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Cadmium and Alzheimer's Disease Mortality in U.S. Adults: Updated Evidence with a Urinary Biomarker and Extended Follow-up Time

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Abstract

Cadmium has been linked to impaired cognitive function in adults and may cause behavioral, physiological and molecular abnormalities characteristic of Alzheimer's disease (AD) in animals. Evidence linking cadmium and AD in humans is limited, but supportive. In the most recent epidemiologic study, blood cadmium in U.S. adults was positively associated with elevated AD mortality 7–13 years later. The association between urinary cadmium – an arguably more appropriate biomarker for studying chronic diseases – and AD mortality has not yet been explored. Further study of cadmium and AD mortality in an independent population, with longer follow-up, and stratified by sex is also needed. We sought to answer these questions using the U.S. National Health and Nutrition Examination Survey (NHANES) (1999–2006 cycles) and NHANES III (interviews in 1988–1994) datasets, separately linked to AD mortality as of 2011. We used survey-weighted Cox regression models predicting age at AD death and adjusted for race/ethnicity, sex, smoking status, education and urinary creatinine. An interquartile range (IQR; IQR=0.51 ng/mL) increase in urinary cadmium was associated with 58% higher rate of AD mortality (hazard ratio (HR) =1.58, 95% CI: 1.20, 2.09. p-value=0.0009, mean follow-up: 7.5 years) in NHANES 1999–2006 participants. In contrast, in NHANES III participants, an IQR (IQR=0.78 ng/mL) increase in urinary cadmium was not associated with AD mortality (HR=0.85, 95% CI: 0.63, 1.17, p-value=0.31, mean follow-up: 13 years). Also in the NHANES III sample however, when the maximum follow-up time was restricted to 12.7 years (i.e. the same as NHANES 1999–2006

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The National Health and Examination Survey (NHANES) was approved by the institutional review board of the National Center for Health Statistics. All NHANES data used in this study are publicly available.

participants) and urinary creatinine adjustments were not made, urinary cadmium was associated with elevated AD mortality (HR=1.11, 95% CI: 1.02, 1.20, p-value=0.0086). Our study partially supported an association between cadmium and AD mortality, but the sensitivity of results to follow-up time and creatinine adjustments necessitate cautious interpretation of the association. Further studies, particularly those on toxicological mechanisms, are required to fully understand the nature of the "cadmium-AD mortality" association.

Keywords

Cadmium; Alzheimer's disease; NHANES; Cognitive function; Heavy metal

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly and the 6th leading cause of death in the United States (Kochanek et al., 2016). Due to the global aging population structure, the prevalence of AD is increasing worldwide, and is projected to affect 13.8 million people (26% of people aged 65 years or older) in 2050 in the U.S. alone (Hebert et al., 2013). Despite this looming public health issue, little is known about AD prevention. A small subset of AD (~5%) is early-onset (before age 55) and has definitive genetic etiologies. The vast majority of AD is late-onset. For this type of AD, age and genetics are risk factors, while education, social engagement and physical activity have been implicated as protective factors (Mayeux and Stern, 2012).

Heavy metals are likely candidates for AD risk factors, because some metals are known to cause cognitive impairment, and many are able to interact with amyloid-beta, producing aggregates similar to neuritic plaques characteristic of AD brains (Roberts et al., 2012). Among the metals studied, cadmium has garnered considerable attention because of widespread exposures through occupation, smoking and foods. Since the 1990s, a number of studies have compared cadmium levels in the liver, blood, cerebral spinal fluids and brain tissues between AD patients and healthy subjects, but the results were inconclusive (Basun et al., 1991, 1994; Gerhardsson et al., 2009; Lee et al., 2012; Lui et al., 1990; Park et al., 2014; Szabo et al., 2015). A few larger epidemiologic studies, on the other hand, have consistently shown an inverse association between urinary or blood cadmium and cognitive functions in adults (Ciesielski et al., 2013; Gao et al., 2008; Viaene et al., 2000). Most recently, increased blood cadmium levels were associated with elevated AD mortality among participants aged 60 years or older in the National Health and Nutrition Examination Survey (NHANES) (1999–2002 cycles) (Min and Min, 2016).

While the study by Min & Min demonstrated an association between blood cadmium and AD, several questions remain. First, whether the association with AD mortality also exists for urinary cadmium is unknown. Urinary cadmium is a widely accepted marker of long-term cadmium exposure, which is an exposure timeframe potentially more relevant to chronic diseases such as AD. Second, the association between cadmium and AD has not been tested for replication in an independent population. Lastly, men and women are exposed to cadmium in different circumstances and they may metabolize cadmium

differently. Whether cadmium's association with AD differs by sex remains unexplored. We sought to answer these questions by first, relating urinary cadmium measured at NHANES baseline interviews (cycle 1999–2000 through cycle 2005–2006) to AD mortality in the next 5 to 13 years. This population is similar to the one Min & Min analyzed in terms of time period, population characteristics and cadmium exposure levels. As an additional verification, we also examined the association between blood cadmium and AD mortality in this population. We then addressed the replication question by testing the association between urinary cadmium and AD mortality in NHANES III participants. NHANES III participants were a representative sample of the US population in 1988–1994, and were generally exposed to higher levels of cadmium. This allowed us to test the cadmium and AD mortality association in another population, and also allowed for a longer mortality follow-up period, addressing the limitation of short follow-up duration Min & Min discussed in their study. Finally, we explored effect modification by sex in both the NHANES 1999–2006 population and the NHANES III population, for both urinary and blood cadmium. Our goal was to verify and expand current evidence on the association between AD mortality and cadmium, a widespread and potentially modifiable environmental exposure.

2. Methods

2.1. Study population

Our primary analysis used data from NHANES cycles 1999/2000 through 2005/2006; and our secondary analysis used data from the NHANES III survey conducted between 1988 and 1994. Both NHANES (1999–2006) and NHANES III are national surveys on the behavioral risk factors, environmental exposures and health status of a representative sample of the civilian, non-institutionalized U.S. population. Details about survey design, participant recruitment and survey contents can be found at the Centers for Disease Control and Prevention (CDC)'s website (http://www.cdc.gov/nchs/nhanes.htm).

For both primary and secondary analyses, participant inclusion criteria were 1) being at least 60 years old at NHANES interview, 2) having complete data on urinary cadmium/blood cadmium and core covariates (sex, race and smoking status) and 3) having known mortality status as of the end of 2011 through linkage to the National Death Index (a national database of death certificates). For the analysis of urinary cadmium and AD in NHANES 1999–2006, 2028 participants were at least 60 years old at interviews and had urinary cadmium data. After excluding 1 participant due to unknown mortality status, and 4 due to missing smoking status, 2023 were included in the analytic sample. For the analysis of blood cadmium and AD in NHANES 1999–2006, 6157 participants were at least 60 years old at interviews and had blood cadmium data. After excluding 3 participants due to unknown mortality status, and 13 due to missing smoking status, 6141 were included in the analytic sample. Finally, for the analysis of urinary cadmium and AD in NHANES III, 5056 participants were at least 60 years old at interviews and had urinary cadmium data. Of these, 59 participants were excluded due to missing urinary creatinine. Additionally, 3 participants were excluded because their urinary cadmium (which was below the limit of detection) could not be estimated using our algorithm (details in the next section) due to missing education. The final analytic sample thus included 4994 individuals.

All participants of NHANES (1999–2006) and NHANES III provided informed consent consistent with the requirements of the National Center for Health Statistics Institutional Review Board. All data used in this analysis are publicly available.

2.2. Urinary and blood cadmium

Blood and urine samples were collected as part of the surveys' examination components. All equipment and containers used for specimen collection, shipping, storage and analysis were screened to ensure they were free of metal contamination. In NHANES 1999–2006, collected urine specimens were frozen and shipped to the Division of Laboratory of Sciences, National Center for Environmental Health, where urinary cadmium was measured by inductively-coupled plasma-mass spectrometry (ICP-MS) with an ELAN® 6100 DRC or an ELAN® DRC II (PerkinElmer, Norwalk, CT). In 1999–2002, urinary cadmium concentrations were corrected for molybdenum oxide interference using the equation "Corrected cadmium = measured cadmium – [(0.00175 measured molybdenum oxide) – 0.0136]" by the National Center for Health Statistics (NCHS); in 2003–2006, no equation was applied because the laboratory-reported values were already corrected for interference. The limit of detection (LOD) for urinary cadmium was 0.057 ng/mL from 1999 to 2002, either 0.057 ng/mL or 0.042 ng/mL in 2003–2004 and 0.042 ng/mL in 2005–2006. Of the 2023 urine samples, 33 (1.7%) had urinary cadmium below the LOD and 2 had urinary cadmium set to 0 by NCHS because of negative values arising from molybdenum correction. For these 35 observations, instead of letting their urinary cadmium equal to LOD/ or 0 as provided by NHANES, we decided to replace these values by the median cadmium estimated from a weighted accelerated failure time (AFT) model, where cadmium was assumed to follow a log-normal distribution, was left-censored at the LOD (or LOD corrected for molybdenum in 1999–2002), and was a function of race/ethnicity, sex, smoking status, education and urinary creatinine (i.e. the same set of covariates included in the model predicting AD mortality). We used the AFT model-based method because recent literature (Kong and Nan, 2016; Nie et al., 2010) shows that the simple substitution method using a constant such as LOD/√2 is biased. The single imputation procedure yields less biased results than the simple substitution method (Atem et al., 2017). In this article we used the AFT model (a Tobit model) to impute the cadmium concentration when it was below the LOD. AFT model fitting was performed in SAS 9.3 (SAS Institute, Cary, NC).

For blood cadmium in NHANES 1999–2006, collected whole blood specimens were likewise frozen before analysis at the Division of Laboratory of Sciences, National Center for Environmental Health. From 1999 to 2002, blood cadmium was measured by atomic absorption spectroscopy with a PerkinElmer SIMAA 6000 spectrometer (PerkinElmer, Norwalk, CT). From 2003 to 2006, blood cadmium was measured by ICP-MS using ELAN series DRC spectrometers (PerkinElmer, Norwalk, CT). NCHS did not indicate the need to calibrate blood cadmium values obtained from the two measurement systems. The LOD from 1999 to 2002 and from 2005 to 2006 was 0.2 ng/mL and the LOD from 2003 to 2004 was 0.14 ng/mL. The blood cadmium levels of 226 (3.6%) participants were below the LOD and they were replaced with cadmium estimated using the same method described for urinary cadmium. Lastly, in NHANES III, urinary cadmium was measured by atomic absorption spectrometry with Zeeman background correction with a Perkin-Elmer model

3030 atomic absorption spectrophotometer (Norwalk, CT). The LOD was 0.03 ng/mL. 126 (2.4%) participants' urinary cadmium levels were undetectable, and these values were also replaced with cadmium estimated using the same method as their NHANES 1999–2006 counterparts.

2.3. Alzheimer's disease mortality

For both NHANES 1999–2006 and NHANES III participants, cases of AD mortality were identified from the NCHS 2011 Public-Use Mortality File linked to NHANES participants. Detailed methodologies for mortality status ascertainment and linkage can be found at the NCHS website (http://www.cdc.gov/nchs/data-linkage/mortality-methods.htm). Briefly, to build the mortality file, NCHS queried the National Death Index for participants' mortality status as of December 31st, 2011. The National Death Index is a centralized database of death certificate records from state vital statistics offices. In the mortality file linked to NHANES, the mortality status, leading cause of death and time from NHANES interview to death or end of follow-up were available. Leading causes of death were identified by NCHS using ICD-10 codes (for deaths after 1999) and ICD-9 codes (for deaths before 1999). We treated those who died of AD as cases, and those who died of other causes or were alive at the end of follow-up as censored observations. We calculated the age at AD death or end of follow-up for each participant, and used that as the outcome in survival analyses.

2.4. Covariates

The same set of covariates was considered for both primary and secondary analyses. Participants' age, smoking status, education level and family income at the time of survey participation and their sex, race/ethnicity were collected with questionnaires at survey interviews. Urinary creatinine, used to account for urine dilution, was measured using the Jaffe reaction with a Beckman Synchron CX3 Clinical Analyzer (Beckman Instruments, Inc., Brea, CA) in NHANES 1999–2006, and a Beckman Synchron AS/ASTRA Clinical Analyzer (Beckman Instruments, Inc., Brea, CA) in NHANES III. Family income was used to calculate the poverty income ratio (PIR), defined as the ratio of family income to the federally-determined poverty threshold. PIR was used to indicate individual socioeconomic status.

2.5. Statistical analysis

Both primary and secondary analyses followed the same analytic procedures outlined below. SAS 9.3 (SAS Institute, Cary, NC) was used for data management and R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) (mainly packages "Survey (version 3.30)" and "Survival (version 2.38)") was used for analysis. First, the distributions of all variables were checked to ensure all values were valid and to gain a preliminary understanding of the data prior to modeling. For descriptive purposes, survey-weighted means/geometric means (standard error/geometric standard error) were reported for continuous variables and survey-weighted proportions were reported for categorical variables. To model the association between cadmium and age at AD death, we used survey-weighted Cox models (also known as "inverse sampling probability weighted Cox regression"), implemented with the function "svycoxph" with the R package "Survey". The survey-weighted Cox model is an extension of the common Cox model and incorporates

complex survey design factors (clusters, strata, and weights) to produce parameter estimates. We chose to model age at AD death, instead of time since NHANES interview as the outcome because a prior simulation study showed that using “time on study” as the time scale could lead to bias in many situations (Thiébaut and Bénichou, 2004). We first fit a model with the cadmium marker only, and then progressively adjusted for potential confounders, including race/ethnicity, sex, smoking status and education. For models concerning urinary cadmium, we additionally adjusted for urinary creatinine to account for urine dilution. The proportional hazard assumption of all full models was checked with Schoenfeld residuals, and the functional forms of covariates were checked with Martingale residuals. Hazard ratios (HRs) and 95% confidence intervals (CIs) for an interquartile range (IQR) increase in urinary or blood cadmium were reported for all models. To explore potentially non-linear relationships between cadmium and log AD hazard ratio, we also modeled cadmium using natural splines with knots at the “5^{th}, 50^{th} and 95^{th} weighted percentiles”, the “10^{th}, 50^{th} and 90^{th} weighted percentiles” and the weighted quartiles in fully-adjusted models. We presented the results of the best-fitted model as dose-response curves.

Because by design, the follow-up duration for the NHANES III sample was much longer (up to 23 years versus up to 12.7 years for the NHANES 1999–2006 sample), we also repeated descriptive analyses and re-ran the Cox regression models in NHANES III after restricting follow-up time to a maximum of 12.7 years. This was achieved by recoding all AD deaths occurring after 12.7 years (i.e. 152 months) to “censored” and recoding their attained age to “baseline age plus 152/12”. For subjects that died of AD within 12.7 years of NHANES III interview, no recoding was performed. Lastly, for subjects that did not die of AD, their attained age was recoded to “baseline age plus 152/12”.

For sensitivity analyses, we checked for residual confounding in the primary analysis (NHANES 1999–2006) by additionally adjusting for PIR and blood lead (separately, not both at the same time) in full models. We also investigated the impact of a longer follow-up time for those who attended earlier NHANES 1999–2006 survey cycles (and were thus exposed to higher levels of cadmium) by adjusting for survey cycle in the full models. To evaluate the impact of left truncation and of the fact that cadmium was measured at different ages for different subjects, we additionally adjusted for baseline age in all full models.

Lastly, effect modification by sex was explored in both the primary (NHANES 1999–2006) and secondary analysis (NHANES III) by analyzing the association between cadmium and AD separately in men and women.

3. Results

3.1. Primary analysis (Cadmium and AD mortality in NHANES 1999–2006)

Subjects in both the urinary cadmium and blood cadmium samples were representative samples of the eligible US population in 1999–2006, and had similar overall distributions of age, follow-up time, sex, race/ethnicity, smoking status and education levels (Table 1). Participants in both samples were on average 71 years old at baseline, largely white (81%) and about 47% were never smokers. Study sample mean urinary (0.40 ng/mL) and blood

(0.51 ng/mL) cadmium levels suggest relatively low cadmium exposure in the general US population.

The distributions of urinary and blood cadmium in each sample followed well-known patterns (Table 2), except that urinary cadmium decreased with age and blood cadmium remained stable with age. In addition, women had significantly lower urinary cadmium levels than men, but the relationship reversed for blood cadmium. As expected, smoking (past or current) and having lower education levels were strongly associated with higher cadmium exposure.

Over a mean follow-up duration of 7.5 years (range: 0 to 12.7), 21 AD deaths occurred in the urinary cadmium sample (weighted AD risk=1.1%) and 76 AD deaths occurred in the blood cadmium sample (weighted AD risk=1.3%) (Table 1). Consistent with such low AD mortality, those who did not die of AD until the end of follow-up had similar characteristics as their respective total samples. In contrast, those who eventually died of AD were significantly older at baseline, more likely to be white and less likely to be current smokers than their non-AD counterparts. There was little sex difference between cases and non-cases. At baseline, AD cases had slightly higher urinary and blood cadmium than non-cases.

In Cox regression models (Table 3), an IQR increase in urinary cadmium (0.51 ng/mL) was associated with 56% higher AD mortality (HR (95% CI) = 1.56 (1.18, 2.07), p-value=0.0016) without adjusting for covariates. Adjustment for race/ethnicity and sex slightly reduced the magnitude of the association, but when smoking status was also included in the model the HR returned to 1.57 (95% CI: 1.20, 2.05). The HR remained essentially unchanged with further adjustment for education and urinary creatinine. In the fully-adjusted model, an IQR increase in urinary cadmium was associated with 58% increase in AD mortality (HR (95% CI) =1.58 (1.20, 2.09), p-value=0.0009).

Compared with urinary cadmium, the association between blood cadmium and AD mortality was weaker in magnitude but still positive (Table 3). Without considering confounders, an IQR increase in blood cadmium (0.36 ng/mL) was associated with 10% higher AD mortality (HR (95% CI) = 1.10 (0.91, 1.32), p-value=0.33). The HR increased with each successive adjustment for covariates. In the final model, an IQR increase in blood cadmium was associated with 22% increase in AD mortality (HR (95% CI) = 1.22 (1.01, 1.48)), and the association was significant (p-value=0.04).

For both urinary cadmium and blood cadmium, additionally adjusting the full models for blood lead, survey cycle and baseline age resulted in similar HR estimates (data not shown). When PIR was additionally included in the full models, the HR per IQR increase in urinary cadmium became slightly smaller (HR (95% CI) =1.52, (1.09, 2.11), p-value=0.01), but the HR for blood cadmium remained similar. Modeling urinary cadmium with natural splines revealed that among participants with urinary cadmium below the 99th percentile (2.6 ng/mL), the predicted log HR of AD mortality increased essentially linearly with urinary cadmium (Figure 1). The linear increase in log HR with increasing blood cadmium was less apparent (Figure 2), consistent with the weaker and less significant hazard ratio associated with blood cadmium when the biomarker was modeled linearly.

3.2. Secondary analysis (Cadmium and AD mortality in NHANES III)

Subjects in the NHANES III sample were comparable to those from NHANES 1999–2006 in terms of baseline age, sex, race/ethnicity and the distributions of urinary cadmium by covariates (Supplementary Tables 1 and 2). However, NHANES III subjects had higher levels of cadmium (geometric mean of urinary cadmium = 0. 60 ng/mL) than those from NHANES 1999–2006. During an average of 13 years (range: 0 to 23) of follow-up, 102 AD deaths occurred (weighted AD risk = 2.2 %). At baseline, the only distinction between AD mortality cases and non-cases was their older age. However, if we examined the AD deaths that occurred within the first 12.7 years of follow-up—the maximum follow-up duration in NHANES 1999–2006—the number of AD deaths reduced to 44 (weighted risk= 0.99 %) (Supplementary Table 3). Although the differences were not statistically significant, those who died of AD within the first 12.7 years from baseline had higher urinary cadmium and were more likely to be white. These patterns were similar to those observed in the NHANES 1999–2006 samples, and suggested that those exposed to higher cadmium likely died of AD earlier, while ethnic minorities died of AD later.

Using similar Cox regression models, we found that urinary cadmium was not associated with AD mortality over the entire NHANES III follow-up period from 1988–1994 to 2011 (i.e. 23 years) (Table 4). However, within the first 12.7 years of follow-up, an IQR increase in urinary cadmium was associated with 11% increase in AD mortality without adjustment for creatinine (HR (95% CI) = 1.11 (1.02, 1.20), p=0.0086). After adjusting for urinary creatinine, however, the HR decreased to 0.82 (95% CI: 0.59, 1.15) and was not statistically significant (p-value=0.23). Results were essentially the same when age was additionally adjusted in full models. Surprisingly, regardless of the duration of follow-up, urinary creatinine was an independent, significant or nearly significant predictor of elevated AD mortality (Per IQR (89.4 mg/dL) increase in urinary creatinine, HR over entire follow-up (95% CI) = 1.49 (0.93, 2.36), p-value=0.08; HR within the first 12.7 years (95% CI) = 2.22 (1.29, 3.80), p-value=0.003).

3.3. Effect measure modification by sex

There was little evidence for different “cadmium-AD mortality” associations between men and women (Supplementary Figure 1), although the HR associated with urinary cadmium was exceptionally high (HR=1.95 (1.21, 3.15), p-value=0.005) among men in the NHANES 1999–2006 sample. However, given the small number of male AD mortality cases in this sample (n= 12), and indeed within each sex stratum, it was difficult to draw any reliable conclusions about effect modification.

4. Discussion

We verified and expanded the current epidemiologic evidence on the association between cadmium and AD mortality by answering three questions. We found that, first, urinary cadmium was significantly associated with elevated AD mortality among participants of NHANES 1999–2006, supporting the positive association between blood cadmium and AD mortality reported by Min & Min. Second, cadmium was positively associated with AD mortality among NHANES III participants, an independent sample, under certain conditions

(within first 12.7 years of follow up and without creatinine adjustment). Lastly, we did not observe sex differences in cadmium's association with AD, but our analysis was limited by power. While our primary analysis supported an association between cadmium and AD mortality, certain intricacies in our results required cautious interpretation of the "Cd-AD mortality" association.

The first intricacy was that the magnitude of cadmium's association with AD mortality differed by cadmium exposure biomarker (urine versus blood measures). In our analysis in NHANES 1999–2006, a larger and more significant HR was observed for urinary cadmium. While the difference did not result in opposite conclusions, it did complicate interpretations of the NHANES 1999–2006 analysis. Our findings are a reminder that both urinary and blood cadmium are imperfect proxies for the underlying cadmium exposure of interest (for example, the total dose of cadmium at the target organ(s) over a lifetime), and care should be taken when deciding which biomarker to use as the "exposure". Traditionally, blood cadmium is thought to represent recent exposure, while urinary cadmium is believed to reflect long-term body burden (Adams and Newcomb, 2014). Under this assumption, an attenuation of the cadmium "effect" is expected with blood cadmium measures, because blood cadmium's variability with day-today exposure is equivalent to increased exposure measurement error. However, the view that blood cadmium represents mainly short-term exposure, while urinary cadmium represents long-term exposure has recently been challenged, especially in low exposure settings such as the U.S. (Bernard, 2016). While maintaining the prevailing interpretation of urinary cadmium versus blood cadmium, Adams and Newcomb also cautioned that a significant portion of current blood cadmium could reflect "long-ago" exposure. On the other hand, Chaumont et al. showed that at low exposure levels, urinary cadmium might not necessarily increase with age and might depend heavily on recent exposures and physiological processes governing cadmium absorption and secretion (Chaumont et al., 2013). Currently, there is no consensus on this new interpretation of urinary cadmium, but as demonstrated by the differing results we obtained for urinary and blood cadmium, reporting disease associations with only one biomarker and omitting another may be misleading. Investigating multiple exposure markers simultaneously may give us a fuller picture and more opportunities to unravel the often complex exposure-disease relationships.

The second complexity in our results was that although the NHANES III sample was largely similar to the NHANES 1999–2006 samples, the cadmium and AD mortality association was only partially replicable in NHANES III. Specifically, in NHANES III the urinary cadmium-AD mortality association was weaker, and more sensitive to urinary creatinine adjustment. In that study sample, urinary creatinine was an independent risk factor of AD mortality and cadmium was not (with urinary creatinine adjustment). One possible explanation for the discrepancy between NHANES (1999–2006) and NHANES III is that the number of AD cases was small in both analyses, and therefore the results may differ simply by chance. Alternatively, the statistical pattern surrounding urinary creatinine adjustment in NHANES III may suggest a more nuanced relationship among urinary creatinine, urinary cadmium and AD mortality. Urinary creatinine has been used in many epidemiologic studies to account for urine dilutions, and we intended to use it for the same purpose in our study. However, for urinary creatinine to be a valid marker of urine dilutions,

it has to be freely filtered by the kidneys and not affected by either cadmium or AD mortality. If these assumptions were violated, including creatinine in a regression model could lead to bias in the HR estimate of cadmium. Moreover, the degree and direction of this bias would have depended on the specific relationships among cadmium, creatinine and AD mortality and would not have been readily predictable. As with other studies, we could not verify whether the assumptions behind using urinary creatinine were met, but violations of the assumptions were not impossible. For example, creatinine is both filtered and secreted by the kidneys, and the proportion secreted increases in the presence of kidney injuries (Shemesh et al., 1985). Because the kidneys are major targets of cadmium toxicity, using creatinine to account for urine dilutions may not be appropriate, particularly if renal injuries are relatively extensive, as in the case of older people chronically exposed to relatively high levels of cadmium. If creatinine was not an appropriate marker to account for urine dilutions in NHANES III, results from models with creatinine were biased, and the amount and direction of such bias were unclear. Results from models without creatinine might be more informative, because assuming that urine dilutions were independent of cadmium and AD mortality, the lack of urine dilution adjustments resulted in non-differential misclassification of cadmium exposure, which in turn would lead to underestimates of the “Cd-AD mortality” association. Unfortunately, no other markers of urine dilutions were available in the NHANES III and NHANES 1999–2006 datasets, and we were unable to evaluate our results against those where urine dilutions were adjusted using alternative means. Nevertheless, our unusual findings regarding creatinine adjustments in NHANES III has added to the growing number of studies questioning the practice of urinary creatinine adjustment (Wagner et al., 2010; Weaver et al., 2011, 2014).

Third, in the NHANES III analysis the association between cadmium and AD mortality became weaker as follow-up duration increased. In the NHANES III sample, within the first 12.7 years since baseline, each IQR increase in urinary cadmium was associated with 11% increase in AD mortality prior to creatinine adjustment. However, if the follow-up duration was extended all the way to the end of 2011, each IQR increase in urinary cadmium was not associated with any changes in AD mortality. If cadmium causes AD, this situation could arise if persons particularly susceptible to cadmium’s “effects” died of AD rapidly soon after baseline and those who remained were less susceptible/more resistant to cadmium toxicity. In this scenario, even though cadmium was associated with elevated AD mortality initially, over a 17–23 years’ period, cadmium was on average not associated with AD. Alternatively, urinary cadmium might simply be a marker of factors associated with approaching AD-related death. In this case, urinary cadmium measured many years before death might not be associated with AD mortality risks. Regardless of the explanation, it should be noted that the 12.7-year cut-off was an arbitrary number based on what happened to be the maximum follow-up time in the NHANES 99–06 samples, the results from which we tried to replicate in the NHANES III population. The significance this coincidental number, if there is any, needs further evaluation.

Clearly, issues discussed above can only be resolved with additional epidemiologic studies with alternate designs, but to fully understand the relationship between cadmium and AD, toxicological studies are needed as well. Currently, a small body of experimental evidence has shown that cadmium treatment could reduce the learning and memory capacities of mice

genetically susceptible to AD (Li et al., 2012). Cadmium-treated animals also had lower levels of brain α-secretase and neutral endopeptidase (Li et al., 2012), as well as higher levels of amyloid precursor protein (Ashok et al., 2015). Together, these conditions caused increased amyloid beta production, decreased amyloid beta degradation, and the formation of larger and more numerous senile plaques. Additionally, cadmium directly promoted amyloid beta aggregation *in vitro* (Notarachille et al., 2014), and caused basal forebrain cholinergic neuron death in cell cultures (Del Pino et al., 2015). These studies have generated important insights about the potential AD-related toxicity of cadmium, but few have showed that cadmium was present in the brain regions relevant to AD development. On its own, little cadmium crosses the blood brain barrier (BBB), except in young animals (Evans and Hastings, 1992; Méndez-Armenta and Ríos, 2007; Shukla et al., 1996). If cadmium causes AD, this means that it may directly induce pathological changes in the brain only when other substances are present to facilitate its entry to the brain, or after the metal or additional factors compromise the BBB (Kim et al., 2013). Alternatively, cadmium may cause AD in a less direct manner. For example, dysfunction of the choroid plexus, a brain region involved in amyloid-beta clearance, has been hypothesized to cause AD (Krzyzanowska and Carro, 2012). Cadmium accumulates in the choroid plexus and damages its structure (Zheng, 2001). Whether these cadmium-induced changes in the choroid plexus eventually lead to AD still awaits investigation. Furthermore, cadmium is known to cause systemic, global changes in DNA methylation (Vilahur et al., 2015) and AD is associated with brain DNA methylation changes (Bakulski et al., 2012). Whether cadmium causes AD through global epigenetic changes could be another hypothesis to explore.

Collectively, existing toxicological and epidemiological studies suggest a link between cadmium and AD, but the results are far from conclusive, and non-causal explanations for our findings are possible. For example, AD patients typically have multiple comorbidities, including kidney injuries that may increase AD-related mortality risks and result in faster excretion of cadmium (ATSDR, 2008). The association between urinary cadmium and AD mortality may reflect this, although the significant association between blood cadmium and AD mortality in the NHANES 99–06 sample makes this explanation less likely. It's also possible that cadmium exposure may only enhance the symptoms of AD or increase its severity. Since those with severe symptoms are more likely to be recorded as dying of AD, we might be capturing this association. We also could not eliminate the potential impact of unmeasured, residual confounding; and so cadmium may simply be a marker of other factors associated with AD mortality.

Other limitations of our study include assessing AD with death certificates and measuring cadmium at only one time point. Death certificates are inaccurate assessments of AD. If among those who died of other causes, AD should have been the true underlying cause of death, and if cadmium was truly associated with AD, the associations we reported were underestimates. Finally, even if we could measure AD and cadmium perfectly, the association with AD mortality could still be biased due to competing risks, where some exposed subjects died of other causes before having a chance to die of AD. The best strategy to partially remedy the competing risk problem is to follow a group of relatively young (perhaps middle-aged) and healthy subjects in future studies and monitor for early cognitive and physiological changes preceding AD and eventual AD incidence.

5. Conclusions

Our findings have added to the current understanding about the relationship between cadmium and AD. Similar to blood cadmium, urinary cadmium was associated with elevated risk of AD mortality in the 5–13 years following exposure assessment among older US adults; and this association was partially replicable in a separate population. Variability in results across NHANES 1999–2006 and NHANES III underscores the importance of defining a relevant window of exposure and follow-up period in future studies. Our study also raises questions about urinary creatinine adjustment, which deserves more thoughtful consideration in future investigations. AD is a devastating disease with no cure and few known modifiable risk factors. If cadmium indeed increases AD risk, changing the environment may be a viable and sustainable effort to reduce AD morbidity and mortality. More epidemiologic and toxicological studies are needed to fully understand the nature of the "cadmium-AD mortality" association, which may open new avenues for AD prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AD	Alzheimer's disease
PIR	Poverty-income ratio
LOD	limit of detection
HR	hazard ratio
NHANES	National Health and Nutrition Examination Survey

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Highlights

- Urine Cd positively associated with Alzheimer's disease mortality in NHANES 99–06
- Urine Cd NOT associated with AD in NHANES III, except under some conditions
- Results partly supportive of "Cd-AD mortality" link
- Inconsistency and insufficient toxicological data suggest cautious interpretation

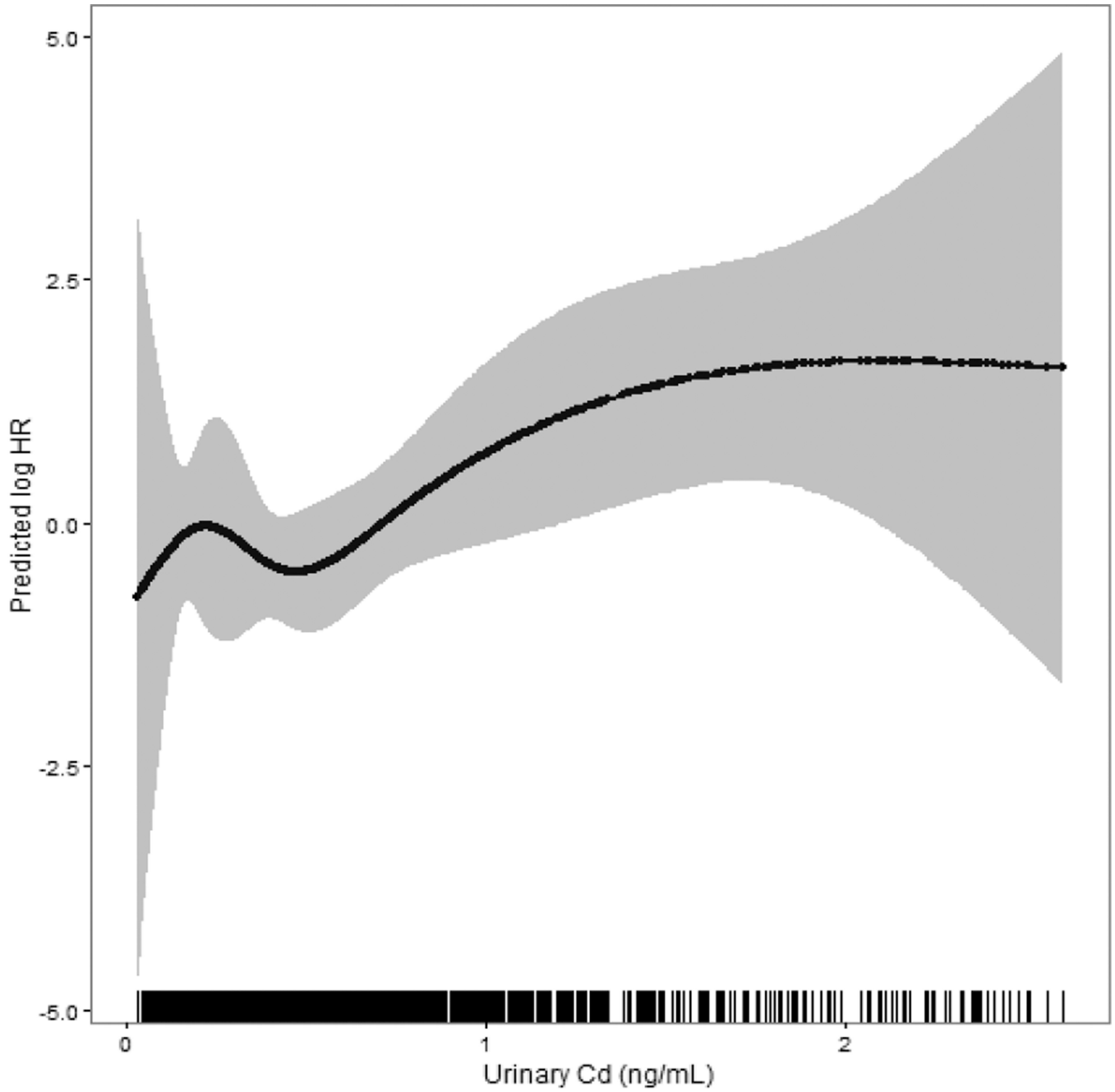

Figure 1. Urinary cadmium and predicted log hazard ratio of AD

The figure was based on a survey-weighted Cox regression model where 1) only data in the lower 99% of urinary cadmium was used (N total=1990; N case=21), 2) urinary cadmium was modeled with natural splines with knots at the 25th, 50th and 75th weighted percentiles and 3) the model was adjusted for sex, race/ethnicity, smoking status, education level and urinary creatinine. We used data in the lower 99th percentile of urinary cadmium because very few observations were present in the upper 1% and the confidence interval for the predicted log HR was large. Including all the data would obscure the presentation of the relationship in the majority (lower 99%) of the data.

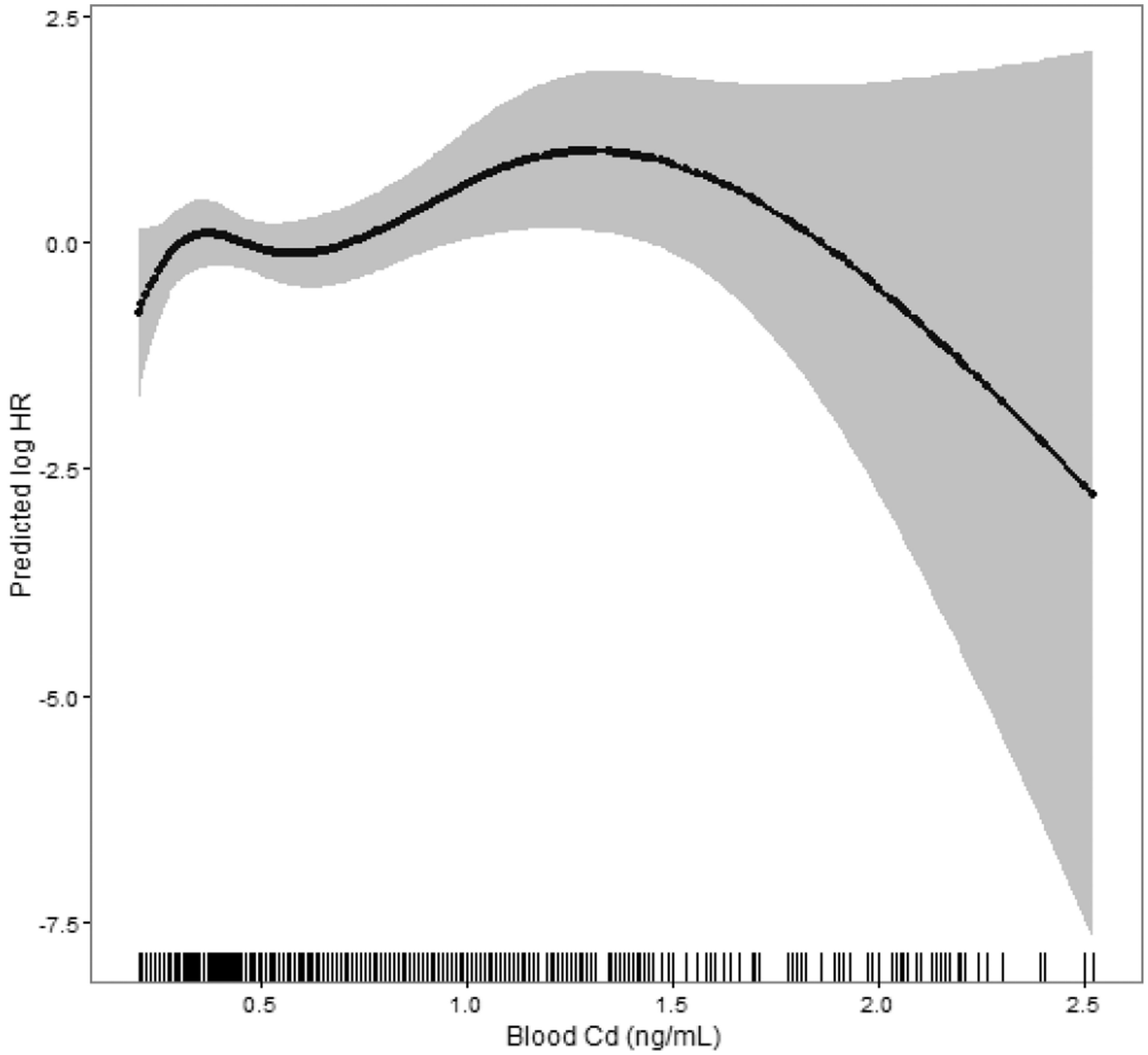

Figure 2. Blood cadmium and predicted log hazard ratio of AD

The figure was based on a survey-weighted Cox regression model where 1) only data in the lower 99% of blood cadmium was used (N total=6062; N case=76), 2) blood cadmium was modeled with natural splines with knots at the 10th, 50th and 90th weighted percentiles and 3) the model was adjusted for sex, race/ethnicity, smoking status and education level. We used data in the lower 99th percentile of blood cadmium because very few observations were present in the upper 1% and the confidence interval for the predicted log HR was large. Including all the data would obscure the presentation of the relationship in the majority (lower 99%) of the data.

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Table 1

Population characteristics of the NHANES 1999–2006 samples [a]

	Urinary Cadmium Sample				Blood Cadmium Sample			
	All	**Non-AD**	**AD**	**p-value** [b]	**All**	**Non-AD**	**AD**	**p-value**
N	2023	2002	21		6141	6065	76	
Age (mean (SE)) (years)	71.1 (0.24)	71.0 (0.24)	78.5 (1.22)	<.0001	71.1 (0.16)	71.0 (0.16)	78.3 (0.79)	<.0001
Urinary cadmium (geometric mean (GSE)) (ng/mL)	0.40 (1.03)	0.40 (1.03)	0.50 (1.28)	0.35	*NA*	*NA*	*NA*	*NA*
Urinary creatinine (geometric mean (GSE)) (mg/dL)	82.5 (1.02)	82.4 (1.02)	93.1 (1.22)	0.54	*NA*	*NA*	*NA*	*NA*
Blood cadmium (geometric mean(GSE)) (ng/mL)	*NA*	*NA*	*NA*	*NA*	0.51 (1.01)	0.51 (1.01)	0.54 (1.06)	0.22
Follow-up time (mean (SE)) (years)	7.5 (0.13)	7.5 (0.13)	5.9 (0.59)	0.009	7.5 (0.12)	7.5 (0.12)	5.9 (0.39)	0.0002
Male	43.9%	43.8%	51.4%	0.51	43.7%	43.8%	39.2%	0.53
Female	56.1%	56.2%	50.0%		56.3%	56.2%	60.6%	
White	81.0%	81.0%	89.9%		82.0%	81.9%	93.9%	
Black	8.6%	8.6%	10.1%	0.03	8.0%	8.1%	5.5%	<.0001
Others	10.4%	10.5%	0.32%		9.9%	10.0%	0.48%	
Never smoker	46.7%	46.7%	50.5%		46.3%	46.1%	60.3%	
Past smoker	41.7%	41.7%	44.4%	0.72	41.3%	51.4%	36.4%	0.02
Current smoker	11.6%	11.7%	5.44%		12.4%	19.5%	3.1%	
Less than high school [c]	29.6%	29.7%	26.0%		29.0%	29.1%	22.7%	
High school graduate or some college	52.1%	52.0%	65.2%	0.37	51.6%	51.4%	62.0%	0.31
College graduate or above	18.2%	18.3%	8.7%		19.4%	19.5%	15.3%	

[a] The complex sampling design was accounted for when computing means, geometric means, standard errors and proportions.

[b] Comparisons were between AD cases and non-AD subjects. For categorical variables, p-values were obtained from Pearson's Chi-square test with Rao & Scott adjustment. For continuous variables, p-values were obtained from survey-weighted generalized linear models. Urinary cadmium, blood cadmium and urinary creatinine were log-transformed in the models.

[c] In the urinary cadmium sample, the education level of 4 participants was missing; all 4 were non-cases. In the blood cadmium sample, the education level of 18 participants was missing; all 18 participants were non-cases.

Table 2

Urinary and blood cadmium levels in population subgroups, NHANES 1999–2006 [a]

	N	Urinary Cadmium (geometric mean (GSE)) (ng/mL)	p-value[b]	N	Blood Cadmium (geometric mean (GSE)) (ng/mL)	p-value
Age(years)						
60–64	479	0.43 (1.06)		1471	0.52 (1.02)	
65–69	427	0.40 (1.06)	0.05	1233	0.48 (1.03)	0.74
70–76	505	0.40 (1.05)		1537	0.51 (1.02)	
77–85	612	0.36 (1.04)		1900	0.52 (1.01)	
Male	1052	0.45 (1.04)	ref	3061	0.48 (1.02)	ref
Female	971	0.36 (1.03)	<.0001	3080	0.52 (1.02)	<.0001
White	1146	0.38 (1.03)	ref	3587	0.50 (1.02)	ref
Black	359	0.50 (1.06)	0.0002	1020	0.48 (1.03)	0.14
Others	518	0.44 (1.08)	0.13	1534	0.54 (1.03)	0.10
Never smoker	948	0.30 (1.03)	ref	2851	0.41 (1.02)	ref
Past smoker	825	0.46 (1.04)	<.0001	2514	0.50 (1.01)	<.0001
Current smoker	250	0.80 (1.05)	<.0001	776	1.18 (1.03)	<.0001
Less than high school [c]	839	0.44 (1.05)	ref	2534	0.57 (1.02)	ref
High school graduate or some college	879	0.40 (1.04)	0.15	2671	0.50 (1.02)	<.0001
College graduate or above	301	0.33 (1.05)	0.0002	918	0.43 (1.02)	<.0001

[a] The complex sampling design was accounted for when computing geometric means and standard errors.

[b] p-values were obtained through survey-weighted generalized linear regression. Urinary and blood cadmium was log-transformed. The p-values for “Age” were from tests for linear trend over the four age categories.

[c] In the urinary cadmium sample, the education level of 4 participants was missing; all 4 were non-cases. In the blood cadmium sample, the education level of 18 participants was missing; all 18 participants were non-cases.

Table 3

Hazard ratios (HR) of AD for each interquartile range (IRQ) increase in cadmium, NHANES 1999–2006

	Urinary Cadmium (N case= 21; N total=2023)		Blood Cadmium (N case=76; N total=6141)	
	HR per IQR (0.51 ng/mL) increase (95% CI)	p-value	HR per IQR (0.36 ng/mL) increase (95% CI)	p-value
Without urinary creatinine adjustment				
Model 1[a]	1.56 (1.18, 2.07)	0.0016	1.10 (0.91, 1.32)	0.33
Model 2	1.51 (1.15, 1.98)	0.002	1.13 (0.94, 1.35)	0.18
Model 3	1.57 (1.20, 2.05)	0.0009	1.20 (0.99, 1.45)	0.05
Model 4[b]	1.56 (1.17, 2.07)	0.002	1.22 (1.01, 1.48)	0.04
With urinary creatinine adjustment (urinary cadmium models only)				
Model 4a	1.58 (1.20, 2.09)	0.0009		

[a]Model 1: Urinary/blood cadmium only
Model 2: Model 1+ race/ethnicity and sex
Model 3: Model 2 + smoking status
Model 4: Model 3 + education
Model 4a (Urinary Cd only): Model 4 +urinary creatinine

[b]When education was additionally included in the regression model, 4 observations were excluded from the urinary cadmium model and 18 were excluded from the blood cadmium model due to missing education. All participants missing in education were non-cases.

Table 4

Hazard ratios (HR) of AD for each interquartile range (IRQ) increase in cadmium, NHANES III

	Urinary Cadmium (Follow-up up to 2011 (23 years)) N case= 102; N total=4994		Urinary Cadmium (Follow-up up to 12.7 years) N case=44; N total=4994	
	HR per IQR (0.78 ng/mL) increase (95% CI)	**p-value**	**HR per IQR (0.78 ng/mL) increase (95% CI)**	**p-value**
Without urinary creatinine adjustment				
Model 1[a]	1.05 (0.92, 1.20)	0.43	1.11 (1.02, 1.21)	0.017
Model 2	1.06 (0.92, 1.22)	0.40	1.13 (1.02, 1.24)	0.012
Model 3	1.02 (0.88, 1.18)	0.81	1.12 (1.04, 1.20)	0.0029
Model 4[b]	1.01 (0.86, 1.18)	0.94	1.11 (1.02, 1.20)	0.0086
With urinary creatinine adjustment				
Model 4a	0.85 (0.63, 1.17)	0.31	0.82 (0.59, 1.15)	0.23

[a]Model 1: Urinary cadmium only
Model 2: Model 1+ race/ethnicity and sex
Model 3: Model 2 + smoking status
Model 4: Model 3 + education
Model 4a: Model 4 +urinary creatinine

[b]When education was additionally included in the model, 29 observations were excluded due to missing education. All 29 participants were non-cases.