ARTICLE

Urinary cadmium concentration and the risk of ischemic stroke

Cheng Chen, PhD, Pengcheng Xun, MD, PhD, Cari Tsinovoi, PhD, Leslie A. McClure, PhD, John Brockman, PhD, Leslie MacDonald, PhD, Mary Cushman, MD, Jianwen Cai, PhD, Lisa Kamendulis, PhD, Jason Mackey, MD, and Ka He, MD, ScD

Correspondence
Dr. He
kahe@indiana.edu

Neurology® 2018;91:e382-e391. doi:10.1212/WNL.0000000000005856

Abstract

Objectives

To examine the association between urinary cadmium levels and the incidence of ischemic stroke and to explore possible effect modifications.

Methods

A case-cohort study was designed nested in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, including 680 adjudicated incident cases of ischemic stroke and 2,540 participants in a randomly selected subcohort. Urinary creatinine–corrected cadmium concentration was measured at baseline. Multivariable-adjusted hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were estimated with the Barlow weighting method for the Cox proportional hazards regression model.

Results

The median urinary cadmium concentration was 0.42 (interquartile range 0.27–0.68) μg/g creatinine. After adjustment for potential confounders, urinary cadmium was associated with increased incidence of ischemic stroke (quintile 5 vs quintile 1: HR 1.50, 95% CI 1.01–2.22, p for trend = 0.02). The observed association was more pronounced among participants in the lowest serum zinc tertile (tertile 3 vs tertile 1: HR 1.82, 95% CI 1.06–3.11, p for trend = 0.004, p for interaction = 0.05) but was attenuated and became nonsignificant among never smokers (tertile 3 vs tertile 1: never smokers: HR 1.27, 95% CI 0.80–2.03, p for trend = 0.29; ever smokers: HR 1.60, 95% CI 1.06–2.43, p for trend = 0.07, p for interaction = 0.51).

Conclusions

Findings from this study suggest that cadmium exposure may be an independent risk factor for ischemic stroke in the US general population. Never smoking and maintaining a high serum zinc level may ameliorate the potential adverse effects of cadmium exposure.

CME Course
NPub.org/cmelist

From the Departments of Epidemiology and Biostatistics (C.C., P.X., C.T., K.H.) and Environmental Health (L.K.), School of Public Health, and Department of Neurology (J.M.), School of Medicine, Indiana University, Bloomington; Department of Epidemiology and Biostatistics (L.A.M.), Dornsife School of Public Health, Drexel University, Philadelphia, PA; Columbia Research Reactor (J.B.), University of Missouri; National Institute for Occupational Safety and Health (L.M.), Centers for Disease Control and Prevention, Washington, DC; Department of Medicine (M.C.), Larner College of Medicine, University of Vermont, Burlington; and Department of Biostatistics (J.C.), University of North Carolina at Chapel Hill.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

BMI = body mass index; **CI** = confidence interval; **HDL** = high-density lipoprotein; **HR** = hazard ratio; **ICP-MS** = inductively coupled plasma mass spectrometry; **LDL** = low-density lipoprotein; **NHANES** = National Health and Nutrition Examination Survey; **REGARDS** = Reasons for Geographic and Racial Differences in Stroke.

Because of its role in initiating atherosclerosis,[1,2] cadmium exposure may be a potential explanation for disparities in stroke rates due to geographic variation and consequently the different levels of exposure in human. Epidemiologic data indicate an association between cadmium exposure and higher blood pressure.[3–6] However, data directly relating cadmium exposure to ischemic stroke risk are sparse, and the findings are inconsistent.[7–9]

Smoking is a major source of cadmium and an important risk factor for stroke in the general population.[10] However, few studies have adjusted for smoking status or considered it as a potential effect modifier when examining the association between cadmium and stroke because of a lack of data.[8] In addition, cadmium shares similar physical and chemical properties with zinc.[11] Their similarity allows cadmium to compete with zinc and replace it in biological systems and to destabilize the functional sites of zinc-containing proteins when zinc is deficient.[11] Because cadmium and zinc share many food sources,[12] increasing attention has been paid to the potential joint health effect of zinc and cadmium.[2] In fact, a significant interaction of zinc and calcium has been demonstrated in animal models.[13,14] However, this interaction has not been confirmed in human studies.

We therefore examined the prospective association of urinary cadmium levels with the incidence of ischemic stroke and explored possible effect modifications of this association.

Methods

Study design and population

The detailed design and methods of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) project have been described previously.[15] In brief, the REGARDS project enrolled and is following a cohort of 30,239 black and white Americans ≥45 years of age. Blacks and individuals residing in the Stroke Belt region (Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee), where the stroke mortality exceeds the rest of United States, were oversampled. Participants were recruited from January 2003 to October 2007 through a combination of mail and telephone contact. Among those consented to the study, information on demographics and risk factors was assessed in a telephone interview, followed by an in-person physical assessment 3 to 4 weeks later in which blood and urine samples, anthropometric measurements, and other risk factor data were collected by standardized protocols. Participants (or their proxies) were contacted every 6 months to ascertain potential strokes. Incident strokes and stroke types were verified by relevant hospital records and adjudicated centrally by neurologists. Additional details are provided elsewhere.[16]

A case-cohort study design was used in this study.[17] The case-cohort sample consists of 2 parts: a random sample from the total REGARDS cohort, called the subcohort, supplemented by all of the incident ischemic stroke cases identified through September 2012 and not sampled in the subcohort. The subcohort (n = 2,666) was selected from the entire REGARDS cohort (n = 29,653) with a fixed sampling probability of 9% in each stratum jointly classified by age (<55, 55–64, 65–74, 75–84, and ≥85, years), sex (male and female), race (black and white), and stroke region residency (Stroke Buckle [coastal plains of North Carolina, South Carolina, and Georgia], the rest of Stroke Belt, and non–Stroke Belt region).[18,19] Through September 2012, 713 incident cases of ischemic stroke were identified. Among those, 63 cases were included in the random subcohort by random selection. Of the 3,316 participants in the case-cohort study, we excluded 156 whose cadmium measurements were not available. Thus, the final dataset includes 680 cases of ischemic stroke and 2,540 participants in the random subcohort (including 60 overlapping incident cases; figure).

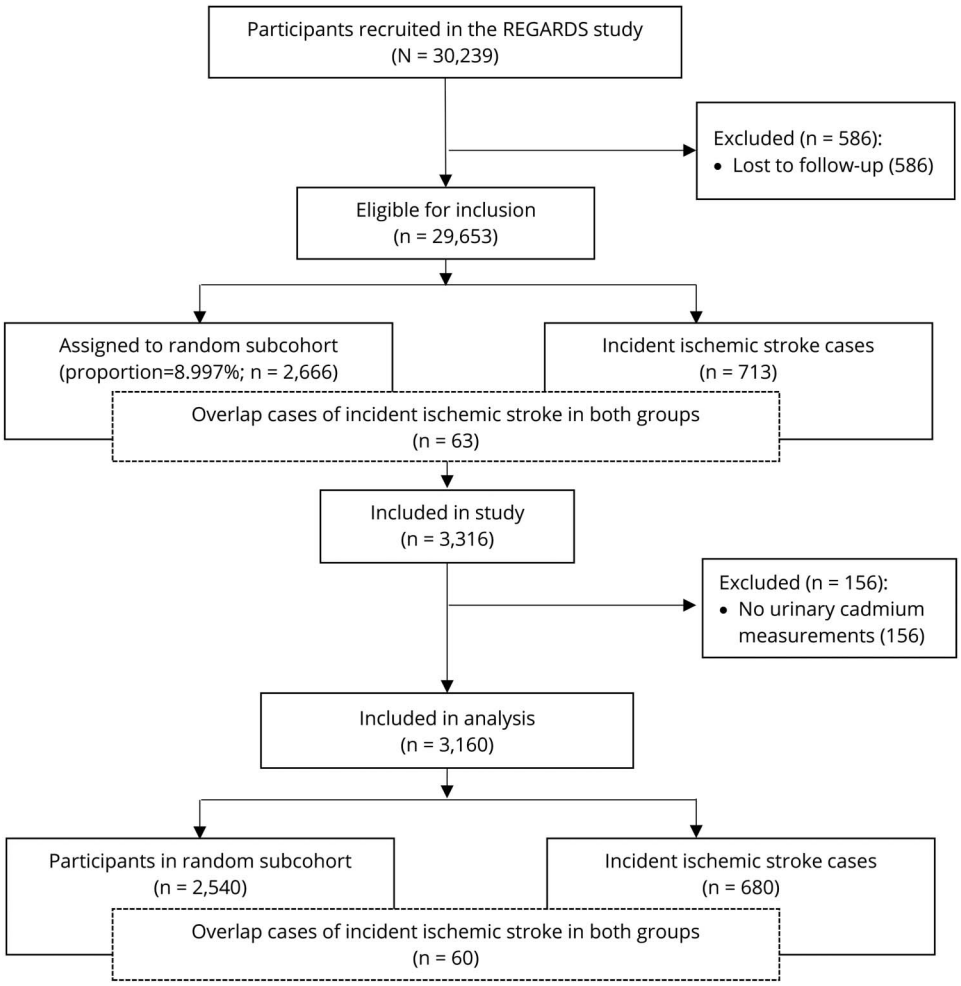
Standard protocol approvals, registrations, and patient consents

Approval was received from the ethics standards committee on human experimentation for all experiments with human participants. Written informed consent was obtained from all study participants (consent for research).

Laboratory analyses

Urine and fasting blood samples were collected, shipped overnight with ice packs to a central laboratory at the University of Vermont, and stored at −80°C.[20] Urinary cadmium was measured via the National Health and Nutrition Examination Survey (NHANES) method with a NEXION 300X quadrupole inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, Waltham, MA) operated with a dynamic reaction cell to eliminate interference from urinary molybdenum.[21,22] Urinary creatinine was assessed with the Modular-P chemistry analyzer from Roche/Hitachi (Tokyo, Japan).[23] Several other elements were also measured, including urinary arsenic (ICP-MS) and serum calcium, magnesium, and zinc concentrations (ICP-MS). Lipid profiles were analyzed with colorimetric reflectance spectrophotometry and C-reactive protein by particle-enhanced immunonephelometry with the BNII nephelometer (N High

Figure Flowchart of subcohort and cases sampling



REGARDS = Reasons for Geographic and Racial Differences in Stroke.

Sensitivity CRP; Dade Behring, Deerfield, IL).[24] Estimated glomerular filtration rate was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[25]

Outcome, exposure, and covariates

The outcome was time to incident ischemic stroke obtained from self-report and adjudicated through hospital records by neurologists. Survival time was defined as the period between the baseline interview and ischemic stroke (first incidence), last follow-up, death, or data freeze (September 2012), whichever came first. The primary exposure was urinary creatinine–corrected cadmium concentration classified in quintiles or as a continuous variable.

Key covariates included age, sex, race (black or white), region (Stroke Buckle, the rest of Stroke Belt, or non–Stroke Belt region), income (US $1,000–$10,000/y), body mass index (BMI; <25.0, 25.0–29.9, or ≥30.0 kg/m^2), smoking status (never, former, current smoker <15 pack-years, current smoker between 15 and 30 pack-years, current smoker between 30 and 50 pack-years, or current smoker ≥50 pack-years), alcohol consumption (never, former, or current drinker), physical activity (none, 1–3, or ≥4 times a week), hypertension (yes or no), history of myocardial infarction (yes or no), history of atrial fibrillation (yes or no), diabetes mellitus (yes or no), dyslipidemia (yes or no), high-density lipoprotein (HDL)/low-density lipoprotein (LDL) cholesterol ratio, C-reactive protein, serum calcium, serum magnesium, and urinary arsenic concentrations. Demographics, income level, smoking status, alcohol consumption, physical activity, and medical history were assessed via self-report. History of myocardial infarction and atrial fibrillation was determined by ECG evidence of the diseases or self-reported history of the diseases. Hypertension was defined as any self-reported use of blood pressure control medication, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg. Diabetes mellitus was defined as any self-reported use of glucose control medication, fasting blood glucose concentration >126 mg/dL, or nonfasting glucose >200 mg/dL. Dyslipidemia was defined as any self-reported use of lipid control medication, triglycerides ≥240 mg/dL, LDL

cholesterol ≥160 mg/dL, or HDL cholesterol ≤40 mg/dL. Weight and height were measured by trained professionals using standardized protocols and were used to calculate BMI (kilograms per meter squared).

Statistical analyses

Baseline characteristics were summarized as mean values with SD or medians with interquartile ranges for continuous variables and frequencies (proportions) for categorical variables. Analysis of variance, Kruskal-Wallis test, or χ^2 test, as appropriate, was used to compare participants' characteristics across quintiles of urinary creatinine–corrected cadmium concentration levels in the subcohort. Covariates significantly associated with cadmium in the subcohort were considered in the main analysis. The Barlow[26] weighting method of the Cox proportional hazards regression model for the case-cohort study was used to examine the association between urinary cadmium levels and the incidence of ischemic stroke. Continuous urinary creatinine–corrected cadmium concentration was used to test for linear trend, with those values above the 95th percentile deleted to reduce the influence of outliers. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) using the lowest quintile of urinary creatinine–corrected cadmium concentration as the referent were estimated in 3 sequential models. Model 1 was adjusted for age, sex, race, age-sex and age-race interactions, and region. Model 2 was in addition adjusted for income, BMI, smoking status, alcohol consumption, and physical activity. Model 3 was further adjusted for diabetes mellitus, HDL/LDL cholesterol ratio, C-reactive protein, serum calcium, and urinary arsenic concentrations.

Several sensitivity analyses were conducted to test the robustness of our findings. First, the concentration of cadmium in spot urine specimens is highly dependent on fluid intake and excretion. Although the cadmium/creatinine ratio was suggested to be a more useful tool, different methods to control for this dilution uncertainty have been suggested in the past.[27] Thus, we performed a sensitivity analysis using original urinary cadmium concentration in micrograms per milliliter as the exposure with urinary creatinine concentration as a covariate in the model, rather than using urinary creatinine–corrected cadmium concentration. Second, noncase participants with prevalent myocardial infarction, atrial fibrillation, and coronary artery disease were excluded to reduce the influence of high-risk individuals. Third, we adjusted separately for never/former/current smokers and for accumulated pack-years. Fourth, arsenic exposure is likely to be a confounder because it often coexists with cadmium in the environment[28] and is associated with increased ischemic stroke risk.[29] Although we intended to present the association between cadmium and ischemic stroke risk independently of arsenic exposure, the confounding effect of arsenic may be small. Thus, we did not adjust for urinary arsenic concentration in a sensitivity analysis. Fifth, kidney function is related to cadmium excretion through urine and the risk of stroke.[30] Therefore, we did a sensitivity analysis with additional adjustment for estimated glomerular filtration rate. Finally, we reported the robust sandwich estimates with adjustment for variance due to the case-cohort study design. To test the robustness of the results, other parametric models, including the Poisson and Weibull regression models, were examined.

In stratified analyses, urinary creatinine–corrected cadmium tertiles were used to ensure sufficient power instead of quintiles along with continuous urinary creatinine–corrected cadmium concentration. Sex (female vs male), race (black vs white), region (Stroke Buckle vs the rest of Stroke Belt vs non–Stroke Belt region), smoking status (never vs ever smokers), and serum zinc (tertiles) were considered as potential effect modifiers. Interaction was tested with continuous urinary creatinine–corrected cadmium concentration, excluding those values above the 95th percentile.

The proportional hazards assumption was assessed by testing the coefficient of the interaction between quintiles of urinary creatinine–corrected cadmium concentration and time.[31] Values of $p \leq 0.05$ (main effect) and ≤ 0.10 (interaction) were considered statistically significant. All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC).

Date availability

The data-sharing plan of the REGARDS study is consistent with NIH policy. Any data not published within this article are available in the REGARDS data repository. Anonymized data may be shared by request from any qualified investigator in compliance with NIH regulations.

Results

Baseline characteristics of participants

In the study population, 53% participants were female and 41% were black with an average age of 66 years. The average follow-up time was 7 years. The median urinary creatinine–corrected cadmium concentration was 0.42 μg/g creatinine (interquartile range 0.27–0.68 μg/g creatinine). Baseline characteristics are shown in table 1. Participants with higher urinary creatinine–corrected cadmium concentration were more likely to be older, female, alcohol drinkers, and physically inactive. They had lower income levels; lower BMI levels; higher levels of HDL/LDL cholesterol ratio, C-reactive protein, and serum calcium; and lower urinary arsenic concentration. They were less likely to be never smokers and to have diabetes mellitus.

Cadmium exposure and incident ischemic stroke

After adjustment for potential confounders, urinary creatinine–corrected cadmium levels were associated with the incidence of ischemic stroke (table 2). A significant linear trend was observed with the use of continuous cadmium concentration. The proportionality assumption was satisfied.

In the sensitivity analyses that model urinary cadmium adjusted for urinary creatinine to control for the dilution uncertainty of spot urine specimens and that exclude noncase

Table 1 Baseline characteristics of the study population by quintiles of urinary creatinine–corrected cadmium levels[a]

			Quintiles of urinary cadmium levels, μg/g creatinine[c]					
	Case-cohort[b]	Random subcohort	Q1, ≤0.24	Q2, 0.25–0.35	Q3, 0.36–0.49	Q4, 0.50–0.77	Q5, ≥0.78	*p* Value
No.	3,160	2,540	508	508	508	509	507	—
Age, y	65.9 ± 9.4	64.9 ± 9.3	63.2 ± 9.1	65.2 ± 9.5	65.7 ± 9.6	65.2 ± 9.2	65.2 ± 8.9	<0.05
Female, %	53.4	54.4	32.9	45.1	57.3	65.0	71.8	<0.05
Black, %	40.9	41.0	42.1	39.4	40.2	40.7	42.8	0.80
Region, %								
Stroke Buckle	21.1	21.0	18.7	21.1	24.0	23.2	18.2	0.09
The rest of Stroke Belt	34.8	34.8	39.4	31.1	34.1	35.0	34.7	
Non–Stroke Belt region	44.1	44.1	41.9	47.8	41.9	41.9	47.1	
Income, US $1,000/y	6.4 ± 2.1	6.5 ± 2.1	7.0 ± 2.0	6.8 ± 2.1	6.3 ± 2.1	6.2 ± 2.1	6.0 ± 2.1	<0.05
BMI, %								
<25.0 kg/m^2	24.6	24.3	17.9	19.2	24.1	23.8	36.5	<0.05
25.0–29.9 kg/m^2	38.0	37.6	37.3	41.8	37.7	39.5	31.7	
≥30.0 kg/m^2	37.4	38.1	44.8	39.0	38.3	36.7	31.9	
Smoking status, %								
Never	45.5	46.2	66.7	57.1	49.4	38.5	19.1	<0.05
Former	39.1	38.8	30.1	37.4	42.3	42.8	41.4	
Current	15.4	15.0	3.2	5.5	8.3	18.7	39.5	
Alcohol consumption, %								
Never	30.7	30.2	34.7	31.7	33.1	26.1	25.4	0.03
Former	18.7	18.2	14.0	18.1	19.3	18.5	21.1	
Current	50.6	51.6	51.4	50.2	47.6	55.4	53.5	
Physical activity, %								
None	33.3	33.2	30.6	27.7	32.5	36.8	38.5	<0.05
1–3 times/wk	35.4	35.3	36.8	38.4	32.9	36.0	32.3	
≥4 times/wk	31.3	31.5	32.6	33.9	34.5	27.1	29.3	
Hypertension, %	61.8	59.0	60.1	54.2	59.8	59.9	60.9	0.19
Myocardial infarction, %	13.8	12.1	10.7	12.2	10.2	11.8	15.5	0.09
Atrial fibrillation, %	10.0	8.9	9.8	9.5	8.9	8.2	8.3	0.88
Diabetes mellitus, %	23.0	22.0	25.7	24.3	21.4	19.9	18.63	<0.05
Dyslipidemia, %	59.4	57.9	60.8	57.8	57.1	56.3	57.6	0.68
HDL/LDL cholesterol ratio	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.5 (0.3–0.6)	0.5 (0.4–0.6)	0.5 (0.4–0.7)	<0.05
C-reactive protein, mg/L	2.4 (1.0–5.0)	2.2 (1.0–4.8)	1.9 (0.9–4.0)	2.1 (0.9–4.8)	2.1 (0.9–4.6)	2.6 (1.1–5.0)	2.6 (1.1–5.9)	<0.05
Serum calcium, mg/dL	1.0 (9.6–10.4)	1.0 (9.6–10.4)	9.9 (9.5–10.3)	9.9 (9.6–10.4)	10.0 (9.6–10.4)	10.1 (9.7–10.4)	10.1 (9.7–10.5)	<0.05

Continued

Table 1 Baseline characteristics of the study population by quintiles of urinary creatinine–corrected cadmium levels[a] *(continued)*

			Quintiles of urinary cadmium levels, μg/g creatinine[c]					
	Case-cohort[b]	Random subcohort	Q1, ≤0.24	Q2, 0.25–0.35	Q3, 0.36–0.49	Q4, 0.50–0.77	Q5, ≥0.78	*p* Value
Serum magnesium, mg/dL	2.0 (1.9–2.1)	2.0 (1.9–2.1)	2.0 (1.9–2.1)	2.0 (1.9–2.1)	2.0 (1.9–2.1)	2.0 (1.9–2.1)	2.0 (1.9–2.1)	0.91
Urinary arsenic, μg/dL	1.0 (0.5–2.0)	1.0 (0.5–2.1)	1.1 (0.6–2.2)	1.0 (0.6–2.3)	1.0 (0.5–2.1)	0.9 (0.5–1.9)	0.9 (0.5–1.9)	<0.05

Abbreviation: BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Q = quintile.
[a] Results are presented by means ± SE, medians (interquartile ranges), or proportions. The *p* values are for any difference across the quintiles of urinary creatinine–corrected cadmium levels in the random subcohort by using analysis of variance, Kruskal-Wallis test, or χ^2 test as appropriate.
[b] Participants in the random subcohort and incident cases of ischemic stroke.
[c] Quintiles of urinary creatinine–corrected cadmium were calculated from the random subcohort (n = 2,540).

participants with prevalent myocardial infarction, atrial fibrillation, and coronary artery disease, the observed associations were slightly attenuated. In the sensitivity analyses that was adjusted separately for never/former/current smokers and for accumulated pack-years, that was not adjusted for urinary arsenic concentration, and that was in addition adjusted for estimated glomerular filtration rate, the results were not materially changed. In addition, the results did not change appreciably with the use of Poisson or Weibull regression models.

Table 3 presents stratified analyses by prespecified factors, including sex, race, region, smoking status, and serum zinc. A significant interaction was observed between continuous urinary creatinine–corrected cadmium concentration and serum zinc levels. The association was attenuated among individuals with high serum zinc levels, and it was more pronounced in the lowest tertile of serum zinc.

In the analyses stratified by smoking status, a significant association remained among ever smokers but was attenuated and became nonsignificant in never smokers, although no significant interaction was found. When ever smokers were further subcategorized as former or current smokers, the association was attenuated presumably because of insufficient power, and the interaction was not statistically significant.

Discussion

Findings from this study add solid evidence to the literature supporting the hypothesis that cadmium exposure may

Table 2 Multivariable-adjusted HRs (95% CIs) of incident ischemic stroke by quintiles of urinary creatinine–corrected cadmium levels[a]

	Quintiles of urinary cadmium levels[b]					*p* Value for linear trend[c]
	Q1	Q2	Q3	Q4	Q5	
Range, μg/g creatinine	≤0.24	0.25–0.35	0.36–0.49	0.50–0.77	≥0.78	—
Median, μg/g creatinine	0.18	0.29	0.41	0.60	1.03	—
Subcohort, n	508	508	508	509	507	—
Cases, n	108	122	132	151	167	—
Model 1[d]	1.00 (Referent)	0.99 (0.73–1.34)	1.09 (0.80–1.47)	1.31 (0.97–1.76)	1.46 (1.08–1.98)	0.0004
Model 2[e]	1.00 (Referent)	1.20 (0.86–0.65)	1.17 (0.83–1.64)	1.25 (0.88–1.77)	1.42 (0.99–2.05)	0.04
Model 3[f]	1.00 (Referent)	1.19 (0.84–1.69)	1.16 (0.81–1.67)	1.27 (0.88–1.84)	1.50 (1.01–2.22)	0.02

Abbreviations: CI = confidence interval; HR = hazard ratio; Q = quintile.
[a] All models were constructed with weighted Cox proportional hazards regression analysis for case-cohort study.
[b] Quintiles of urinary creatinine–corrected cadmium were calculated from the random subcohort (n = 2,540).
[c] The *p* value for linear trend was examined by use continuous urinary creatinine–corrected cadmium with those values above the 95th percentile deleted.
[d] Model 1 was adjusted for age, sex, race (black or white), age-sex and age-race interactions, and region (Stroke Buckle, the rest of Stroke Belt, or non–Stroke Belt region).
[e] Model 2 was in addition adjusted for income (US $1,000–$10,000/y), body mass index (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), smoking status (never, former, current smoker <15 pack-years, current smoker between 15 and 30 pack-years, current smoker between 30 and 50 pack-years, and current smoker ≥50 pack-years), alcohol consumption (never, former, and current drinker), and physical activity (none, 1–3 times/wk, or ≥4 times/wk).
[f] Model 3 was in addition adjusted for diabetes mellitus (yes or no), high-density lipoprotein/low-density lipoprotein cholesterol ratio, C-reactive protein, serum calcium, and urinary arsenic concentrations.

Table 3 Associations (HRs [95% CIs]) between urinary creatinine–corrected cadmium levels and incident ischemic stroke stratified by some prespecified factors[a]

	Urinary cadmium (mean ± SD), μg/g creatinine	Events, n	Participants, n	Tertiles of urinary cadmium levels, μg/g creatinine[b]			*p* Value for linear trend[c]
				T1, ≤0.31	T2, 0.32–0.55	T3, ≥0.56	
All participants	0.55 ± 0.51	680	3,160	1 (Referent)	1.16 (0.88–1.52)	1.33 (0.99–1.79)	0.02
Sex							
Female	0.64 ± 0.50	330	1,686	1 (Referent)	0.72 (0.45–1.16)	1.22 (0.77–1.94)	0.35
Male	0.45 ± 0.51	350	1,474	1 (Referent)	1.57 (1.12–2.22)	1.36 (0.89–2.08)	0.02
***p* for interaction**	—	—	—		0.30		
Race							
Black	0.56 ± 0.62	281	1,293	1 (Referent)	0.96 (0.61–1.50)	1.30 (0.81–2.09)	0.37
White	0.55 ± 0.42	399	1,867	1 (Referent)	1.32 (0.92–1.88)	1.42 (0.95–2.11)	0.02
***p* for interaction**	—	—	—		0.59		
Region							
Stroke Buckle	0.54 ± 0.40	150	667	1 (Referent)	0.93 (0.43–1.98)	0.99 (0.45–2.17)	0.37
Rest of Stroke Belt	0.55 ± 0.52	230	1,100	1 (Referent)	0.95 (0.59–1.54)	1.35 (0.83–2.21)	0.05
Non–Stroke Belt region	0.55 ± 0.55	300	1,393	1 (Referent)	1.54 (1.01–2.36)	1.49 (0.94–2.36)	0.45
***p* for interaction**	—	—	—		0.95		
Smoking status							
Never smoker	0.41 ± 0.31	288	1,437	1 (Referent)	0.99 (0.66–1.46)	1.27 (0.80–2.03)	0.29
Ever smoker	0.67 ± 0.61	392	1,723	1 (Referent)	1.53 (1.01–2.31)	1.60 (1.06–2.43)	0.07
***p* for interaction**	—	—	—		0.51		
Former	0.57 ± 0.56	278	1,236	1 (Referent)	1.33 (0.84–2.11)	1.32 (0.83–2.11)	0.37
Current	0.93 ± 0.64	114	487	1 (Referent)	3.85 (0.68–21.71)	4.66 (0.86–25.17)	0.22
***p* for interaction**	—	—	—		0.75		
Zinc[b]							
Tertile 1	0.55 ± 0.44	277	1,188	1 (Referent)	1.56 (0.96–2.53)	1.82 (1.06–3.11)	0.004
Tertile 2	0.54 ± 0.48	216	987	1 (Referent)	0.98 (0.60–1.60)	0.97 (0.55–1.71)	0.49
Tertile 3	0.57 ± 0.61	187	985	1 (Referent)	1.14 (0.65–2.00)	1.55 (0.88–2.73)	0.06
***p* for interaction**	—	—	—		0.05		

Abbreviations: CI = confidence interval; HR = hazard ratio; T = tertile.
[a] All models were constructed with weighted Cox proportional hazards regression analysis for case-cohort study with adjustment for the covariates in model 3 (table 2) except the potential modifier and its related variables.
[b] Tertiles of urinary creatinine–corrected cadmium and serum zinc were calculated from the random subcohort (n = 2,540).
[c] *p* Value for linear trend was examined by use of continuous urinary creatinine–corrected cadmium with those values above the 95th percentile delete.

increase the incidence of ischemic stroke in the US population.

Results of this study are consistent with 1 previous study that measured blood cadmium[8] but not 2 other studies that assessed dietary cadmium intake.[7,9] The inconsistent findings from the dietary studies may be explained by the low correlation between biomarker-measured cadmium levels and dietary estimate of cadmium intake for several reasons. For example, dietary cadmium estimated via food frequency questionnaires cannot capture the cadmium exposure related to smoking or environment, which is captured by measuring cadmium levels in blood or urine.[32] In addition, dietary estimate is prone to subjective measurement error, while direct

measurement of levels in blood or urine may provide more objective assessment of the body burden.[32] In addition, biomarker-measured levels takes bioavailability into account, but dietary estimates do not.[32]

Of note, some previous studies focused on cadmium and the incidence or mortality from all types of strokes. One meta-analysis published in 2013 pooled results from 5 longitudinal and cross-sectional studies and found no association between cadmium exposure and all stroke risk.[33] It is possible that any likely association between cadmium exposure and the risk of ischemic stroke may be attenuated by including hemorrhagic stroke in the analysis because cadmium exposure may not be related to hemorrhagic stroke.[34] The null association found in the meta-analysis may also be explained by the limited number of primary studies and the heterogeneity resulting from different study designs and outcome definitions.[33]

Because cigarette smoking is a major source of cadmium exposure in the general population and an established risk factor for ischemic stroke,[10] the average pack-years of cigarette smoking was taken into account in our main analyses to reduce the possibility of residual confounding. This has not been accounted for in the previous studies. In stratified analyses, the association between cadmium exposure and the incidence of ischemic stroke was retained among ever smokers but substantially attenuated among never smokers, although the test for interaction was not statistically significant. Besides the relatively low level of cadmium exposure among never smokers, the disparity could be explained by the fact that never smokers generally have healthier lifestyles, including a healthy diet. Stroke risk may be reduced by stroke-beneficial nutrients from the diet, given that never smokers in the general population are exposed to cadmium mainly through diet.[8] For instance, food items that contribute to cadmium exposure (e.g., vegetables, seafood, and peanuts) are also high in nutrients beneficial to cerebrovascular health.[35] Thus, never smokers may be less susceptible to cadmium toxicity at low to moderate exposure levels because of the protection of dietary factors. Indeed, higher urinary cadmium concentration among never smokers was correlated with the higher adherence to a Mediterranean diet (data not shown), which has been shown to lower the risk of ischemic stroke among never smokers.[36] In contrast, ever smokers are exposed to cadmium through both cigarette smoking and diet, so they have relatively high exposure levels. Because the residual confounding may not be completely removed in the analysis, further studies restricted to never smokers or that collect detailed information on smoking such as quit-years of former smokers, smoking history, or biomarker of smoking (e.g., nicotine levels) are warranted.

Cadmium and zinc are 2 metallic elements in group IIB that share similar physical and chemical properties.[11] Zinc plays a critical role in major metabolic pathways either as a cofactor or to stabilize enzyme structures.[11] Their similarity allows cadmium to compete with zinc in metabolism when zinc is deficient and to destabilize the functional sites of zinc-containing proteins, which are the most abundant among all cofactor-containing proteins.[11] Because cadmium and zinc have many common food sources,[12] increasing attention has been paid to the potential joint health effect of zinc and cadmium.[2] In laboratory studies, the impairment of endothelial cell monolayer caused by cadmium toxicity is protected by zinc through prevention of cadmium-induced de-endothelialized area formation, detachment of [^{3}H] thymidine-labeled endothelial cells from the monolayer, and a decrease in the incorporation of [^{3}H]thymidine into the acid-insoluble fraction of the growing cells.[37] Zinc also suppressed cadmium-induced nonspecific cell damage marked by the activity of lactate dehydrogenase in the medium.[37] In mouse models, supplementation of zinc significantly prevented cadmium-induced changes in serum concentrations of lipid compounds and indexes of lipid peroxidation, suggesting that enhanced dietary zinc intake may protect against oxidative damage to cell membranes induced by cadmium toxicity.[13] In addition, zinc supplementation inhibited cadmium accumulation and cadmium-induced apoptotic cell death by 1.2- to 2.0-fold and decreased the production of reactive oxygen species by 1.1- to 2.0-fold in HeLa cells and bovine aorta endothelial cells.[14] In the present study, we observed that the association between cadmium and incident ischemic stroke was substantially attenuated among individuals with high serum zinc levels, which supports an inhibitory effect of zinc on cadmium toxicity in human.

The observed association between cadmium and ischemic stroke in this study was not materially modified by sex, race, or region. Some previous studies reported that the effect of cadmium on cardiovascular outcomes was more evident in women than men[8,38,39] because of the higher exposure levels and the greater susceptibility to cadmium-induced health effect in women.[40] However, no published study focusing on ischemic stroke has observed effect modification by sex.

To the best of our knowledge, this study is the first longitudinal study linking urinary cadmium to the risk of ischemic stroke. Previous studies focused mainly on all strokes as a result of a lack of information on stroke types or measured cadmium in diet. Studies have suggested that urinary cadmium reflects long-term exposure and total body burden,[41] which is crucial for us to understand the health effect of cadmium exposure in the general population. Of note, the present study is the first to provide evidence supporting the inhibitory effect of zinc on cadmium toxicity in human. This finding is of great potential clinical and public health significance because accumulated cadmium in the human body is difficult to excrete actively.[42] In addition, detailed information on smoking was collected in the present study, which enabled us to reduce the residual confounding and to study the effect modification by smoking status.

One concern of the present study is that urinary cadmium concentration was measured only at baseline, so the possible

within-person variation may affect our findings.[43] However, 1 time point measurement of urinary cadmium is considered to reflect long-term body burden because of its decades-long half-life.[44] In addition, no evidence indicates that the within-person variation in urinary cadmium is differential in this population. Thus, our findings based on the baseline cadmium exposure should not be substantially biased. The distribution of cadmium concentration in the present study is right-skewed. Because we compared the quintiles of cadmium levels, the results were less likely to be affected by extreme values. The linear trend tested using continuous cadmium levels with the exclusion of values above the 95th percentile reduces the influence of some extreme values (i.e., outliers), although the sample size was slightly reduced. In addition, similar to other observational studies, the residual confounding from dietary and environmental factors cannot be completely ruled out, although the main and sensitivity analyses showed consistent results after adjustment for available important factors, including other biomarker-measured trace minerals and heavy metals. In addition, the potential competing risk of death is not negligible in this study; thus, we used a cause-specific hazard model. Furthermore, the model used in the present study may be overspecified/underspecified because the adjustment scheme was based on ad hoc methods.

This large prospective study provides solid evidence supporting the hypothesis that cadmium exposure is an independent risk factor for ischemic stroke in black and white Americans with a low to moderate level of exposure. Findings also suggest that the adverse effect of cadmium exposure may be ameliorated by being a never smoker and maintaining a high serum zinc level.

Author contributions

Ka He: study concept and design, acquisition of data. Cheng Chen and Pengcheng Xun: analysis and interpretation of data. Cheng Chen: draft manuscript, statistical analysis. Ka He, Pengcheng Xun, Cari Tsinovoi, Leslie A. McClure, John Brockman, Leslie MacDonald, Mary Cushman, Jianwen Cai, Lisa Kamendulis, and Jason Mackey: critical revision of manuscript of intellectual content.

Acknowledgment

The authors thank the other investigators, the staff, and the participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at regardsstudy.org.

Study funding

This work was supported by a research grant from the NIH (R01ES021735). The REGARDS research project is supported by cooperative agreement U01 NS041588 from the National Institute of Neurologic Disorders and Stroke, NIH, Department of Health and Human Service. Representatives of the funding agency have been involved in the review of the manuscript but not directly involved in the collection, management, analysis, or interpretation of the data. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute for Occupational Safety and Health, the National Institute of Neurologic Disorders and Stroke, and the NIH.

Disclosure

C. Chen, P. Xun, C. Tsinovoi, L. McClure, and J. Brockman are supported by NIH grant R01ES021735. L. MacDonald works at the National Institute for Occupational Safety and Health. M. Cushman and J. Cai are supported by NIH grant R01ES021735. L. Kamendulis and J. Mackey report no disclosures relevant to the manuscript. K. He is supported by NIH grant R01ES021735. Go to Neurology.org/N for full disclosures.

Received November 6, 2017. Accepted in final form April 13, 2018.

References

1. Messner B, Bernhard D. Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance. Biometals 2010;23:811–822.
2. Messner B, Knoflach M, Seubert A, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. Arterioscler Thromb Vasc Biol 2009;29:1392–1398.
3. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Guallar E. Cadmium exposure and hypertension in the 1999–2004 National Health and Nutrition Examination Survey (NHANES). Environ Health Perspect 2008;116:51–56.
4. Pizent A, Jurasovie J, Telisman S. Blood pressure in relation to dietary calcium intake, alcohol consumption, blood lead, and blood cadmium in female nonsmokers. J Trace Elem Med Biol 2001;15:123–130.
5. Staessen JA, Kuznetsova T, Roels HA, Emelianov D, Fagard R. Exposure to cadmium and conventional and ambulatory blood pressures in a prospective population study. Am J Hypertens 2000;13:146–156.
6. Eum KD, Lee MS, Paek D. Cadmium in blood and hypertension. Sci Total Environ 2008;407:147–153.
7. Julin B, Bergkvist C, Wolk A, Åkesson A. Cadmium in diet and risk of cardiovascular disease in women. Epidemiology 2013;24:880–885.
8. Barregard L, Sallsten G, Fagerberg B, et al. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish adults: the Malmo Diet and Cancer Study. Environ Health Perspect 2016;124:594–600.
9. Julin B, Wolk A, Thomas LD, Åkesson A. Exposure to cadmium from food and risk of cardiovascular disease in men: a population-based prospective cohort study. Eur J Epidemiol 2013;28:837.
10. Kernan WN, Ovbiagele B, Black HR, et al. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2014;45:2160–2236.
11. Tang L, Qiu R, Tang Y, Wang S. Cadmium-zinc exchange and their binary relationship in the structure of Zn-related proteins: a mini review. Metallomics 2014;6:1313–1323.
12. Pang Y, Peng RD, Jones MR, et al. Metal mixtures in urban and rural populations in the US: the Multi-Ethnic Study of Atherosclerosis and the Strong Heart Study. Environ Res 2016;147:356–364.
13. Rogalska J, Brzóska MM, Roszczenko A, Moniuszko-Jakoniuk J. Enhanced zinc consumption prevents cadmium-induced alterations in lipid metabolism in male rats. Chem Biol Interact 2009;177:142–152.
14. Szuster-Ciesielska A, Stachura A, Słotwińska M, et al. The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. Toxicology 2000;145:159–171.
15. Howard VJ, Cushman M, Pulley L, et al. The Reasons for Geographic and Racial Differences in Stroke Study: objectives and design. Neuroepidemiology 2005;25:135–143.
16. Howard VJ, Kleindorfer DO, Judd SE, et al. Disparities in stroke incidence contributing to disparities in stroke mortality. Ann Neurol 2011;69:619–627.
17. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika 1986;73:1–11.
18. Cushman M, Jenny NS, Zakai NA, et al. N-terminal pro-B-type natriuretic peptide and stroke risk: the Reasons for Geographic and Racial Differences in Stroke Cohort. Stroke 2014;45:1646–1650.
19. Cai J, Zeng D. Sample size/power calculation for case-cohort studies. Biometrics 2004;60:1015–1024.
20. Kabagambe EK, Judd SE, Howard VJ, et al. Inflammation biomarkers and risk of all-cause mortality in the Reasons for Geographic and Racial Differences in Stroke Cohort. Am J Epidemiol 2011;174:284–292.

21. Jarrett JM, Xiao G, Caldwell KL, Henahan D, Shakirova G, Jones RL. Eliminating molybdenum oxide interference in urine cadmium biomonitoring using ICP-DRC-MS. J Anal At Spectrom 2008;23:962–967.
22. McElroy JA, Kruse RL, Guthrie J, Gangnon RE, Robertson JD. Cadmium exposure and endometrial cancer risk: a large Midwestern U.S. population-based case-control study. PLoS One 2017;12:e0179360.
23. Aaron KJ, Campbell RC, Judd SE, Sanders PW, Muntner P. Association of dietary sodium and potassium intakes with albuminuria in normal-weight, overweight, and obese participants in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Clin Nutr 2011;94:1071–1078.
24. Cushman M, McClure LA, Howard VJ, Jenny NS, Lakoski SG, Howard G. Implications of increased C-reactive protein for cardiovascular risk stratification in black and white men and women in the US. Clin Chem 2009;55:1627–1636.
25. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612.
26. Barlow WE. Robust variance estimation for the case-cohort design. Biometrics 1994; 50:1064–1072.
27. Johner SA, Boeing H, Thamm M, Remer T. Urinary 24-h creatinine excretion in adults and its use as a simple tool for the estimation of daily urinary analyte excretion from analyte/creatinine ratios in populations. Eur J Clin Nutr 2015;69: 1336–1343.
28. Sorahan T. Lung cancer mortality in arsenic-exposed workers from a cadmium recovery plant. Occup Med (Lond) 2009;59:264–266.
29. Tsinovoi CL, Xun P, McClure LA, et al. Arsenic exposure in relation to ischemic stroke: the Reasons for Geographic and Racial Differences in Stroke study. Stroke 2018;49:19–26.
30. Muntner P, Judd SE, McClellan W, Meschia JF, Warnock DG, Howard VJ. Incidence of stroke symptoms among adults with chronic kidney disease: results from the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. Nephrol Dial Transpl 2012;27:166–173.
31. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994;81:515–526.
32. He K. Trace elements in nails as biomarkers in clinical research. Eur J Clin Invest 2011;41:98–102.
33. Tellez-Plaza M, Jones MR, Dominguez-Lucas A, Guallar E, Navas-Acien A. Cadmium exposure and clinical cardiovascular disease: a systematic review. Curr Atheroscler Rep 2013;15:356.
34. Lee MS, Park SK, Hu H, Lee S. Cadmium exposure and cardiovascular disease in the 2005 Korea National Health and Nutrition Examination Survey. Environ Res 2011; 111:171–176.
35. Lakkur S, Judd SE. Diet and stroke: recent evidence supporting a Mediterranean-style diet and food in the primary prevention of stroke. Stroke 2015;46:2007–2011.
36. Tsivgoulis G, Psaltopoulou T, Wadley VG, et al. Adherence to a Mediterranean diet and prediction of incident stroke. Stroke 2015;46:780–785.
37. Toshiyuki K, Atsushi M, Chika Y, Michiko S, Hiroshi K. Zinc protection against cadmium-induced destruction of the monolayer of cultured vascular endothelial cells. Toxicol Lett 1993;66:247–255.
38. Peters JL, Perlstein TS, Perry MJ, McNeely E, Weuve J. Cadmium exposure in association with history of stroke and heart failure. Environ Res 2010;110:199–206.
39. Qian L, Nishijo M, Nakagawa H, et al. Relationship between urinary cadmium and mortality in habitants of a cadmium-polluted area: a 22-year follow-up study in Japan. Chin Med J 2011;124:3504–3509.
40. Vahter M, Åkesson A, Lidén C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. Environ Res 2007;104:85–95.
41. Tellez-Plaza M, Navas-Acien A, Menke A, Crainiceanu CM, Pastor-Barriuso R, Guallar E. Cadmium exposure and all-cause and cardiovascular mortality in the US general population. Environ Health Perspect 2012;120:1017.
42. Nordberg GF, Kjellström T. Metabolic model for cadmium in man. Environ Health Perspect 1979;28:211.
43. Akerstrom M, Sallsten G, Lundh T, Barregard L. Associations between urinary excretion of cadmium and proteins in a nonsmoking population: renal toxicity or normal physiology? Environ Health Perspect 2013;121:187–191.
44. Nordberg G. Handbook on the Toxicology of Metals. 3rd ed. Amsterdam: Academic Press; 2007.