort, visits 1 and 5 (includes values from visits 1 and 5, average time span of 35 days), and (iii) WTC-HP cohort, visits 1 and 2 (includes all values from both visit, average time span of 180 days).

The difference in U-Cd_{cr} between visits was expressed two ways using the absolute value of: (i) the absolute difference ($\pm$ SD) in U-Cd_{cr} between two consecutive visits or (ii) the relative difference (also known as the percent change) in U-Cd_{cr} between those two visits. Both samples from WTC-HP were used and only samples 1 and 2 were chosen from the HOPE study for these calculations. In accordance with the criteria presented by Rosner,²⁷

we considered the reproducibility to be good when $0.40 \leq \text{ICC} < 0.75$ and excellent when $\text{ICC} \geq 0.75$.

To investigate factors influencing variability of U-Cd_{cr} , we used a forward selection procedure for the WTC-HP cohort, as follows. Suppose y_{1l} and y_{2l} were log-transformed U-Cd_{cr} on visit 1 and visit 2, respectively, the new dependent variable was absolute value of $y_{2l} - y_{1l}$. As independent variables, we used the difference in time of sampling, number of days between visits, and difference in frequency of consumption of foods or beverages (Supplementary Table 1) self-reported before the visit. The frequency of consumption of a specific food/beverage item was calculated in two ways, either as the frequency (i) over the 3 days preceding the visit or (ii) on the day before the visit. Demographic variables such as sex, age, and so on remain constant at the two visits; thus, they had no effect on within-person variability. At the time of the study there were only four individuals currently smoking and therefore the number of cigarettes recently smoked was not an influential variable in this analysis.

All statistical analyses were performed using SAS 9 (Cary, NC, USA). To calculate ICC, we used a SAS 9 macro that allows for covariate adjustment.²⁸

RESULTS

Descriptive Statistics and Urinary Markers

Demographic characteristics are summarized in Table 1. The respective mean (min–max) age for the WTC-HP and HOPE cohorts was 31 (26–38) and 50 (38–65) years. Participants in the WTC-HP had higher BMI than HOPE participants and included former and current smokers, in addition to never-smokers.

Median concentrations of urinary markers, stratified by age, smoking status, sex, BMI, and sample number, are reported in Table 2. On average, the mean U-Cd_{cr} was not significantly different between the WTC-HP and the HOPE cohort ($U\text{-test } P=0.23$). U-Cd_{cr} increased with age and was higher among females in the WTC-HP cohort ($U\text{-test } P<0.01$) but not in the HOPE cohort ($U\text{-test } P=0.30$). In addition, current smokers had higher U-Cd_{cr} levels than former smokers who in turn had higher levels than never-smokers ($U\text{-test } P=0.04$ between current smokers and former/never-smokers). In WTC-HP, women who reported at least one pregnancy had a higher U-Cd_{cr} than women with zero pregnancies, although not significantly ($U\text{-test } P=0.18$). Participants with $\text{BMI} < 25$ had a significantly higher U-Cd_{cr} levels in the HOPE, but not in the WTC-HP cohort ($U\text{-test } P=0.02$ and $P=0.51$, respectively). Median values in different strata for U-Cd ($\mu\text{g/l}$) and creatinine (g/l) are presented in Supplementary Table 2.

Factors Associated with U-Cd_{cr}

In multiple regression models using the log-transformed mean U-Cd_{cr} for each individual, demographic and experimental parameters were examined in both cohorts (Supplementary Table 3). No significant correlation with age was observed in the HOPE cohort, where all participants were 26–38 years old. In the WTC-HP cohort, each additional year of age was associated with a 3% increase in U-Cd_{cr} . In sex-stratified analyses, this association with age

was significant in men ($P < 0.01$) but not in women ($P = 0.30$) (data not shown). Female participants exhibited significantly higher levels of U-Cd_{cr} in the WTC-HP (+101%, $P < 0.01$) but not in the HOPE cohort (+13%, $P = 0.25$), which consisted of nulliparous younger women (Supplementary Table 3). Each additional pregnancy was associated with a 20% increase in U-Cd_{cr} ($P < 0.01$). Higher levels of U-Cd_{cr} were measured in both former and current smokers compared with never-smokers (+54% and +115%, respectively, $P < 0.01$). Unadjusted regression models showed a 2% increase in U-Cd_{cr} for every additional year of second-hand smoke exposure during adult life ($P < 0.01$, data not shown), but when other covariates were added to the model (age, sex, smoking history, pregnancies, and so on), this association became nonsignificant ($P = 0.87$) (Supplementary Table 3). BMI was inversely associated with U-Cd_{cr} in the HOPE cohort ($P < 0.01$) but not associated in the WTC-HP cohort ($P = 0.27$). Little evidence was observed for an association between the time of sampling and U-Cd_{cr} levels ($P = 0.18$).

ICCs of Multiple Measurements of U-Cdcr

For the HOPE cohort, the overall unadjusted ICC (95% CI) for U-Cd_{cr} was 0.76 (0.61, 0.88) when taking into account only the first four measurements collected within a few days of each other and 0.66 (0.48, 0.81) when the fifth measurement collected a month later was also used. The ICC of samples from the WTC-HP cohort was 0.78 (0.67, 0.85) for the two measurements collected 6 months apart (Table 3). When we excluded urine samples because of high or low creatinine levels, there was a minimal impact on the ICCs (Supplementary Table 4).

In stratified analyses, the HOPE U-Cd_{cr} ICCs were generally lower when we took into account all five measurements (spanning two menstrual cycles, for women) than when only the first four measurements (all within one menstrual cycle, for women) were used (Table 3). ICCs were generally in the range of 0.6–0.8 across the different strata in both cohorts, suggesting that smoking status, sex, or age had little influence on the ICC. A few of the groups in the HOPE cohort had lower ICCs but the sample size was small ($n < 10$) and the lower ICCs were not replicated in the WTC-HP cohort. Overall, the ICCs we obtained for repeated spot urine measures, on average 6 months apart (WTC-HP), were comparable to, if not greater than, the ICC measures obtained from repeated first morning urine samples, collected within a few days of each other (HOPE cohort, samples 1–4).

Characterizing Intra-Individual Variability of U-Cdcr

Figure 1a demonstrates the distribution of mean U-Cd_{cr} values in the two cohorts using all five samples in the HOPE cohort and two samples in the WTC-HP cohort. Figure 1b and c show the distribution of the relative and absolute change between consecutive visits (samples 1 and 2 for both cohorts). The HOPE cohort included a larger percentage of people with lower absolute and relative change (Figure 1b and c). Overall, the frequency distributions in Figure 1 spanned a similar range between the HOPE and the WTC-HP cohorts. The three table insets in Figure 1 show the individuals with the highest values. The highest overall mean U-Cd_{cr} values came from the WTC-HP cohort, with four out of five participants being either former ($n = 3$) or current ($n = 1$) smokers. Those with the highest absolute change between two measurements also came from the WTC-HP cohort, with three

individuals that were never-smokers, one former and one current smoker. Those with high relative change included individuals from both cohorts, with mean U-Cd_{cr} both above and below the mean values in each cohort. There is some overlap across these three inset tables but there also are individuals who appear in only one of the tables. Additional results describing relative and absolute variability of U-Cd_{cr} across different sample strata are shown in Supplementary Table 5 and indicate that the mean absolute difference is 0.05–0.15 $\mu\text{g/g}$ and the mean relative difference is generally 30–40%, regardless of the cohort.

For the WTC-HP cohort, we investigated factors that might explain the absolute difference in U-Cd_{cr} across the two measurements; none of the experimental or dietary factors was statistically significant in regression models.

DISCUSSION

The current study reports limited temporal variability and good to excellent reproducibility in U-Cd_{cr} levels in two distinct populations. The first group had repeated first morning urine samples from 22 individuals collected within days (ICC = 0.76) or weeks (ICC = 0.66) of one another. The second group included fasting spot-urine samples from 78 individuals collected 6 months apart (ICC = 0.78). Although most individuals experienced changes in their U-Cd_{cr} not larger than 0.10 $\mu\text{g/g}$, a few participants exhibited greater variability between time points (Figure 1). None of the dietary or experimental factors that we collected were able to account for the observed temporal variability.

A recent Spanish study reported ICC = 0.72 in U-Cd_{cr} from 83 individuals using first morning urine samples collected a year apart; levels of U-Cd_{cr} were comparable to our study.¹⁹ A Swedish study²⁰ investigating U-Cd_{cr} in samples collected 1 week apart reported ICC = 0.70 for spot urine samples, results which are comparable to the ICC values reported here, and a higher ICC for first morning void samples (ICC = 0.89). The mean cadmium levels reported by the Swedish group were lower than in our study, 0.09 vs 0.19 $\mu\text{g/g}$ in men and 0.16 vs 0.44 $\mu\text{g/g}$ in women for spot urine samples, and 0.08 vs 0.24 $\mu\text{g/g}$ in men and 0.17 vs 0.20 $\mu\text{g/g}$ in women for first morning void samples. The levels of U-Cd_{cr} we report here (geometric means 0.20–0.23 $\mu\text{g/g}$ in HOPE and WTC-HP, respectively) are comparable to those observed in the US NHANES 2011–2012 (geometric mean 0.18 $\mu\text{g/g}$).¹ A previous study conducted in the United States (141 women sampled 3–9 months apart)¹⁰ reported similar U-Cd_{cr} levels, but the ICC was lower (0.50) than that reported in our samples. The authors had indicated that their samples were not collected explicitly for trace metal analysis and thus there may have been potential for contamination of the urine collection containers.¹⁰ The most recent study on this topic was conducted on 11 men in China¹⁷ and reported an ICC of 0.68 for first morning void and 0.53 for spot samples. Although the authors do not explicitly note what measures were taken to account for MoO interference, it should be noted that the method they indicate they used was reported in an earlier publication (not referenced by the Chinese study) to use He gas in the collision cell.^{22,29} The measures of interference correction (in particular, the gas used in the Chinese study) would be noteworthy, because they report highly variable urinary Mo in the same samples, which could have had an effect on the accuracy of their results.¹⁷ Our analysis included re-preparation and analysis of at least 5% of all samples, and the use of 10% H₂/He gas in the

collision cell allowed for removal of MoO interference. Our results demonstrated high reproducibility, even at these low levels of U-Cd_{cr} ($P = 0.90$).

Other previous studies of temporal variability in U-Cd_{cr} relied on populations with mean levels $>1 \mu\text{g/g}$, with some as high as $10 \mu\text{g/g}$.^{21,30,31} Those studies tended to show lower correlation across repeated samples ($P=0.6-0.7$),²¹ relative difference $\sim 100\%$,³¹ and a coefficient of variation $\sim 35\%$.³⁰ However, as the levels of U-Cd increase, there is greater likelihood of renal impairment, which is known to influence excretion of cadmium.²⁵ Taken as a whole, the literature points towards relatively strong temporal stability of U-Cd_{cr} from either spot urine samples or first morning voids when levels are below $1-2 \mu\text{g/g}$, over a period of at least several months.

In comparing our two sets of samples (HOPE and WTC-HP), measures of temporal stability were similar even though the HOPE samples were first morning urines collected days or weeks apart and the WTC-HP samples were spot urines in fasting patients, collected on average 6 months apart. The distribution of the relative and absolute differences in U-Cd_{cr} levels for repeated samples were similar as depicted in Figure 1. ICCs were comparable whether samples were collected days apart (ICC = 0.76) or 6 months apart (ICC = 0.78); the ICC dropped in the HOPE cohort when samples were collected weeks apart (ICC = 0.66) but the CIs continued to overlap.

In accordance with previous reports,^{12,25,31} higher levels of U-Cd_{cr} were associated with age and being female in the WTC-HP cohort (Supplementary Table 3). However, age and being female were not associated with U-Cd_{cr} in the HOPE cohort, although the age range was tight (26–38 years) and none of the women had experienced childbirth. Others have shown that the increase in cadmium body burden for females is highly influenced by parity;¹² we also see that the levels of U-Cd_{cr} in women were associated with the number of pregnancies in the WTC-HP cohort. We also report an inverse association between U-Cd_{cr} levels and BMI, in accordance with previous reports,³² which could be at least partially a result of the relationship between BMI, muscle mass, and urinary creatinine.^{33–35}

Smoking was also associated with participants' mean U-Cd_{cr} in the WTC-HP cohort (participants from the HOPE cohort never smoked). Current smokers ($n = 4$) displayed a 115% increase in U-Cd_{cr} and former smokers displayed a 54% increase, compared with never-smokers. When we analyzed the smoking characteristics of the participants with the five highest mean U-Cd_{cr} values, we found that three were former smokers and one was a current smoker. Many studies have reported similar findings of elevated U-Cd_{cr} for current and former smokers,^{7–11} although a few studies reported no differences between former smokers and never-smokers.^{21,25,36} It is possible that this observation can be attributed to the number of years having smoked or the number of years elapsed since quitting; the former smokers in the WTC-HP cohort smoked for on average 18.23 ± 11.67 years and stopped smoking on average 16.5 ± 12.2 years earlier ($n=24$).

In examining the relative and absolute differences in U-Cd_{cr} between visits, a small number of individuals demonstrated sizeable changes ($>0.30 \mu\text{g/g}$). These results may hint at a role for recent exposures or other factors contributing to the changes in U-Cd_{cr}. However, we

examined changes in the dietary diaries up to 3 days before sample collection, as well as changes in time of day that the samples were collected, and we were unable to identify any association with the change in U-Cd_{cr}. However, the validity of food questionnaires in determining U-Cd levels is controversial.³⁷ Future investigations should attempt to shed light on why some individuals exhibit greater changes in their U-Cd_{cr} levels over time.

In interpreting data from exposure studies, it is important to note that temporal stability in U-Cd_{cr} levels does not necessarily imply that the biomarker directly reflects historical exposures. Recent reports have highlighted how the urinary excretion of Cd can be influenced by altered kidney functionality.²⁵ Further reports of co-excretion of cadmium and proteins in the urine^{36,38–40} might indicate why U-Cd has been associated with diseases characterized by increased albuminuria or proteinuria such as cardiovascular or bone diseases, even at very low levels of U-Cd.⁴⁰ These findings should warn researchers that even if levels of U-Cd_{cr} remain stable in healthy individuals for long periods of time.⁴¹ Hence, the risk for reverse causality exists in cross-sectional or case-control studies when U-Cd is measured when a disease is already present. Therefore, the reinforced observation that U-Cd_{cr} shows limited temporal variability, even when measured in spot urine samples several months apart, suggests that the ideal study design for epidemiologic investigation is the prospective cohort study, in which urine cadmium can be measured before onset of the disease of interest in banked samples, avoiding the use of samples collected at the same time as diagnosis.

This study has several strengths and advantages. First, samples were collected from two distinct populations, inclusive of both sexes and smoking status. Even though the WTC-HP cohort is represented by police, fire, and emergency first responders who were exposed to the 9–11 WTC disaster, their U-Cd levels were within the normal range of the general population¹ and the sample was limited to those without cancer, diabetes, or kidney disease. Therefore, observations from this study can, in principle, be translated to the non-occupationally exposed general population. Second, temporal variability in U-Cd_{cr} was monitored across both short and long time frames. Third, dietary intake variables as well as other demographic characteristics were collected in an attempt to isolate the factors contributing to the observed temporal variability. Fourth, the analysis was performed with consumables that were screened to minimize potential contamination and the analysis method included numerous QC samples to provide confidence in the accuracy of results both within and between analytical batches.

Despite the strengths of the study, there are also some potential areas for future optimization. First, we were unable to measure markers of kidney function (such as glomerular filtration rate or low-molecular protein excretion) or directly determine levels of Cd accumulated in the kidneys. It has been reported that changes in urinary function (urinary flow rate with or without body weight adjustment) can influence biomarker concentrations such as U-Cd concentration.⁴² It is possible that these factors may contribute to the observed temporal variability in the U-Cd_{cr} levels of some individuals, although creatinine correction should help mitigate this concern. It was also not possible to collect a 24-h urine sample, to compare with first morning and spot urine. However, few epidemiologic cohorts include 24-

h urine samples in their biobanks, justifying our focus on first morning and fasting spot urines.

CONCLUSIONS

In conclusion, we report that U-Cd_{cr} was relatively stable, showing good to excellent reproducibility across time points up to 6 months apart in individuals of both sexes. These findings are relevant to those with U-Cd_{cr} concentrations <1 µg/g and suggest that a single fasting spot or first morning urine sample is adequate for estimating urine cadmium in epidemiologic studies when collected before onset of disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Frequency distribution of (a) mean U-Cd_{cr}, (b) absolute, and (c) relative difference between samples 1 and 2 in the HOPE ($n=21$) and World Trade Center-Health Program (WTC-HP) ($n=78$) cohorts. The mean time difference between sample 1 and 2 was 1.8 ± 0.9 and 180 ± 34 days, respectively. Bars represent the percent frequency of values falling into respective bins, with respect to each cohort total n . For each panel the five participants with the largest values are highlighted in the insets and details are shown in the adjoining tables. (a) The mean of five and two visits is shown for HOPE (white bars) and WTC-HP (black bars) cohort, respectively. (b) The frequency of absolute difference, calculated as the absolute value of the U-Cd_{cr} at visit 2 minus the value at visit 1, is shown for the HOPE (white bars) and WTC-HP cohort (black bars). (c) The relative difference, calculated as the absolute value of the percent of value at visit 2 with respect to the value at visit 1, is shown for the HOPE (white bars) and WTC-HP cohort (black bars).

Table 1

Demographic information for the HOPE and WTC-HP cohorts.

	HOPE cohort	WTC-HP cohort		
	All (never-smokers)	Never-smokers	Former smokers	Current smokers
<i>n</i>	22	52	22	4
Men (<i>n</i> (%))	9 (41)	34 (65)	13 (59)	2 (50)
Age (years (min–max))	30.5 (26–38)	50.6 (38–61)	50.8 (43–65)	47.8 (42–55)
BMI (kg/m ² ±SD)	26.5±6.5	30.8±5.3	29.0±7.0	32.3±7.1
Smoking (pack-years±SD)	—	—	15.5±13.5	19.0±17.1
Second-hand smoke (years±SD) ^a	—	5.1±8.6	12.4±16.8	13.3±17.5
No. of pregnancies (mean±SD)	0	0.9±1.2	0.8±0.9	0.5±0.7

Abbreviations: BMI, body mass index; HOPE, Home Observation of Perinatal Exposure; WTC-HP, World Trade Center-Health Program.

^a Defined as the number of years of self-reported exposure during adult age (>18 years) at either home or work, whichever was higher.

Table 2Median (IQR) levels of U-Cd_{cr} measured in the HOPE and WTC-HP cohorts.

	<i>N</i>		U-Cd _{cr} (µg Cd/g creatinine)	
	HOPE	WTC-HP	HOPE	WTC-HP
All	22	78	0.19 (0.16)	0.21 (0.20)
<i>Age (years)</i>				
21–30	13	—	0.20 (0.16)	—
31–40	9	3	0.18 (0.14)	0.12 (0.05)
41–50	—	36	—	0.18 (0.16)
51–60	—	36	—	0.27 (0.27)
>60	—	3	—	0.27 (0.09)
<i>Smoking status</i>				
Never-smokers	22	52	0.19 (0.16)	0.17 (0.16)
Former smokers	—	22	—	0.30 (0.25)
Current smokers	—	4	—	0.55 (0.48)
<i>Sex</i>				
Males	9	49	0.17 (0.14)	0.16 (0.09)
Females	13	29	0.21 (0.16)	0.37 (0.33)
<i>Number of pregnancies^a</i>				
0	13	16	0.19 (0.16)	0.34 (0.31)
1–2	0	10	—	0.36 (0.41)
3	0	3	—	0.55 (0.29)
<i>BMI</i>				
<25	10	13	0.27 (0.25)	0.22 (0.35)
25–30	8	25	0.16 (0.10)	0.20 (0.17)
>30	4	40	0.13 (0.12)	0.20 (0.21)
<i>Urine sample #</i>				
1	22	78	0.18 (0.17)	0.22 (0.25)
2	22	78	0.20 (0.15)	0.20 (0.18)
3	22	—	0.20 (0.20)	—
4	22	—	0.16 (0.15)	—
5	22	—	0.21 (0.13)	—

Abbreviations: BMI, body mass index; HOPE, Home Observation of Perinatal Exposure; IQR, interquartile range; U-Cd_{cr}, creatinine-adjusted urinary cadmium; WTC-HP, World Trade Center-Health Program.

^aReported for females only.

Table 3

ICC (95% CI) of repeated measurements of U-Cd_{cr} in the HOPE and WTC-HP cohorts for total sample population and different strata.

U-Cd _{cr} change	HOPE, visits 1 through 4 ^a		HOPE, visits 1 and 5 ^b		WTC-HP ^c	
	n _{ind} (n _{obs}) ^d	ICC (95% CI)	n _{ind} (n _{obs}) ^d	ICC (95% CI)	n _{ind} (n _{obs}) ^d	ICC (95% CI)
All	22 (88)	0.76 (0.61, 0.88)	22 (44)	0.66 (0.48, 0.81)	78 (156)	0.78 (0.67, 0.85)
<i>Age (years)</i>						
21–30	13 (42)	0.79 (0.59, 0.90)	13 (26)	0.65 (0.41, 0.83)	0	—
31–40	9 (36)	0.67 (0.38, 0.87)	9 (18)	0.63 (0.35, 0.84)	3 (6)	0.63 (0.08, 0.97)
41–50	0	—	0	—	36 (72)	0.80 (0.65, 0.89)
51–60	0	—	0	—	36 (72)	0.72 (0.55, 0.85)
>60	0	—	0	—	3 (6)	0.00
<i>Sex</i>						
Males	9 (36)	0.73 (0.45, 0.92)	9 (18)	0.48 (0.20, 0.81)	49 (98)	0.69 (0.51, 0.81)
Females	13 (42)	0.72 (0.50, 0.89)	13 (26)	0.67 (0.44, 0.86)	29 (58)	0.58 (0.29, 0.78)
<i>Smoking status</i>						
Never	22 (88)	0.76 (0.61, 0.88)	22 (44)	0.66 (0.48, 0.81)	52 (105)	0.70 (0.55, 0.82)
Former	0	—	0	—	22 (44)	0.79 (0.59, 0.91)
Current	0	—	0	—	4 (8)	0.84 (0.37, 0.98)
<i>BMI^e</i>						
<25	10 (40)	0.78 (0.55, 0.91)	10 (20)	0.70 (0.45, 0.87)	10 (20)	0.75 (0.46, 0.92)
25–30	8 (32)	0.53 (0.22, 0.82)	8 (16)	0.33 (0.10, 0.70)	28 (56)	0.77 (0.58, 0.89)
>30	4 (16)	0.43 (0.08, 0.86)	4 (8)	0.35 (0.06, 0.82)	37 (74)	0.78 (0.63, 0.88)

Abbreviations: BMI, body mass index; CI, confidence interval; HOPE: Home Observation of Perinatal Exposure; ICC, intra-class correlation; WTC-HP, World Trade Center-Health Program.

^aShort-term variability. Time between visit 1 and 4 was on average 6 days. Includes all samples from HOPE visits 1 to 4.

^bMedium-term variability. Time between visit 1 and 5 was on average 35 days. Includes samples from HOPE visits 1 and 5.

^cLong-term variability, time between visit 1 and 2 was on average 180 days. Includes samples from both WTC-HP visits.

^dNumber of individuals (n_{ind}) and samples (n_{obs}).

^eBMI missing for three participants of the WTC-HP cohort.