Applying Cadmium Relative Bioavailability to Assess Dietary Intake from Rice to Predict Cadmium Urinary Excretion in Nonsmokers

Di Zhao,† Rong-Yan Liu,† Ping Xiang,† Albert L. Juhasz,‡ Lei Huang,† Jun Luo,† Hong-Bo Li,*† and Lena Q. Ma†§

†State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, People’s Republic of China
‡Future Industries Institute, University of South Australia, Mawson Lakes, South Australia 5095, Australia
§Soil and Water Science Department, University of Florida, Gainesville, Florida 32611, United States

Supporting Information

ABSTRACT: Dietary Cd intake is often estimated without considering Cd bioavailability. Measured urinary Cd for a cohort of 119 nonsmokers with rice as a staple was compared to predicted values from rice-Cd intake with and without considering Cd relative bioavailability (RBA) in rice based on a steady state mouse kidney bioassay and toxicokinetic model. The geometric mean (GM) of urinary Cd and β2-microglobulin was 1.08 and 234 μg g⁻¹ creatinine. Applying Cd–RBA in foods to aggregate Cd intake (41.5 ± 12.4, 48.0 ± 9.3, 48.8 ± 21.3% for rice, wheat, and vegetables), rice was the largest contributor (71%). For 63 participants providing paired urine and rice samples, the predicted GM of urinary Cd at 4.14 μg g⁻¹ based on total Cd in rice was 3.5 times that of measured value at 1.20 μg g⁻¹, while incorporating Cd–RBA to assess rice-Cd intake made the two closer with GM at 1.07 μg g⁻¹. The cohort findings were extended to a national scale, with urinary Cd for nonsmokers from rice Cd intake was mapped at province/city levels after considering rice Cd–RBA. Therefore, incorporating Cd bioavailability to assess dietary Cd intake is a valuable tool to accurately estimate human Cd exposure and associated health risk.

INTRODUCTION

Cadmium is a ubiquitous environmental pollutant, posing a threat to human health. Due to rapid industrialization and urbanization, China faces challenges of soil contamination with heavy metals. Recent national survey shows that 19.4% of agricultural soils is contaminated, with Cd being the priority contaminant.¹ Epidemiological studies have shown the association between prolonged chronic low Cd exposure and cardiovascular disease, decreased bone density, and cancer.²,³ In addition, higher exposure may result in renal damage, proximal tubular reabsorptive dysfunction, and fractures of long bones in the skeleton.⁴,⁵ Among different pathways, smoking is an important contributor to Cd exposure in smokers, whereas dietary intake is the primary contributor for nonsmokers. In China, recent studies showed elevated Cd concentrations in dietary staples, including rice, wheat, and vegetables due to anthropogenic impacts on farmland soils and/or alternative cultivation practices.⁶ Although upland cultivation reduces arsenic accumulation in rice compared to paddy cultivation, dryland farming facilitates Cd accumulation in rice.⁷ Under anaerobic conditions (paddy rice), Cd readily precipitates as sulfides whereas aerobic cultivation (wheat) favors Cd uptake by plants.⁷ Therefore, food Cd issue is becoming a serious problem especially when paddy soil is cocontaminated by Cd and As. Considerable variability has been reported due to genetic, soil, water, and food processing factors.⁸,⁹ A market basket survey of 2629 individual food samples from 31 provinces of China showed the highest Cd at 17 μg g⁻¹ fw,¹⁰ whereas Zhu et al.¹¹ reported 65% of rice samples from rural areas of southern China contained Cd concentrations exceeding the current Chinese rice standard of 0.20 μg g⁻¹.¹² The data suggest that a proportion of the population may be exposed to unacceptable Cd concentrations through rice consumption. Urinary Cd has been widely used as a biomarker for long-term human exposure to Cd. Following absorption, Cd accumulates in the kidney cortex with a half-life of 10–30 years.⁷ However, population-based studies show that Cd kidney burden is proportional to Cd excreted in urine, making creatinine-corrected urinary Cd a suitable biomarker of Cd body burden.¹³ Epidemiologic studies have shown adverse renal
effects from Cd exposure, such as elevated excretion of low molecular weight proteins (e.g., β2-microglobulin) due to impairment on renal tubular reabsorption function and lower estimated glomerular filtration.\textsuperscript{14,15} In addition, nonrenal effects, including increased risk of hypertension, poor neurodevelopment, cancer and cardiovascular disease, and osteoporosis have also been associated with elevated urinary Cd.\textsuperscript{13,16,17}

To protect against kidney damage, the World Health Organization (WHO) established a urinary Cd threshold of 5.24 μg g\textsuperscript{-1} creatinine, whereas the European Food Safety Authority (EFSA) established a value of 1 μg g\textsuperscript{-1} creatinine.\textsuperscript{18}

In addition to urinary Cd measurement, assessment of dietary Cd intake provides a mechanism to predict Cd body burden and potential health effects. Food questionnaires are frequently utilized to estimate food consumption with dietary Cd concentrations being sourced from the literature or measured based on market basket surveys.\textsuperscript{19,20} However, while numerous studies have examined Cd levels in foods,\textsuperscript{10,21} there is a paucity of direct evidence showing the relationship between food consumption and Cd body burden, partially due to a lack of paired food Cd and urinary Cd data for a studied cohort, although toxicokinetic models were developed to link Cd intake with urinary Cd.\textsuperscript{22} In addition, dietary Cd intake is often calculated using total Cd concentration in foods and consumption rate without considering Cd bioavailability (i.e., the fraction of Cd in food that absorbed into the systemic circulation following oral ingestion).\textsuperscript{8} Currently, there is no report of Cd bioavailability in foods. In addition, as detailed by Begg et al.\textsuperscript{23} and Reeves and Chaney,\textsuperscript{24} essential elements (e.g., Zn, Fe, and Ca) influence Cd absorption, thereby impacting exposure predictions based on total Cd concentration. We hypothesized that incorporating Cd bioavailability in foods to assess dietary Cd intake provides a valuable tool to accurately estimate human Cd exposure using urinary Cd as the biomarker.

Accordingly, the aim of this study was to test the hypothesis. First, paired rice Cd and urinary Cd data were included to assess Cd exposure in a Cd-impacted cohort from Yixing, Jiangsu Province, China. The location was selected as elevated Cd concentrations have been reported in the river sediments (up to 5000 μg g\textsuperscript{-1} Cd) and farmland soil (0.53–5.92 μg g\textsuperscript{-1} Cd) due to the increased use of Cd pigments for pottery production.\textsuperscript{25} The specific objectives of the cohort study were to (1) develop a mouse model to determine Cd relative bioavailability (RBA, relative to absorption of Cd chloride) in Cd-contaminated foods; (2) refine exposure associated with food consumption after incorporating Cd–RBA into Cd intake calculations; (3) predict urinary Cd in nonsmokers from rice consumption with or without incorporating Cd–RBA in rice using a toxicokinetic model; and (4) compare the predicted urinary Cd with measured values for the cohort. The derived relationships from the cohort findings were utilized to predict urinary Cd for nonsmokers at a national scale of China to identify the national spatial distribution for Cd exposure, which was based on rice consumption, rice–Cd concentration and Cd–RBA.

## MATERIALS AND METHODS

**Study Location and Cohort.** The study area was located in Yixing city, Jiangsu Province, China (Supporting Information (SI) Figure S1), which is famous for producing dark-red enamel pottery with 252 ceramic workshops and factories within ~205 km\textsuperscript{2}.\textsuperscript{25} The raw material purple clay is available only in the study area, which is characterized by high mineral content (quartz, kaolin, and mica with high concentrations of Fe oxide). However, due to over exploitation, the local clay resources are becoming scarce, leading to the mining ban by local government in 2005. As a result, enamels and pigments containing Fe, Mn, Cr, and Cd are now added into common clays to produce desired colors. However, the practice results in the discharge of wastewater into nearby rivers, which contains elevated concentrations of heavy metals, increasing Cd concentration in river sediments up to 5000 μg g\textsuperscript{-1} Cd.\textsuperscript{25} In addition, as local farms utilize river water for irrigation, increasing Cd concentration in farmland soils has also been observed with 0.15–0.40 in 2014 versus 0.53–5.92 μg g\textsuperscript{-1} Cd in 2012.\textsuperscript{25}

Furthermore, small farms often coexist with pottery production facilities in the study area (Figure S1). Local villagers grow cereals (rice in summer and wheat in winter) and vegetables in farmlands surrounded by ceramic workshops and factories. Therefore, there is a high possibility of elevated Cd concentrations in foods from the district and elevated dietary Cd intake by the residents. As a major dietary staple, local rice is predominantly consumed by local residents while wheat is predominantly exported to other regions of China.

The study cohort consisted of 119 nonsmokers (aged 14–97 years, 37 males, and 82 females) living in two villages of the area. Association between urinary Cd concentrations and hypertension/impaired kidney function in cohorts from the district has been reported.\textsuperscript{26} Based on dietary surveys and interviews, >80% of the cohort consumed local rice while others consumed market rice. All participants provided written consent and the study was approved by Nanjing University.

**Analyses of Cd Concentrations in Urine, Food, and Environmental Samples.** First morning urine samples were obtained from the 119 participants in May, 2015. First morning urine is more concentrated in Cd and has been correlated with 24 h urine collection.\textsuperscript{27} Urine samples were filtered (0.45 μm) and immediately stored at 4 °C prior to analysis. Polished rice (local rice, n = 53; market rice, n = 10) and wheat grain (whole grain with bran; n = 23) were obtained from the participants, while fresh vegetables (courgette, long bean, loofah, okra, eggplant, radish, potato, pumpkin vines, amaranth, water spinach, taro, pakchoi, and leek; n = 43) were obtained from the participants (n = 31) or bought from local vegetable markets (n = 12), producing 63 paired samples of rice and urine. All food was washed and cooked with Milli-Q water, and then freeze-dried prior to analysis. In addition, environmental samples including drinking water (n = 33), river water (n = 11), farmland soil (n = 10), riverside soil (n = 3), indoor housedust (n = 10), and ceramic clay samples (n = 8) were collected for analysis. Detailed sampling information is provided in the SI.

Cadmium concentration in urine was determined using inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, PerkinElmer) following dilution with 0.1 M HNO\textsubscript{3}. Urine creatinine was determined using the Jaffe reaction method while β\textsubscript{2}-microglobulin was measured using the Latex enhanced immuno-turbidimetry method. Cadmium concentration in food and environmental samples was determined using ICP-MS following digestion using USEPA Method 3050B and expressed based on dry weight basis (dw).

**Assessment of Cd Relative Bioavailability in Foods.** Human exposure to Cd may be influenced by Cd concentration in the media as well as its bioavailability. To refine Cd exposure, Cd relative bioavailability (RBA, relative to cadmium chloride, CdCl\textsubscript{2}) in representative rice (n = 10; 0.29–1.09 μg g\textsuperscript{-1} Cd

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addition, Cd accumulation in mouse kidneys showed a linear relationship was used, between Cd in urine and in kidney cortex. Statistical estimates between Cd in urine following food and CdCl₂ exposure; and Cd

A steady state multiple dosing approach via feed was utilized. Specifically, cooked rice, wheat, or vegetables were combined with basal mouse feed (1:1 w/w dw) and fed to mice over a 10 day period with Cd accumulation in the kidneys being used as the end point of Cd exposure. Detailed exposure parameters are provided in Table S2.

Following exposure, mice were sacrificed to collect the kidneys, which were immediately stored at −80 °C prior to freeze-drying. Cadmium concentration was determined using ICP-MS following digestion using USEPA Method 3050B.

Cd–RBA was calculated using the following equation (eq 1) by comparing the dose normalized Cd–kidney accumulation from food to CdCl₂, which was amended into mouse chow and food mixture (0.2–1.0 μg g⁻¹ dw). This soluble salt was chosen for Cd–RBA calculations as it is the reference compound that has been used to determine Cd toxicological reference value. In addition, Cd accumulation in mouse kidneys showed a linear response to CdCl₂ dose level (Figure S2).

Cd relative bioavailability (%) = \left( \frac{\text{kidney Cd}_\text{food}}{\text{Cd dose}_\text{food}} \times \frac{\text{Cd dose}_\text{CdCl₂}}{\text{kidney Cd}_\text{CdCl₂}} \right) \times 100

(1)

where, kidney Cd_food and kidney Cd_CdCl₂ are Cd concentration in mouse kidneys following food and CdCl₂ exposure; and Cd dose_food and Cd dose_CdCl₂ are Cd dose level from food and CdCl₂.

Predicting Urinary Cd from Rice Cd Intake. Following determination that rice was the most important contributor to aggregate Cd intake, a one-compartment toxicokinetic model, linking dietary Cd intake to urinary Cd, was used to predict urinary Cd concentration for an individual of 60 kg BW and predict urinary Cd concentration for an individual of 60 kg BW and 50 years age. Age 50 was chosen because urinary Cd reaches maximum at this age. Mapping spatial distribution of urinary Cd at provincial level was performed using Geographic Information System to identify hotspots of Cd exposure. In addition, for Hunan Province with high urinary Cd concentration, maps showing spatial distribution of urinary Cd at city-level were also developed.

QA/QC and Data Processing. Quality assurance and control were conducted using standard reference materials for rice (GSB-21, Chinese Geological Reference Materials) and urine (ZK018-1 and ZK018-2, Chinese Academy of Preventive Medicine). The accuracy of USEPA Method 3050B was acceptable with measured Cd concentration of 0.012 ± 0.002 vs 0.012 ± 0.003 μg g⁻¹ in rice GSB-21, and 5.20 ± 0.50 and 14.8 ± 0.83 vs 5.34 ± 0.48 and 15.5 ± 1.35 μg L⁻¹ in urine ZK018-1 and ZK018-2. The limit of detection (LOD), calculated as 3 times the standard deviation of the blank values, was 0.005 μg L⁻¹. All urine samples contained Cd levels > LOD. During urine creatinine analyses, standard creatinine solutions (0–200 μmol L⁻¹, n = 10) were measured, with 85–115% recoveries (averaging 100 ± 8%). During Cd analyses using ICP-MS, spiked and check samples (1–10 μg Cd L⁻¹) were included every 20 samples, with 101 ± 6.5% and 99 ± 8.3% recoveries (n = 30).

Geometric means (GM) were used to summarize the quantitative variables. All graphs were created using SigmaPlot (version 12.5, Systat Software Inc., San Jose, CA). Urinary Cd and β2-microglobulin were adjusted by creatinine and expressed as μg g⁻¹ creatinine. Differences in Cd concentration between local and market rice, Cd–RBA between individual rice, wheat, or vegetable samples, and difference between measured and predicted urinary Cd were assessed using variance analysis based on Tukey’s multiple comparisons using SAS (version 9.1.3), with p value <0.05 being considered significant.

RESULTS AND DISCUSSION

Urinary Cd and β2-Microglobulin Concentrations in Studied Cohort. Urinary Cd concentrations of the cohort ranged from 0.20 to 5.41 μg g⁻¹ creatinine, with a GM of 1.08
± 0.90 μg g⁻¹ creatinine (Table 1). The GM was lower than the WHO urinary Cd threshold at 5.24 μg g⁻¹ creatinine but similar

Table 1. Geometric Mean of Urinary Cd and β2-Microglobulin Concentration by Age, Sex, and Occupation, and Rice Consumption in the Studied Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Urinary Cd</th>
<th>β2-microglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Group</td>
<td>119</td>
<td>1.08 ± 0.90</td>
<td>234 ± 1161</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>4</td>
<td>0.56 (0.32–1.01)</td>
<td>85.0 (80.3–93.2)</td>
</tr>
<tr>
<td>30–40</td>
<td>5</td>
<td>0.84 (0.32–1.70)</td>
<td>92.5 (68.5–120)</td>
</tr>
<tr>
<td>40–50</td>
<td>17</td>
<td>1.05 (0.32–2.11)</td>
<td>148 (54.9–877)</td>
</tr>
<tr>
<td>50–60</td>
<td>35</td>
<td>1.19 (0.24–5.41)</td>
<td>199 (43.9–1471)</td>
</tr>
<tr>
<td>60–70</td>
<td>28</td>
<td>1.06 (0.28–4.56)</td>
<td>227 (24.2–3359)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>30</td>
<td>1.12 (0.20–3.99)</td>
<td>430 (34.4–3892)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>37</td>
<td>0.90 (0.32–4.56)</td>
<td>327 (24.2–10490)</td>
</tr>
<tr>
<td>female</td>
<td>82</td>
<td>1.17 (0.20–5.41)</td>
<td>208 (34.4–3892)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pottery worker</td>
<td>48</td>
<td>1.02 (0.24–3.15)</td>
<td>143 (24.2–1034)</td>
</tr>
<tr>
<td>nonpottery worker</td>
<td>71</td>
<td>1.12 (0.20–5.41)</td>
<td>352 (68.5–10490)</td>
</tr>
<tr>
<td>Rice consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>locally grown</td>
<td>87</td>
<td>1.20 (0.24–5.41)</td>
<td>195 (24.2–10490)</td>
</tr>
<tr>
<td>market</td>
<td>32</td>
<td>0.80 (0.20–2.60)</td>
<td>439 (43.9–3892)</td>
</tr>
</tbody>
</table>

Table 1. Geometric Mean of Urinary Cd and β2-Microglobulin Concentration by Age, Sex, and Occupation, and Rice Consumption in the Studied Cohort

to the EFSA threshold at 1 μg g⁻¹ creatinine.18 Compared to urinary Cd concentrations from a cohort of 12,000 residents from mining and smelting impacted regions in China (~4.8 μg g⁻¹ creatinine),20 urinary Cd concentrations in this study were significantly lower. However, its health risk cannot be overlooked, as recent epidemiological studies suggest that prolonged exposure to Cd at low levels can also cause adverse health effects.2,30

In the cohort, several factors were identified to influence urinary Cd concentration including age and gender. Urinary Cd concentration showed an increasing trend with age (GM of 0.56, 0.84, 1.05, and 1.19 μg g⁻¹ creatinine for subgroups of age <30, 30–40, 40–50, and 50–60, respectively) (Figure 1A), and was higher in females than males (GM of 1.17 and 0.90 μg g⁻¹ creatinine) (Table 1), consistent with other report.20 The increased urinary Cd concentration with age possibly reflects increased Cd body burden due to its long half-life (10–30 years). In addition, age-related changes in renal physiology such as lower tubular reabsorption capacity is also an important factor.31 Lower Fe storage in females has been suggested for gender differences in urinary Cd concentration, as Fe deficiency increases Cd absorption from foods due to up-regulation of Fe transporters, which also transport Cd.32

In addition, urinary Cd concentration was significantly higher (p < 0.05) in participants consuming local rice compared to market rice (GM of 1.20 and 0.80 μg g⁻¹ creatinine) (Table 1). However, there was no significant difference (p > 0.05) among subgroups of “occupationally exposed” pottery and nonpottery workers (GM of 1.02 and 1.12 μg g⁻¹ creatinine), illustrating that non-dietary pathway was an insignificant Cd exposure route.

β2-microglobulin concentrations in the cohort were 24.2–3892 μg g⁻¹ creatinine (GM of 234 μg g⁻¹ creatinine) with 12.6% exceeding the reference value of 1000 μg g⁻¹ creatinine for renal dysfunction (Figure 1B).29 Similar to urinary Cd concentration, GM of β2-microglobulin increased from 85.0 to 430 μg g⁻¹ for <30 to >70 age groups. However, significantly higher values were observed in males than females (p = 0.01; GM of 327 and 208 μg g⁻¹ creatinine). The association between urinary Cd and β2-microglobulin was insignificant (p = 0.18), however, there was a greater chance of having β2-microglobulin >1000 μg g⁻¹ creatinine for participants with urinary Cd > 1 μg g⁻¹ creatinine than those <1 μg g⁻¹ creatinine (14% vs 9.8%). Elevated urinary Cd (>1 μg g⁻¹ Cd creatinine) has been associated with elevated urinary excretion of β2-microglobulin.15 However, at lower urinary Cd concentration, proteinuria may not result from Cd toxicity but as a result of normal physiological changes such as diuresis, body position, and exercise.32,33 Thus, interpretation of adverse effects of Cd on kidney function at low level of Cd exposure should be viewed with caution.

Cadmium Concentrations in Food and Environmental Samples. Significantly higher Cd concentrations (p < 0.05) were observed in local rice (0.02–1.09 μg g⁻¹ with GM of 0.24 μg g⁻¹) compared to market rice (0.01–0.17 μg g⁻¹ with GM of 0.05 μg g⁻¹) (Figure 2A). Among local rice (n = 53), 72% exceeded the Chinese Cd limit of 0.20 μg g⁻¹, whereas all market rice was below the limit (Figure 3A). Similarly, high Cd concentrations (0.002–3.71 μg g⁻¹ with GM of 0.26 μg g⁻¹) have been observed in rice produced from locations impacted by mining and smelting activities in China.9

Elevated Cd concentrations were also observed in wheat (0.11–2.03 μg g⁻¹), all exceeding the Chinese Cd threshold value of 0.10 μg g⁻¹ (Figure 3B). Higher Cd concentrations in

Figure 1. Variation of urine Cd levels (A) and β2-microglobulin concentration (BMG; B) in different age groups from a ceramic town, China. The dash line indicates urinary Cd and β2-microglobulin of 1 μg kg⁻¹ creatinine and 1000 μg kg⁻¹ creatinine, above which a large burden of adverse health effects may occur.
wheat than rice were probably attributed to different water regimes under summer rice-winter wheat rotation. Under anaerobic conditions of paddy rice, Cd readily precipitates as sulfides whereas aerobic cultivation of wheat favors Cd uptake by plants. However, differences may also be attributed to processing factor, that is, rice samples were polished and wheat grains were not. Polishing is an important practice to decrease Cd concentration in both rice and wheat grains as Cd often concentrates in the bran. In addition, Cd concentrations in vegetables (0.02–3.64 μg g⁻¹ dw) were analyzed, averaging 0.62 ± 0.95 μg g⁻¹ dw (Figure 3C). Among different species, leek (2.79 ± 0.83 μg g⁻¹, n = 2) and pakchoi (1.62 ± 1.22 μg g⁻¹, n = 9) contained higher Cd concentrations. These values are similar to those reported by Luo et al., who reported that leafy greens may contain elevated Cd compared to other vegetables (0.40–3.66 μg g⁻¹ dw).

The main source of Cd for rice, wheat, and vegetables in the study area was presumably due to soil irrigation with Cd-contaminated river water. Elevated Cd concentrations (5–5030 μg L⁻¹) were detected in river water (n = 11) (Figure 3F), possibly from wastewater discharge from ceramic clay processing factories. Pigments containing high Cd concentrations (e.g., Cd yellow and Cd red containing 48 845 and 39 820 μg g⁻¹ Cd) have been observed in the district, whereas Cd concentrations in riverside and farmland soil were 3.97–108 and 0.45–7.01 μg g⁻¹, respectively (Figure 3DE).

**Cadmium Relative Bioavailability in Food Items.** When considering human exposure to Cd, it is influenced by both Cd bioavailability and total Cd concentration in the foods. However, a dearth of information is available regarding Cd bioavailability in foods. In this study, Cd–RBA in rice (n = 10), wheat (n = 8), and vegetables (n = 6) was assessed after developing a steady state mouse kidney bioassay. In rice, Cd–RBA varied from 16.9 ± 9.9% to 57.4 ± 9.7% (Figure 2C), however, a strong positive correlation was observed between Cd–RBA and Cd concentration in rice (R² = 0.6) (Figure 2B). Unlike rice, Cd–RBA in wheat varied from 37.4 ± 2.0% to 67.6 ± 2.6%, but was independent of Cd concentration (Figure 2C and Figure S3A). The most variation in Cd–RBA was observed for vegetables (Figure 2C), with high Cd–RBA in leek and amaranth (78.0 ± 17.6 and 67.2 ± 21.0%), moderate in pumpkin vines, pakchoi, and water spinach (44.3 ± 9.5, 44.0 ± 8.5, and 41.3 ± 8.6), and low in taro (17.7 ± 2.6%).

Although this was the first report to assess Cd–RBA in foods using an in vivo bioassay, previous in vitro studies on rice show similar strong positive correlation between Cd bioaccessibility and Cd concentration (R² = 0.92–0.98). Increasing Cd–RBA with Cd concentration may suggest availability of transport sites for Cd-like molecules in mouse, and thus as more Cd becomes available in food, uptake rates increase substantially. Alternatively, elimination or distribution dynamics within mice may be altered at high Cd concentrations in food, altering the relationship between kidney Cd content and bioavailability. Notwithstanding, the observed linear relationship between Cd–RBA and Cd concentration allows its use to predict Cd–RBA in rice samples outside the model, which may

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*Figure 2.* Concentration of Cd in local and market rice samples collected from the cohort study (A), relationship between Cd relative bioavailability based on a mouse kidney bioassay and Cd concentration for 10 rice from the cohort study (B), and Cd relative bioavailability based on a mouse steady state kidney bioassay in 10 rice, 8 wheat, and 6 vegetables from the cohort study after 10 day diet exposure (C). Boxes represent the 25–75th percentiles while solid and dashed lines in boxes denote the median and mean values, respectively. Error bars in A represent the 5th and 95th percentiles, and time signs show outliers, whereas bar in B and C represents the mean and standard deviation of three replicates. Means marked with different letters indicate significant (p < 0.05) differences.
then be incorporated into Cd intake calculations and urinary Cd predictions.

**Contribution of Food and Environmental Samples to Cd Exposure in Humans.** To quantify the influence of food and environmental sources to aggregate Cd exposure, food Cd intake calculated based on average Cd concentration, Cd–RBA, and consumption rate for rice, wheat, and vegetables was compared to Cd intake via drinking water and ingestion of soil and housedust (Table 2). When calculating Cd intake via soil ingestion, the average Cd–RBA in four selected soils (64.8 ± 2.3%) determined using a mouse kidney assay was included, whereas Cd–RBA in housedust and drinking water was assumed at 70 and 100%, respectively. Considering all pathways, overall Cd exposure was 7.37 μg kg⁻¹ bw per week, higher than the JECFA (Joint FAO/WHO Expert Committee on Food Additives) threshold of 5.8 μg kg⁻¹ bw per week. Rice represented the largest source of Cd exposure, accounting for 71.1%, followed by wheat (19.9%), and vegetables (8.41%), with little contribution from drinking water, soil, or housedust (0.55%). Similarly, large contribution of Cd intake by foods from homegrown rice and vegetables has been reported by others (70–99.5%).

**Prediction of Urinary Cd Based on Rice Cd Intake for Studied Cohort.** After identifying rice as the major Cd exposure source, a one-compartment toxicokinetic model was utilized to link the rice–Cd intake to the urinary Cd for the 63 participants providing paired urine and rice samples to refine Cd exposure assessment by including rice Cd–RBA.

<table>
<thead>
<tr>
<th>food/environmental sample</th>
<th>total Cd (μg g⁻¹)</th>
<th>Cd RBA (%)</th>
<th>intake rate (week⁻¹)</th>
<th>DI (μg Cd kg⁻¹ BW week⁻¹)</th>
<th>contribution to overall Cd intake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rice</td>
<td>0.30</td>
<td>41.5</td>
<td>2.5 kg⁶</td>
<td>5.24</td>
<td>71.1</td>
</tr>
<tr>
<td>wheat</td>
<td>0.90</td>
<td>48.0</td>
<td>0.2 kg⁶</td>
<td>1.47</td>
<td>19.9</td>
</tr>
<tr>
<td>vegetables</td>
<td>0.04 fw</td>
<td>48.8</td>
<td>2.0 kg⁸ te</td>
<td>0.62</td>
<td>8.41</td>
</tr>
<tr>
<td>water</td>
<td>0.06 μg L⁻¹</td>
<td>100</td>
<td>14 L⁻¹</td>
<td>0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>soil</td>
<td>3.01</td>
<td>64.8⁷</td>
<td>0.7 g⁷</td>
<td>0.02</td>
<td>0.27</td>
</tr>
<tr>
<td>housedust</td>
<td>1.69</td>
<td>70.0⁸</td>
<td>0.7 g⁸</td>
<td>0.01</td>
<td>0.14</td>
</tr>
</tbody>
</table>

⁶The bioavailability data for housedust was obtained from Turner et al., while data for soil were determined using an in vivo mouse bioassay similar to foods. ⁷Intake rates of rice, wheat, and vegetables were obtained from Li et al. ⁸Drinking water or house dust ingestion rates were obtained from Chen et al. ⁹The data for soil ingestion rate was obtained from Luo et al.
Based on total Cd concentration in rice, predicted urinary Cd concentrations from the model were 0.12–33.0 μg g⁻¹ creatinine, with a GM of 4.14 μg g⁻¹ creatinine. This value was 3.5-fold greater than the measured GM of 1.20 μg g⁻¹ creatinine (0.28–5.41 μg g⁻¹ creatinine) (Figure 4A). However, and urinary Cd concentration or hormone-related cancers. This provided a mechanism to assess the potential long-term health effects associated with Cd exposure, particularly in China where consumption of the staple diet is the predominant Cd exposure source for nonsmokers.

However, when predicted urinary Cd with inclusion of rice Cd–RBA was compared to measured values for individuals, good correlation between the data sets was not observed (Figure S4). Several factors contributed to the inconsistency. The one-compartment toxicokinetic model assumes that food habits of the study participants are stable, but there is considerable variation in food intake during lifetime. Another uncertainty is that the model was derived for older women, but the population of our study included both women and men with women generally having higher gastrointestinal Cd absorption than men. Furthermore, parameters such as Cd biological half-life significantly affect the target dose of Cd in the human body, which varies considerably among individuals (10–30 years). Also, the nutrition status of individual participants is another uncertainty to this model. All these factors would eventually lead to large uncertainty in urinary Cd prediction from rice–Cd intake after considering Cd–RBA, causing poor correlation between measured and predicted urinary Cd for individuals. However, when taking individuals as a group, there was strong agreement between GM values of measured and predicted urinary Cd after applying rice Cd–RBA into dietary Cd intake (Figure 4A), suggesting incorporating Cd bioavailability to assess dietary Cd intake is a valuable tool to accurately estimate human Cd exposure and associated health risk.

**Modeling Urinary Cd for Rice Consumers at a National Scale.** To extend cohort findings to a national scale, Cd concentrations in rice and consumption rates were obtained for 23 provinces and 96 cities across China (Table S4). Cd–RBA was predicted from Cd concentration using the established relationship (Figure 2B), and was utilized to map spatial distribution of urinary Cd concentrations. Predicted urinary Cd concentration at province level showed considerable variation from 0.0001 (Ningxia province) to 4.77 μg g⁻¹ creatinine (Hunan province) (Figure S5), with populations in southern China having significantly higher urinary Cd concentrations than those living in northern China (Figure 4B). In Hunan province, where mining and smelting activities impact Cd contamination in rice, significantly higher urinary Cd concentrations were predicted as a consequence of high Cd in local rice (Figure S6). In addition, considerable variation in urinary Cd concentration was also observed among different cities in the same province. For example, significantly higher urinary Cd concentrations were predicted for Henyang compared to other cities in Hunan province (32.2 vs 0.34–10.9 μg g⁻¹ creatinine) due to differences in Cd concentrations, Cd–RBA, and consumption rates in rice (Figure S7).

Few cohort studies have measured urinary Cd concentrations at a national scale. For example, urinary Cd at 0.22–0.66 μg g⁻¹ creatinine (arithmetic mean of 0.40 μg g⁻¹) in a general population (n = 896; age >18 years, average age 49 years) with low Cd exposure from Changshu city, Jiangsu province has been observed. This is similar to the prediction value of 0.23 μg g⁻¹ creatinine for populations in Changshu based on the toxicokinetic model after incorporation of Cd–RBA, indicating the model can be an accurate tool in large scale exposure analysis for general populations having rice–Cd intake being the dominant contributor to Cd exposure. However, in Cd-
contaminated Dayu county, Jiangxi province, control group \((n = 44)\) consuming low Cd–rice \((0.05 \mu g \text{ g}^{-1})\) has urinary Cd concentrations of 2.87 \(\mu g \text{ g}^{-1}\) creatinine, whereas urinary Cd concentration is 25.8 \(\mu g \text{ g}^{-1}\) creatinine for Cd-exposure group \((n = 32)\) consuming rice with 0.59 \(\mu g \text{ g}^{-1}\) Cd.\(^{39}\) This is higher than the predicted value of 0.44 and 2.12 \(\mu g \text{ g}^{-1}\) creatinine in this study for populations consuming rice of 0.06–0.07 and 0.23 \(\mu g \text{ g}^{-1}\) in the district, respectively, suggesting underestimation for populations living in highly Cd-contaminated districts due to overlooking the contribution of other dietary and nondietary pathways to overall Cd intake.\(^{40}\) For accurate prediction of Cd body burden for populations living in Cd-concentrations of 2.87 \(\mu g \text{ g}^{-1}\) exposure from a smelting-impacted area in southeast China has incidence of osteoporosis at 2.4% associated with chronic Cd than the predicted value of 0.44 and 2.12 \(\mu g \text{ g}^{-1}\) and nondietary pathways to overall Cd intake.\(^{40}\) For accurate prediction of Cd body burden for populations living in Cd-contaminated areas, other dietary pathways (e.g., wheat and vegetables) and nondietary pathways (e.g., smoking) should be assessed.

Urinary Cd has been associated with increased diabetes, hypertension, and even cardiovascular disease mortality.\(^{4,5}\) The incidence of osteoporosis at 2.4% associated with chronic Cd exposure from a smelting-impacted area in southeast China has been correlated with urinary Cd < 2.0 \(\mu g \text{ g}^{-1}\) creatinine.\(^{41}\) Considering the high predicted urinary Cd concentration of 4.77 \(\mu g \text{ g}^{-1}\) creatinine in Hunan province with 67 million population, ~1.6 million population may be at risk of osteoporosis due to rice consumption based on the low incidence of 2.4%. These findings suggest that ingestion of rice can be a health risk for populations in China, especially in southern China. Increased attention should be paid on food safety where both Cd concentration and Cd–RBA are important. Furthermore, strategy should be developed to modulate dietary Cd exposure via decreasing Cd–RBA in rice.

**ASSOCIATED CONTENT**

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b00940.

Detailed information on food samples used for a mouse model bioassay (Tables S1–2), participants in the intervention study (Table S3), Cd concentrations in rice based on literature (Table S4), location of the study area (Figure S1), linear dose response of Cd accumulation in mouse kidneys or liver following CdCl\(_2\) exposure (Figure S2), relationship between Cd–RBA and Cd in wheat or vegetable (Figure S3), predicted urinary Cd concentration against measured urinary Cd for individual participants (Figure S4), values of rice Cd based on literature and predicted urinary Cd (Figure S5), spatial distribution of rice Cd across China (Figure S6), and spatial distribution of predicted urinary Cd in Hunan province (Figure S7) (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

*Phone/fax: +86 025 8968 0637; e-mail: lqma@ufl.edu; hongboli@nju.edu.cn.*

**ORCID**

Jun Luo: 0000-0002-3480-8900

Lena Q. Ma: 0000-0002-8463-9957

**Notes**

The authors declare no competing financial interest.

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