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Associations of blood lead with estimated glomerular filtration rate using MDRD, CKD-EPI and serum cystatin C-based equations

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Abstract

Background. Low-level lead exposure is widespread and has been implicated as a chronic kidney disease (CKD) risk factor. However, studies evaluating associations of lead dose with newer, potentially more accurate, estimates of kidney function, in participants with a wide range of glomerular filtration rates (GFRs), are scarce.

Methods. We compared associations of blood lead and estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and cystatin C single variable, multivariable and combined creatinine/cystatin C equations in 3941 adults who participated in the 1999–2002 National Health and Nutrition Examination Survey cystatin C subsample.

Results. Geometric mean blood lead was 1.7 μg/dL. After multivariable adjustment, differences [95% confidence interval (CI)] in mean eGFR for a doubling of blood lead were −1.9 (−3.2, −0.7), −1.7 (−3.0, −0.5) and −1.4 (−2.3, −0.5) mL/min/1.73 m^2, using the cystatin C single variable, multivariable and combined creatinine/cystatin C equations, respectively, reflecting lower eGFR with increased blood lead. The corresponding differences (95% CI) were −0.9 (−1.9, 0.02) and −0.9 (−1.8, 0.01) using the creatinine-based MDRD and CKD-EPI equations, respectively. In participants aged ≥60 years, differences in mean eGFR ranged from −3.0 to −4.5 mL/min/1.73 m^2, and odds of reduced eGFR (<60 mL/min/1.73 m^2) were increased for all estimates of GFR.

Conclusions. These results support the inclusion of cystatin C-based eGFR in future lead research and provide additional evidence for environmental lead exposure as a CKD risk factor.

Keywords: blood lead; kidney function; lead exposure; NHANES

Introduction

Recent research suggests that environmental lead exposure increases risk for chronic kidney disease (CKD), even at the lower levels currently observed in the USA and other developed countries [1–8]. The association between lead exposure and CKD has been observed in prospective studies, in a variety of populations, and is consistent with experimental and mechanistic evidence [2, 3, 5, 9–14]. Environmental lead exposure remains widespread globally [6, 7, 15]. Moreover, lead accumulated in bone from past exposure remains a source of current endogenous exposure [16]. The increasing prevalence of CKD [17] and the fact that lead exposure is preventable and treatable with chelation in selected settings [18] highlight the need to fully characterize kidney risk from lead exposure. In such research, accurate assessment of kidney function is essential to avoid kidney disease misclassification resulting in underestimation of risk. Equations to estimate glomerular filtration rate (GFR) are the most common method for assessing kidney function clinically and in large epidemiologic studies, where GFR assessment with an exogenous filtration marker is not possible. Ongoing efforts to improve the accuracy of these approaches have resulted in new serum creatinine-based equations and equations incorporating serum cystatin C. However, publications utilizing these newer techniques in research on the impact of lead on the kidney are scarce.

The objective of this study was to evaluate these recently developed GFR-estimating approaches in lead research. Therefore, we compared associations of blood lead level with estimated glomerular filtration rate (eGFR) calculated with recently developed equations to associations using the Modification of Diet in Renal Disease (MDRD) equation [20, 21], a serum creatinine-based equation routinely used in clinical practice and research. We used four new

approaches: the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [22], developed to be more accurate than the MDRD equation at higher GFRs, and three serum cystatin C-based equations: (i) cystatin C only; (ii) cystatin C, age, sex and race and (iii) cystatin C, age, sex, race and serum creatinine [23]. We used data from US adults who participated in the cystatin C subsample of the 1999–2002 National Health and Nutrition Examination Survey (NHANES). To our knowledge, this is the first study to evaluate associations of lead dose with these potentially more accurate eGFR approaches.

Materials and methods

Study population

NHANES 1999–2002 was conducted using a complex multistage sampling design to obtain a representative sample of the noninstitutionalized, civilian US population [24]. The study protocols were approved by the National Center for Health Statistics Institutional Review Board. All participants provided oral and written consent.

In 2006, cystatin C was assayed on stored serum samples from all NHANES 1999–2002 participants aged ≥60 years as well as on a 25% random sample of those aged 12–59 years [25, 26]. The younger group was supplemented with all individuals with a serum creatinine >1.2 mg/dL (SI conversion: multiply by 88.4 for micromoles per liter) in males and >1.0 mg/dL in females [25, 26]. Of 4563 adults aged ≥20 years with cystatin C measures available, we excluded pregnant women and those missing blood lead levels and other variables of interest, leaving 3941 participants with complete data.

Blood lead measurement

Blood lead was measured at the Centers for Disease Control and Prevention's National Center for Environmental Health [24]. Lead was measured in whole blood together with cadmium using a Perkin-Elmer Model SIMAA 6000 simultaneous multielement atomic absorption spectrometer with Zeeman background correction. Strict quality control procedures were followed including confirmation that collection and storage materials were not contaminated. The limit of detection was 0.3 μg/dL [27]; results were below the limit of detection in 0.5% of participants in our study population. For these values, a level equal to the limit of detection divided by the squared root of two was imputed [28, 29]. National Institute of Standards and Technology whole-blood standard reference materials were used for external calibration. The interassay coefficients of variation ranged from 3.1 to 7.0% for concentrations ranging from 2.1 to 29.3 μg/dL [30, 31].

Estimates of GFR

Serum creatinine was measured using a kinetic rate Jaffé method with a Hitachi Model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) [24]. Serum creatinine concentrations were calibrated to standard creatinine [32]. The interassay coefficients of variation were 2.7 and 2.2% at mean creatinine concentrations of 1.67 and 6.51 mg/dL, respectively, for 1999–2000 [33] and 4.4 and 1.5% at mean creatinine concentrations of 0.68 and 7.0 mg/dL, respectively, for 2001–2002 [34]. Serum cystatin C was measured using an automated particle-enhanced nephelometric assay (Dade Behring N Latex Cystatin C run on a Dade Behring Nephelometer II; Siemens Healthcare Diagnostics, Deerfield, IL) [24]. The assay range was 0.23–7.25 mg/L. The interassay coefficients of variation were 5.05 and 4.87% at mean cystatin C concentrations of 0.97 and 1.90 mg/L, respectively [35]. The following equations were used to estimate GFR:

- MDRD eGFR = $175 \times (\text{serum creatinine})^{-1.154} \times \text{age}^{-0.203} \times 1.212$ (if black) $\times$ 0.742 (if female) [20]
- CKD-EPI eGFR = $141 \times \min(\text{Scr}/\kappa, 1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.159$ (if black) $\times$ 1.018 (if female), where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1 and max indicates the maximum of Scr/κ or 1 [22]
- Cystatin C single variable eGFR = $76.7 \times \text{serum cystatin C}^{-1.19}$ [23]
- Cystatin C multivariable eGFR = $127.7 \times (\text{serum cystatin C})^{-1.17} \times \text{age}^{-0.13} \times 1.06$ (if black) $\times$ 0.91 (if female) [23]
- Combined cystatin C/creatinine eGFR = $177.6 \times \text{serum creatinine}^{-0.65} \times \text{serum cystatin C}^{-0.57} \times \text{age}^{-0.20} \times (0.82$ if female) $\times (1.11$ if black) [23]

Other variables

Information on age, sex, race/ethnicity, education, smoking, income and alcohol consumption was based on self-report [24]. Body mass index was calculated by dividing measured weight in kilograms by measured height in meters squared. Serum cotinine was measured by an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method. Hypertension was defined as a mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥90 mmHg, based on three blood pressure measurements obtained during the medical examination, or a self-reported physician diagnosis. Diabetes mellitus was defined as a fasting glucose ≥126 mg/dL, a non-fasting glucose ≥200 mg/dL or a self-reported physician diagnosis.

Statistical analysis

Data were obtained from the NHANES Web site [24] and merged and analyzed using STATA 10 (StataCorp, College Station, TX). Statistical analyses were performed using the survey commands in STATA 10 with specific weights for the cystatin C subsample to account for the complex sampling design.

Distribution of blood lead was right skewed and log-transformed for the analyses. Tertile cut-points were based on weighted distributions in the whole study population. In separate linear regression models for each equation, differences in mean eGFR were estimated comparing each of the two higher tertiles of blood lead to the lowest tertile and for doubling of lead levels. These linear regression models were conducted in all participants and in those <60 and ≥60 years of age. In separate logistic regression models for each equation, odds ratios for reduced eGFR (<60 mL/min/1.73 m^2) were estimated only in participants ≥60 years because few participants <60 years of age had reduced eGFRs (103 participants with the MDRD equation and 53–60 participants with the other equations). Model adjustment was based on biological plausibility and known lead and kidney confounders. Adjustment for cadmium was performed since this is another proximal tubule nephrotoxicant for which exposure is common environmentally. P-values for linear trend in logistic and linear regression models were obtained by including blood lead tertiles coded as ordinal variables; results obtained by entering blood lead as tertile medians were similar. To assess the dose–response relationship in a flexible manner, we also estimated odds ratios for reduced eGFR by modeling blood lead levels with restricted quadratic splines with knots at the 5th, 50th and 95th percentiles. Spline models were conducted only in participants ≥60 years due to the small number of participants <60 years of age with eGFR <60 mL/min/1.73 m^2.

Results

The geometric mean blood lead was 1.7 μg/dL for all participants, 2.2 μg/dL for participants aged ≥60 years and 1.6 μg/dL for participants aged <60 years. Corresponding values for blood cadmium were 0.45, 0.50 and 0.44 μg/L, respectively. Median eGFR levels were lowest using the MDRD equation (Table 1). The weighted prevalence of reduced eGFR (<60 mL/min/1.73 m^2) was 8.4% for the MDRD equation, 6.5% for the CKD-EPI equation and 6.6%, 8.6% and 6.4% for the three cystatin C equations, respectively, consistent with previous prevalence estimates [17]. Geometric mean [95% confidence interval (CI)] blood lead levels for participants with cystatin C multivariable eGFR <60 and ≥60 mL/min/1.73 m^2 were 2.3 (2.1, 2.5) and 1.7 (1.6, 1.7) μg/dL, respectively. Mean blood lead levels were also significantly higher in participants with reduced kidney function using the other equations

Table 1. Median (interquartile range) levels of blood lead and eGFR by participant characteristics using different estimating equations (BMI, body mass index)[a]

Characteristic	*n* (weighted %)	Blood lead level (μg/dL)[a]	MDRD	CKD-EPI	Cystatin C single variable	Cystatin C multivariable	Cystatin C/ creatinine
Overall	3941 (100.0)	1.7 (1.1, 2.5)	85.0 (73.1, 99.6)	94.5 (80.0, 108.6)	95.7 (80.5, 109.8)	93.4 (77.1, 108.4)	93.8 (80.2, 108.3)
Age <60 years	1332 (77.3)	1.6 (1.0, 2.3)	89.3 (77.4, 103.4)	100.8 (87.1, 112.6)	100.0 (86.9, 113.4)	98.7 (85.8, 113.5)	99.5 (87.4, 112.6)
Age ≥60 years	2609 (22.7)	2.2 (1.6, 3.1)	70.5 (59.0, 82.5)	73.4 (60.3, 85.0)	76.7 (63.0, 89.3)	70.7 (57.7, 82.0)	73.2 (60.9, 84.2)
Male	2010 (49.2)	2.1 (1.4, 3.0)	86.2 (74.1, 99.8)	94.4 (81.0, 107.5)	93.1 (79.5, 104.7)	96.2 (79.7, 110.9)	95.0 (82.0, 108.0)
Female	1931 (50.8)	1.4 (0.9, 2.1)	83.5 (71.3, 99.6)	94.5 (78.6, 109.5)	97.1 (81.5, 113.4)	91.5 (74.2, 106.1)	92.7 (78.3, 108.5)
White	2113 (72.8)	1.7 (1.1, 2.4)	82.2 (70.9, 93.5)	91.4 (77.6, 104.2)	93.1 (78.6, 106.3)	90.8 (75.0, 103.3)	90.7 (78.0, 102.8)
Black	705 (10.2)	1.7 (1.2, 2.8)	97.7 (79.7, 111.1)	104.7 (84.5, 121.1)	103.1 (84.7, 117.3)	106.9 (86.1, 123.2)	104.4 (87.5, 120.2)
Mexican American	861 (6.9)	1.7 (1.1, 2.8)	102.0 (86.8, 117.9)	111.8 (97.1, 121.8)	108.0 (93.1, 121.4)	109.4 (93.0, 122.9)	112.4 (96.3, 125.9)
Other race/ ethnicity	262 (10.0)	1.9 (1.0, 2.7)	92.9 (77.6, 109.9)	102.7 (86.2, 115.2)	100.0 (84.7, 115.3)	98.0 (83.1, 116.1)	103.5 (85.5, 117.9)
<High school	1482 (21.5)	1.9 (1.3, 3.1)	91.3 (74.6, 105.8)	99.0 (80.2, 115.0)	93.1 (75.8, 108.0)	90.3 (71.3, 109.6)	94.9 (78.1, 113.2)
High school graduation	934 (27.5)	1.9 (1.2, 2.8)	85.4 (72.4, 100.3)	95.7 (79.2, 109.4)	93.1 (79.5, 106.3)	91.4 (75.6, 104.5)	92.2 (78.8, 108.6)
>High school	1525 (51.0)	1.5 (1.0, 2.2)	83.0 (72.9, 95.3)	93.2 (80.1, 105.2)	98.6 (82.6, 111.5)	96.2 (80.6, 110.7)	93.8 (81.1, 106.7)
BMI <25 kg/m^2	1209 (36.2)	1.7 (1.1, 2.7)	86.1 (73.8, 100.4)	96.1 (81.8, 110.5)	100.0 (85.8, 115.3)	98.1 (82.9, 114.5)	98.1 (84.2, 110.8)
BMI 25–29 kg/m^2	1504 (34.1)	1.8 (1.1, 2.6)	83.2 (72.0, 97.9)	92.7 (77.9, 106.7)	94.4 (79.5, 108.0)	93.3 (77.5, 107.0)	92.0 (78.6, 107.3)
BMI ≥30 kg/m^2	1228 (29.7)	1.6 (1.0, 2.3)	85.5 (73.1, 101.2)	94.0 (80.0, 108.1)	89.3 (74.9, 103.1)	86.8 (71.0, 101.8)	90.2 (77.8, 105.6)
Never smoker	1921 (49.1)	1.4 (0.9, 2.1)	83.9 (72.1, 98.9)	94.4 (78.7, 108.8)	98.6 (81.5, 113.4)	96.1 (78.2, 111.8)	94.6 (80.2, 110.4)
Former smoker	1330 (26.4)	1.8 (1.3, 2.7)	80.7 (70.5, 91.4)	89.1 (76.5, 101.4)	94.4 (79.5, 108.0)	91.2 (75.2, 103.7)	90.7 (77.1, 102.2)
Current smoker	690 (24.5)	2.1 (1.5, 3.2)	91.1 (77.9, 104.5)	102.1 (88.0, 115.8)	91.8 (79.5, 103.1)	91.7 (77.5, 105.7)	97.6 (83.7, 110.7)
Cotinine <0.3 ng/mL[a]	2717 (60.6)	1.5 (1.0, 2.3)	82.6 (70.9, 95.9)	91.4 (77.3, 104.9)	97.1 (81.5, 111.5)	94.5 (76.7, 108.8)	92.4 (78.6, 106.8)
Cotinine 0.3–2.9 ng/mL[a]	333 (9.3)	1.4 (1.0, 2.3)	88.3 (72.6, 99.2)	100.0 (81.7, 111.8)	95.7 (80.5, 109.8)	96.2 (80.1, 113.5)	98.6 (81.0, 110.3)
Cotinine 3.0–99.0 ng/mL[a]	275 (8.4)	2.0 (1.2, 2.7)	90.5 (76.4, 105.0)	102.1 (84.9, 116.7)	100.0 (79.5, 113.4)	98.6 (79.9, 116.9)	98.2 (83.5, 117.0)
Cotinine ≥100 ng/mL[a]	616 (21.6)	2.2 (1.6, 3.4)	90.4 (76.7, 103.7)	100.8 (85.0, 112.9)	89.3 (79.5, 100.0)	90.7 (77.4, 103.2)	94.7 (82.5, 108.0)
Never alcohol drinker	1383 (28.5)	1.4 (1.0, 2.1)	83.7 (70.0, 98.9)	93.2 (76.0, 106.5)	89.3 (73.2, 106.3)	85.7 (68.5, 105.2)	89.5 (73.9, 107.5)
Former alcohol drinker	501 (7.9)	1.9 (1.3, 2.7)	84.1 (71.1, 99.5)	88.6 (75.0, 102.2)	89.3 (74.0, 104.7)	86.3 (67.9, 103.7)	88.7 (72.9, 104.9)
Current alcohol drinker	2057 (63.5)	1.8 (1.2, 2.6)	85.6 (73.8, 100.0)	95.8 (82.0, 110.3)	97.1 (84.7, 111.5)	96.9 (82.0, 110.7)	95.6 (83.3, 109.2)
Diabetes	547 (6.8)	2.0 (1.2, 2.8)	81.2 (63.8, 104.4)	86.2 (67.0, 104.9)	82.6 (65.6, 104.7)	75.9 (62.7, 103.0)	83.5 (65.2, 103.0)
No diabetes	3394 (93.2)	1.7 (1.1, 2.5)	85.1 (73.4, 99.5)	94.8 (80.4, 108.7)	95.7 (81.5, 109.7)	94.5 (78.4, 109.1)	94.1 (81.0, 108.4)
Hypertension	2210 (35.3)	1.9 (1.3, 2.7)	78.3 (65.5, 91.1)	84.5 (69.0, 98.8)	85.8 (71.6, 100.0)	81.6 (65.7, 97.4)	84.4 (69.8, 98.0)
No hypertension	1731 (64.7)	1.6 (1.0, 2.4)	88.6 (76.4, 102.0)	99.8 (85.6, 111.8)	100.0 (85.8, 113.4)	98.5 (84.5, 113.5)	99.3 (86.1, 112.1)

[a]Conversion factors for units: to convert lead to micromoles per liter, multiply by 0.0483; to convert cotinine to nanomoles per liter, multiply by 5.68. Kidney outcomes in mL/min/1.73 m^2.

(data not shown). All eGFR measures were highly correlated, particularly for eGFR levels calculated with the same serum measure: correlations between eGFR calculated using the MDRD equation and eGFR using CKD-EPI, cystatin C single variable, cystatin C multivariable and cystatin C/creatinine equations were 0.95, 0.71, 0.72 and 0.93, respectively (Appendices 1–3).

In the overall sample, multivariable adjusted differences (95% CI) in mean eGFR for a doubling of blood lead were −1.9 (−3.2, −0.7), −1.7 (−3.0, −0.5) and −1.4 (−2.3, −0.5) mL/min/1.73 m^2 for the cystatin C single variable, cystatin C multivariable and cystatin C/creatinine equations, respectively (Table 2). The corresponding differences (95% CI) were −0.9 (−1.9, 0.02) and −0.9 (−1.8, 0.01) using the creatinine-based MDRD and CKD-EPI equations, respectively. For comparison with studies that used serum creatinine and cystatin C as kidney outcomes without incorporating them into estimating equations, fully adjusted mean differences (95% CI) for a doubling of blood lead for serum creatinine and cystatin C were 0.05 (0.02, 0.07) mg/dL and 0.04 (0.02, 0.07) mg/L, respectively. The difference in mean level (95% CI) for a doubling of blood lead using the traditional Cockcroft–Gault equation for creatinine clearance [36] was −2.2 (−3.5, −0.8); however, median creatinine clearance was 107.7 mL/min indicating a substantial overestimation of GFR. After correction for body surface area, an approach reported to be more accurate [37] and providing a more comparable result, the difference in mean level (95% CI) was −1.4 (−2.4, −0.3) although the median (98.3 mL/min/1.73 m^2) remained higher than those using the eGFR equations.

In participants aged ≥60 years, differences in mean eGFR for a doubling of blood lead ranged from −3.0 to −4.5 mL/min/1.73 m^2 across equations (Table 2). In younger participants, the differences were smaller (range −0.2 to −2.2 mL/min/1.73 m^2) and, although not statistically significant, were larger for the cystatin C single and multivariable estimates.

The adjusted odds ratios (95% CI) for reduced eGFR (<60 mL/min/1.73 m^2) for increasing blood lead levels in

Table 2. Differences (95% confidence interval) in mean eGFR (mL/min/1.73 m^2) by blood lead levels[a]

Blood lead, μg/dL[b]	Mean eGFR in all participants	All participants[a]	Age <60[a]	Age ≥60[a]
MDRD				
Tertile 1 (≤1.3)[c]	91.4	0.00 (reference)	0.00 (reference)	0.00 (reference)
Tertile 2 (>1.3–2.2)[c]	84.5	−1.7 (−3.7, 0.2)	−1.1 (−3.5, 1.3)	−3.8 (−5.8, −1.9)
Tertile 3 (>2.2)[c]	83.2	−2.4 (−4.5, −0.3)	−0.8 (−3.4, 1.7)	−7.1 (−9.5, −4.8)
P trend		0.03	0.5	<0.001
Doubling of lead level		−0.9 (−1.9, 0.02)	−0.2 (−1.2, 0.9)	−3.3 (−4.8, −1.9)
CKD-EPI				
Tertile 1 (≤1.3)[c]	99.8	0.00 (reference)	0.00 (reference)	0.00 (reference)
Tertile 2 (>1.3–2.2)[c]	91.2	−1.3 (−2.9, 0.3)	−0.9 (−2.8, 1.0)	−3.1 (−5.0, −1.2)
Tertile 3 (>2.2)[c]	88.4	−1.8 (−3.7, 0.1)	−0.3 (−2.7, 2.0)	−6.1 (−8.3, −3.9)
P trend		0.07	0.7	<0.001
Doubling of lead level		−0.9 (−1.8, 0.01)	−0.2 (−1.2, 0.8)	−3.0 (−4.2, −1.8)
Cystatin C single variable				
Tertile 1 (≤1.3)[c]	100.6	0.00 (reference)	0.00 (reference)	0.00 (reference)
Tertile 2 (>1.3–2.2)[c]	93.7	−1.6 (−4.2, 1.0)	−1.2 (−4.3, 2.0)	−4.5 (−6.7, −2.3)
Tertile 3 (>2.2)[c]	88.2	−3.3 (−5.3, −1.4)	−2.2 (−4.9, 0.4)	−7.8 (−10.3, −5.2)
P trend		0.001	0.09	<0.001
Doubling of lead level		−1.9 (−3.2, −0.7)	−1.3 (−2.8, 0.3)	−4.5 (−5.6, −3.3)
Cystatin C multivariable				
Tertile 1 (≤1.3)[c]	98.8	0.00 (reference)	0.00 (reference)	0.00 (reference)
Tertile 2 (>1.3–2.2)[c]	91.4	−1.2 (−3.6, 1.2)	−1.0 (−3.9, 2.0)	−3.7 (−5.7, −1.8)
Tertile 3 (>2.2)[c]	86.9	−2.9 (−4.7, −1.1)	−1.9 (−4.5, 0.6)	−6.8 (−9.0, −4.6)
P trend		0.003	0.1	<0.001
Doubling of lead level		−1.7 (−3.0, −0.5)	−1.1 (−2.7, 0.4)	−4.0 (−5.0, −2.9)
Cystatin C/creatinine				
Tertile 1 (≤1.3)[c]	100.6	0.00 (reference)	0.00 (reference)	0.00 (reference)
Tertile 2 (>1.3–2.2)[c]	91.8	−1.7 (−3.6, 0.3)	−1.2 (−3.6, 1.2)	−4.2 (−6.0, −2.4)
Tertile 3 (>2.2)[c]	88.7	−2.8 (−4.3, −1.2)	−1.3 (−3.3, 0.7)	−7.6 (−9.8, −5.4)
P trend		0.001	0.2	<0.001
Doubling of lead level		−1.4 (−2.3, −0.5)	−0.7 (−1.7, 0.4)	−3.9 (−5.2, −2.7)

[a] Models adjusted for survey year, age (years modeled as restricted cubic spline with five knots), sex, race/ethnicity, body mass index (kg/m^2), education (<high school, high school, >high school), smoking status (never, former, current), cotinine category, alcohol intake (never, former, current), hypertension (yes, no), diabetes mellitus (yes, no) and blood cadmium (ln μg/L).
[b] Blood lead levels (ug/dL). Conversion factors for units: to convert lead to micromoles per liter, multiply by 0.0483.
[c] Blood lead levels (μg/dL).

participants ≥60 years of age were similar for all equations (Table 3 and Figure 1). In models adjusted for all covariates except cadmium, differences in mean kidney outcome and odds ratios for reduced kidney function were consistent with fully adjusted models but were generally stronger.

Discussion

In this large representative sample of US adults, higher blood lead levels were associated with lower eGFR levels and reduced eGFR (<60 mL/min/1.73 m^2) with all equations examined. Mean differences in eGFR by blood lead levels were larger with cystatin C compared to creatinine equations in analyses in all participants reflecting results in those <60 years of age. For all equations, differences in eGFR levels with increasing blood lead levels were larger for participants ≥60 years of age. In this age group, mean eGFR differences for a doubling in blood lead levels ranged from −3.0 mL/min/1.73 m^2 with the CKD-EPI equation to −4.5 mL/min/1.73 m^2 with the cystatin C single variable equation. Odds of reduced eGFR for a doubling of blood lead level, examined in participants ≥60 years, were consistently increased with all equations.

Lead is a widespread environmental toxicant [15, 38]. In the human body, lead accumulates in bone and the biological half-life is on the order of decades [16]. Thus, although exposure to lead has decreased in developed countries after the institution of public health measures banning lead in gasoline, paint and solder, the body burden of lead resulting from past exposures remains an important source of endogenous exposure [16, 18]. Moreover, exogenous exposure continues to occur through folk remedies, glazed pottery, industrial sources, lead paint, active smoking and exposure to secondhand smoke [6, 39, 40]. Certain populations are disproportionately exposed to lead, especially workers in occupations such as construction and residents in low socioeconomic status communities [6]. Globally, exposure remains higher in developing countries [41–43]. Given the magnitude of exposure, the impact of lead dose on kidney function is a substantial public health concern.

Our results are consistent with publications in other NHANES analyses using the MDRD equation to estimate GFR [7, 8]. The CKD-EPI equation was recently published [22], and to our knowledge, there are no publications examining associations between blood lead and GFR estimated with this equation. A few studies have examined associations between blood lead and kidney function using serum cystatin C or single variable cystatin C-based eGFR equations [1, 4, 44, 45]. In a cross-sectional study of Swedish women, higher blood lead levels were associated with lower serum cystatin C-based eGFR [4, 46].

Table 3. Odds ratios (95% confidence interval) for reduced eGFR (<60 mL/min/1.73 m^2) by blood lead levels for participants ≥60 years of age[a]

	Cases/ noncases (weighted %)	Odds ratios
MDRD		
≤1.3[b]	78/358 (20.6)	1.00 (reference)
>1.3–2.2[b]	179/655 (23.8)	1.29 (0.87, 1.93)
>2.2[b]	391/948 (31.2)	1.90 (1.26, 2.87)
P trend		0.002
Doubling of lead level		1.38 (1.17, 1.63)
CKD-EPI		
≤1.3[b]	76/360 (19.6)	1.00 (reference)
>1.3–2.2[b]	164/670 (21.1)	1.14 (0.76, 1.71)
>2.2[b]	382/957 (29.2)	1.78 (1.18, 2.69)
P trend		0.003
Doubling of lead level		1.37 (1.15, 1.62)
Cystatin C single variable		
≤1.3[b]	68/368 (15.8)	1.00 (reference)
>1.3–2.2[b]	146/688 (19.2)	1.25 (0.86, 1.82)
>2.2[b]	332/1007 (24.4)	1.57 (1.01, 2.46)
P trend		0.040
Doubling of lead level		1.41 (1.17, 1.70)
Cystatin C multivariable		
≤1.3[b]	97/339 (22.4)	1.00 (reference)
>1.3–2.2[b]	214/620 (27.3)	1.48 (1.04, 2.12)
>2.2[b]	423/916 (33.0)	2.02 (1.28, 3.17)
P trend		0.004
Doubling of lead level		1.53 (1.31, 1.80)
Cystatin C/creatinine		
≤1.3[b]	70/366 (17.4)	1.00 (reference)
>1.3–2.2[b]	163/671 (20.6)	1.33 (0.95, 1.86)
>2.2[b]	354/985 (27.2)	2.00 (1.29, 3.08)
P trend		0.003
Doubling of lead level		1.46 (1.21, 1.75)

[a] Models adjusted for survey year, age (years modeled as restricted cubic spline with five knots), sex, race/ethnicity, body mass index (kg/m^2), education (<high school, high school, >high school), smoking status (never, former, current), cotinine category, alcohol intake (never, former, current), hypertension (yes, no), diabetes mellitus (yes, no) and blood cadmium (ln μg/L).

[b] Blood lead levels (μg/dL). Conversion factors for units: to convert lead to micromoles per liter, multiply by 0.0483.

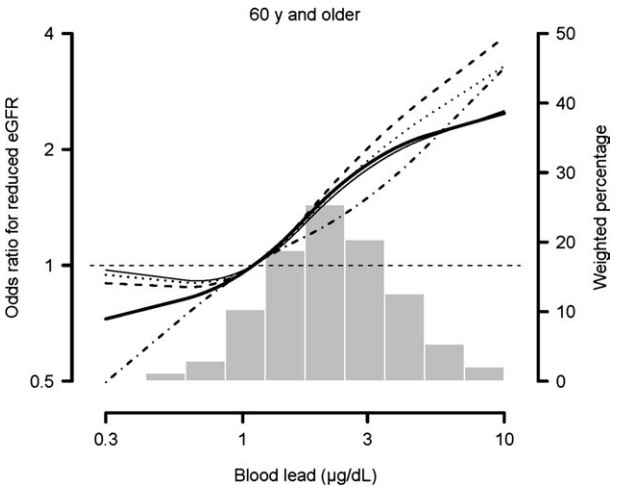

Fig. 1. Odds ratios for reduced eGFR with blood lead levels modeled with restricted quadratic splines with knots at the 5th, 50th and 95th percentiles. MDRD: thick solid line; CKD-EPI: thin solid line; cystatin C single variable: dashed and dotted line; cystatin C multivariable: dashed line; cystatin C/creatinine combined: dotted line.

Associations were comparable to estimates using creatinine clearance as the kidney outcome [4]. An association between blood lead level and serum cystatin C was observed in Belgian adolescents [1]. In US adolescents, blood lead levels were associated with decreased cystatin C-based eGFR [47] levels; the association with creatinine-based eGFR was not statistically significant [44]. In a cross-sectional study of European children, on the other hand, higher blood lead levels were associated with lower serum cystatin C and creatinine levels and these paradoxical associations were attributed to hyperfiltration [45].

Strengths of our study include those related to NHANES data: a relatively large sample size; representation of the US noninstitutionalized civilian population; high-quality, standardized laboratory procedures and extensive quality control. This is also one of the few data sets to date that includes serum creatinine and cystatin C and blood lead. Limitations include lack of GFR measurement using an exogenous filtration marker. The GFR-estimating equations used in this study have important limitations and differences. The MDRD equation systematically underestimates GFR at higher levels; the CKD-EPI equation was developed to be more accurate in this range [22]. Both equations use serum creatinine, which is generated from muscle metabolism and overestimates GFR in individuals with low muscle mass such as the elderly [19]. Cystatin C is a 120-amino acid cysteine protease inhibitor that is freely filtered at the glomerulus and reabsorbed and catabolized in the proximal tubules [19]. It is produced and secreted by all nucleated cells [19]; the resulting lack of muscle mass confounding may increase its accuracy as a kidney filtration marker [48, 49]. Research to assess accuracy of cystatin C-based eGFR is ongoing [50, 51]. Associations between cystatin C and age, sex, race, nutritional factors, body composition and inflammatory markers, that persisted after adjustment for GFR, have recently been reported [35, 52]. Thus, the use of multivariable equations that incorporate age, sex and race as well as equations that use both creatinine and cystatin C may provide more accurate estimations of GFR [23, 51].

Second, reverse causation, specifically increased blood lead levels as a result of reduced kidney excretion, cannot be excluded due to the cross-sectional study design. However, the temporal relation between lead exposure and CKD onset and/or progression is a critical factor in determining causality. Longitudinal data in both CKD patient and general populations have reported lead dose to be a predictor of kidney function decline for follow-up periods as long as 4 to >6 years, respectively [2, 3, 5, 9–11]. Further, reverse causality should be most prominent in populations with CKD. However, analyses to address this in the Normative Aging Study population found that blood lead was positively associated with serum creatinine even over the normal range where a substantial decrease in lead excretion is unlikely [3, 5]. In addition, the impact of lead chelation on kidney function in CKD patients provides evidence against reverse causality [10]. Third, cumulative lead dose could not be analyzed. Blood lead reflects current exogenous exposure as well as endogenous exposure from accumulated body burden. Bone lead is a better marker of cumulative

lead exposure [16] but has never been measured in NHANES. Fourth, our study may be subject to survival bias, due to increased mortality of CKD patients, and to other selection biases which could underestimate the effect of lead on kidney function, such as exclusion of institutionalized participants and need for mobility to attend the examination portion of the NHANES evaluation. The specific criteria used to derive the cystatin C subsample could also result in selection bias. Finally, residual confounding by recently reported factors, including nutritional factors and inflammatory markers, whose associations with cystatin C persist after adjustment for GFR, may also affect our study [35, 52].

In conclusion, in this large representative sample of US adults, higher blood lead levels were associated with lower eGFR and increased odds of reduced eGFR, irrespective of the endogenous marker of GFR and estimating equation used. In all participants, larger differences in mean eGFR for a doubling of blood lead were observed with the cystatin C equations. In participants aged $\geq$60 years, the association between lead and reduced eGFR was observed throughout the range of blood lead levels with no apparent threshold. Given the global burden of CKD, it is essential to conduct research on potential risk factors that are common and preventable, including lead exposure. These results support the inclusion of cystatin C-based eGFR in future lead research and provide additional evidence for environmental lead exposure as a CKD risk factor.

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Appendix

Appendix 1. Correlation coefficients for eGFR by equation in all participants ($n = 3941$)[a]

	MDRD	CKD-EPI	Cystatin C single variable	Cystatin C multivariable	Cystatin C/creatinine
CKD-EPI	0.95				
Cystatin C single variable	0.71	0.76			
Cystatin C multivariable	0.72	0.79	0.97		
Cystatin C/creatinine	0.93	0.94	0.90	0.92	

[a]P-value <0.001 for all correlations.

Appendix 2. Correlation coefficients for eGFRs by equation in participants aged ≥60 years ($n = 2609$)[a]

	MDRD	CKD-EPI	Cystatin C single variable	Cystatin C multivariable	Cystatin C/creatinine
CKD-EPI	0.96				
Cystatin C single variable	0.72	0.76			
Cystatin C multivariable	0.73	0.76	0.98		
Cystatin C/creatinine	0.94	0.94	0.91	0.92	

[a]P-value <0.001 for all correlations.

Appendix 3. Correlation coefficients for eGFRs by equation in participants aged <60 years ($n = 1332$)[a]

	MDRD	CKD-EPI	Cystatin C single variable	Cystatin C multivariable	Cystatin C/creatinine
CKD-EPI	0.94				
Cystatin C single variable	0.58	0.62			
Cystatin C multivariable	0.60	0.64	0.96		
Cystatin C/creatinine	0.92	0.91	0.84	0.86	

[a]P-value <0.001 for all correlations.