Mineral Dietary Supplement To Decrease Cadmium Relative Bioavailability in Rice Based on a Mouse Bioassay

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ABSTRACT: To determine the effectiveness of mineral dietary supplements to modulate cadmium (Cd) exposure, an in vivo mouse bioassay was conducted to determine Cd relative bioavailability (Cd-RBA) in Cd-contaminated rice (0.80 mg Cd kg⁻¹) with and without Zn, Fe, or Ca supplements as nitrate or chloride salts. Without mineral supplements, Cd-RBA was 43 ± 5.3% based on average Cd accumulation in the liver plus kidneys as the end point. Among Ca(NO₃)₂, Zn(NO₃)₂, and Fe(NO₃)₂ supplements, 150–5000 mg kg⁻¹ Ca was the most effective in reducing rice Cd-RBA by 31–80% to 8.5–29%, while 30–200 mg kg⁻¹ Zn supplements was ineffective, with Cd-RBA being 33–57%. Low Fe at <40 mg kg⁻¹ had little impact on rice Cd-RBA (39–47%), while high Fe at 80–200 mg kg⁻¹ decreased Cd-RBA by 37% to 26–27%. The ineffectiveness of Zn supplements in reducing Cd-RBA was probably due to coinciding 8.3- and 3.1-fold increases in Zn accumulation in mouse kidneys and liver with Zn supplements, while Ca and Fe supplements led to much-smaller increases in Ca and Fe accumulation in mouse tissues (1.3–1.6 fold). In addition, compared to Ca(NO₃)₂ supplements, Cd-RBA values determined with CaCl₂ supplements were significantly higher (25–67% versus 8.5–29%), suggesting that chloride enhanced Cd-RBA. Results of this study have important implications for developing effective dietary strategies to reduce dietary Cd exposure and the associated health risks in humans.

INTRODUCTION

Cadmium (Cd) is a toxic and bioaccumulative metal that has been released into the environment during mining and smelting processes. Following absorption by humans, Cd has a long half-life of 10–30 years, thereby adversely impacting human health.¹ While various adverse health effects occur following Cd exposure, its impacts on the kidneys and bone have been observed with low-dose Cd exposure in adults.²³

Cadmium exposure in humans occurs via dietary and nondietary pathways, with diet being the main source of Cd exposure in nonsmokers. Among foods, rice is a main contributor to dietary Cd intake, especially in Southeast Asia, where rice is a staple. It has been estimated that rice consumption contributed 77% of total Cd exposure in rural areas of south China, determined based on a survey of 753 individuals.⁴ Large variations in rice Cd concentrations have been reported for different agricultural areas, with Cd concentrations being up to 7.0 mg kg⁻¹ in the vicinity of Dabaoshan mine, Guangdong Province, China.¹ However, following consumption, only the fraction of Cd that is absorbed into the circulatory system across the gastrointestinal barrier (i.e., bioavailable) poses potential health risks to humans, highlighting the need to determine Cd bioavailability in rice to evaluate its health risk. To assess its health risk to humans, Cd relative bioavailability (RBA) is commonly used by comparing Cd accumulation in animal tissues (e.g., liver and kidneys) following solid matrices (e.g., soil and food) exposure to that of a soluble reference (e.g., Cd chloride) via oral route.⁷–¹₀ Although limited information is available regarding Cd-RBA in rice for human health risk assessment, an in vivo mouse bioassay was used to determine rice Cd-RBA based on Cd accumulation in kidneys following a 10-day exposure.⁷ As detailed in Zhao et al.,⁷ Cd-RBA in 10 rice samples containing

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0.3–1.1 mg kg$^{-1}$ Cd varied from 17–57%; thus, it is important to determine Cd-RBA in rice to accurately assess the health risk associated with rice consumption.

Because the diet pathway is the main source of Cd exposure for humans, developing strategies to decrease Cd-RBA in rice may be important to lower Cd exposure and its associated health effects. Because Cd utilizes the same intestinal transporters as Zn, Fe, and Ca in both animals and humans, studies have shown that nutritional status of the individual may significantly affect Cd absorption in the gastrointestinal tract. For example, Reeves and Chaney showed that increasing dietary Fe from marginal to adequate (13.9 versus 39.1 mg kg$^{-1}$) decreased Cd accumulation in rat tissues by 2.5-fold. Similarly, increasing Ca concentrations from marginal to adequate (2540 versus 4710 mg kg$^{-1}$) decreased Cd absorption by 50%. Another study showed that rats fed with marginal amounts of Zn, Fe, and Ca (6.3, 8.9, and 2421 mg kg$^{-1}$) retained 8 times more Cd than those fed with adequate amounts (30, 32, and 4737 mg kg$^{-1}$). Thus, mineral supplements may potentially decrease Cd absorption from food and its accumulation in tissues, i.e., Cd bioavailability. Unlike Zn, Fe, and Ca, Cl enhances plant Cd uptake by complexing with Cd, thereby increasing its availability. Similarly, Cl could complex with Cd in the gastrointestinal tract, increasing Cd absorption by animals and humans. However, to date, few studies have been conducted using in vivo animal bioassays to determine Cd-RBA in foods. More importantly, there is a lack of information on the influence of dietary minerals and Cl on Cd bioavailability in staple foods such as rice. Because rice is often poor in mineral nutrients including Zn, Fe, and Ca compared with other cereals, it was hypothesized that mineral dietary supplements would decrease Cd-RBA, while dietary Cl might increase Cd-RBA in rice.

Therefore, the aim of this study was to evaluate the effects of mineral nutrients (Zn, Fe, and Ca) and Cl supplements on Cd-RBA in rice based on an in vivo mouse bioassay model. Different concentrations of Zn, Fe, and Ca as nitrate or chloride salts were amended into a Cd-contaminated rice from field, which was fed to mice to investigate their influences on Cd-RBA. Results of this study have important implications for developing effective dietary strategies to reduce Cd exposure and its associated health risk in humans.

## MATERIALS AND METHODS

**Rice Samples.** A representative Cd-contaminated white rice sample (~5 kg polished grains) was collected from a Cd-contaminated district of Yixing, Jiangsu Province, China to evaluate the effects of mineral nutrients on Cd-RBA in rice. Due to processing of clay with Cd pigments, local dark-red enameled pottery factories discharged Cd-contaminated water to surrounding rivers, causing Cd contamination in paddy field and elevated Cd levels in rice grains. Detailed information on Cd contamination source from the district can be found in Zhao et al. After being taken to the lab, the rice grains were washed with Milli-Q water three times, freeze-dried, ground to a fine powder, and thoroughly mixed prior to determination of Cd, Zn, Fe, and Ca concentrations using inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, PerkinElmer) or inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300, PerkinElmer) following triplicate digestion using United States Environmental Protection Agency (USEPA) Method 3050B. The average Cd concentration in the rice was 0.80 ± 0.01 mg kg$^{-1}$ (mean ± standard deviation, n = 3). Concentrations of Zn, Fe, and Ca in the rice were 19.1 ± 0.17, 4.01 ± 0.06, and 102 ± 0.69 mg kg$^{-1}$, respectively (Table 1), being significantly lower than those reported for wheat grain (42.3–55.2, 14.9–42.8, and 300–426 mg kg$^{-1}$) and vegetables (33.8–43.4, 65.2–413, and 1288–26930 mg kg$^{-1}$) from the same district, suggesting that rice was poor in mineral nutrients.

In addition, a control rice sample with low Cd concentration (4.0 ± 0.3 μg kg$^{-1}$) was collected from a local market in Yixing. This sample had similar Zn, Fe, and Ca concentrations (17.8 ± 1.2, 3.7 ± 0.3, and 118 ± 12 mg kg$^{-1}$, respectively) as the Cd-contaminated rice. The level of 4.0 μg Cd kg$^{-1}$ was the Cd background value because this was the lowest Cd concentration detected. The control rice was amended with CdCl$_2$ to serve as a reference to calculate Cd-RBA in the Cd-contaminated rice. CdCl$_2$ was chosen as the reference material due to its high solubility and has been commonly used as a reference material in other studies. The similar Zn, Fe, and Ca concentrations between the Cd-contaminated rice and control rice would eliminate the influence of matrices on Cd absorption following Cd-contaminated rice exposure and CdCl$_2$ exposure via spiked control rice.

### Table 1. Composition after Zn(NO$_3$)$_2$, Fe(NO$_3$)$_2$, Ca(NO$_3$)$_2$, or CaCl$_2$ Supplements into a Cd-Contaminated Rice Sample at Five Levels (mg kg$^{-1}$)

<table>
<thead>
<tr>
<th>dietary variable</th>
<th>Cd (mg kg$^{-1}$)</th>
<th>Zn (mg kg$^{-1}$)</th>
<th>Fe (mg kg$^{-1}$)</th>
<th>Ca (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonsupplemented rice</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Zn(NO$_3$)$_2$, 30 ppm</td>
<td>0.80</td>
<td>30</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Zn(NO$_3$)$_2$, 60 ppm</td>
<td>0.80</td>
<td>60</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Zn(NO$_3$)$_2$, 200 ppm</td>
<td>0.80</td>
<td>200</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Zn(NO$_3$)$_2$, 400 ppm</td>
<td>0.80</td>
<td>400</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Zn(NO$_3$)$_2$, 1000 ppm</td>
<td>0.80</td>
<td>1000</td>
<td>4.01</td>
<td>102</td>
</tr>
<tr>
<td>rice + Fe(NO$_3$)$_2$, 6 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>6</td>
<td>102</td>
</tr>
<tr>
<td>rice + Fe(NO$_3$)$_2$, 12 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>12</td>
<td>102</td>
</tr>
<tr>
<td>rice + Fe(NO$_3$)$_2$, 40 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>40</td>
<td>102</td>
</tr>
<tr>
<td>rice + Fe(NO$_3$)$_2$, 80 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>80</td>
<td>102</td>
</tr>
<tr>
<td>rice + Fe(NO$_3$)$_2$, 200 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>200</td>
<td>102</td>
</tr>
<tr>
<td>rice + Ca(NO$_3$)$_2$, 150 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>150</td>
</tr>
<tr>
<td>rice + Ca(NO$_3$)$_2$, 300 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>300</td>
</tr>
<tr>
<td>rice + Ca(NO$_3$)$_2$, 1000 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>1000</td>
</tr>
<tr>
<td>rice + Ca(NO$_3$)$_2$, 5000 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>5000</td>
</tr>
<tr>
<td>rice + CaCl$_2$, 150 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>150</td>
</tr>
<tr>
<td>rice + CaCl$_2$, 300 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>300</td>
</tr>
<tr>
<td>rice + CaCl$_2$, 1000 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>1000</td>
</tr>
<tr>
<td>rice + CaCl$_2$, 2000 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>2000</td>
</tr>
<tr>
<td>rice + CaCl$_2$, 5000 ppm</td>
<td>0.80</td>
<td>19.1</td>
<td>4.01</td>
<td>5000</td>
</tr>
<tr>
<td>mouse feed</td>
<td>0.09</td>
<td>42.0</td>
<td>222</td>
<td>15687</td>
</tr>
</tbody>
</table>
Nutrient- and Cd-Amended Rice. Prior to assessing the influence of mineral dietary supplements on rice Cd-RBA using an in vivo mouse bioassay, mineral nutrients including Zn, Fe, and Ca were incorporated into the Cd-contaminated rice sample (0.80 ± 0.01 mg Cd kg⁻¹) by cooking with nutrient solutions. Briefly, stock solutions containing 1 g L⁻¹ Zn as Zn(NO₃)₂, 10 g L⁻¹ Fe as Fe(NO₃)₂, and 100 g L⁻¹ Ca as Ca(NO₃)₂ and CaCl₂ were prepared. Rice amended with Ca(NO₃)₂ and CaCl₂ were compared to assess the influence of Cl on Cd-RBA in rice. Chloride salts of Fe and Zn were not used because our study showed Ca supplement as Ca(NO₃)₂ was the most effective in reducing rice Cd-RBA compared with Fe(NO₃)₂ and Zn(NO₃)₂. For each nutrient, five concentrations in cooked rice were obtained by cooking rice with nutrient solutions diluted from stock solutions, i.e., 30, 60, 200, 400, and 1000 mg kg⁻¹ Zn; 6, 12, 40, 80, and 200 mg kg⁻¹ Fe; and 150, 300, 1000, 2000, and 5000 mg kg⁻¹ Ca (Table 1). The supplement gradient was set based on Zn, Fe, and Ca levels in natural foods. The lowest supplement concentration at 30, 6, and 150 mg kg⁻¹ Zn, Fe, and Ca were ~1.5 fold higher than background values in rice (19.1, 401, and 102 mg kg⁻¹ Zn, Fe, and Ca), while the highest concentration represented the maximum concentrations reported in foods.¹⁵ For each supplement level, ~180 g of rice was cooked with 360 mL of diluted nutrient solutions using an electric cooker (CFXB50YB7F-65, Supor, China) for 20 min, followed by a 10 min postcooking period. Following cooking, rice samples were freeze-dried and milled into powders to homogenize the nutrient-amended rice. Milli-Q water was added into the powder to make a paste by kneading before being freeze-dried again. In addition, CdCl₂ was added into the control rice powder to make a paste by kneading before being freeze-dried.

Cd Relative Bioavailability in Rice Using a Mouse Bioassay. Following preparation, the nutrient- and CdCl₂-amended rice samples were administered to mice (female Balb/c) to assess the influence of nutrient supplements on Cd-RBA in rice. Mice weighing 18–20 g were purchased from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China), which were acclimated for 1 week under standard conditions. Following acclimation, mice were randomly assigned to individual plastic cages with three mice per group. Mouse feed was removed and replaced with the amended rice. Each mouse received ~4 g of nutrient- or CdCl₂-amended rice daily at 9:00 am over a 10-day period. The Cd exposure via rice consumption over a 10-day period minimized the variability in Cd accumulation between replicated mice compared to a single gavage dose. Therefore, three mice per treatment was adequate for this experiment. Control mice received Cd-contaminated rice daily without nutrient supplement (0.80 ± 0.01 mg Cd kg⁻¹). In addition, control rice sample containing 4.0 μg kg⁻¹ Cd was administered to three mice to determine background values of Cd accumulation in mouse tissues.

After 10 days of exposure, rice consumption was determined as the difference in rice supplied and remaining. Following overnight fasting and determination of body weights, mice were sacrificed to collect the liver and kidneys, which were immediately stored at −80 °C separately prior to freeze-drying. Mice fed Cd-contaminated rice amended with different levels of nutrients had similar body weights over the 10-day period (Figure S1). Cadmium concentrations in the liver and kidneys were determined using ICP-MS separately following digestion using USEPA Method 3050B (Figure S2), while Zn, Fe, and Ca concentrations were determined using ICP-OES.

Cadmium-dose levels from CdCl₂ exposure were calculated as the product of rice consumption and the difference in Cd concentrations between CdCl₂-amended rice and control rice (4.0 ± 0.3 μg kg⁻¹), while Cd concentrations in mouse liver plus kidneys due to CdCl₂ exposure were calculated as the difference between mice receiving CdCl₂-amended rice and control rice. Cadmium dose levels from consumption of the Cd-contaminated rice were calculated as the product of rice consumption and Cd concentration in the rice (0.80 ± 0.01 mg kg⁻¹ Cd), while Cd concentrations in mouse liver plus kidneys were determined after adjustment for background Cd concentrations.

Previous studies have shown that following absorption, Cd distribution in mouse kidneys or liver varied depending on dosing levels, with ratio of Cd accumulation in kidneys versus liver decreasing with increasing Cd dosing level.⁹,¹⁰ This may introduce variability in Cd-RBA determination when individual end points were used. As a result, average Cd concentrations in kidneys plus liver has been used to determine Cd-RBA in contaminated soils to overcome the concentration-dependent variability of Cd distribution in kidneys or liver.⁹,¹⁰ As such, the average Cd accumulation in the liver plus kidneys was used as the end point to determine Cd-RBA. Cadmium-RBA in the contaminated rice was calculated as the ratio of dose normalized Cd accumulation in the liver plus kidneys following the contaminated rice exposure to that following CdCl₂-spiked control rice exposure (eq 1):

\[
\text{Cd relative bioavailability (‰) = } \frac{\text{organ Cd} \times \text{Cd dose}]_{\text{CdCl}_2 \text{-spiked rice}}_{\text{organ Cd}}_{\text{CdCl}_2 \text{-spiked rice}}_{\text{Cd dose}]_{\text{contaminated rice}}}_{100}
\]

where organ Cdcontaminated rice and organ CdCdCl₂-spiked rice are Cd concentrations in mouse liver plus kidneys following the Cd-contaminated rice and CdCl₂-spiked rice exposure, respectively, and Cd dose]CdCl₂-spiked rice and Cd dose]CdCl₂-spiked rice are Cd dose level from the Cd-contaminated rice and CdCl₂-spiked rice exposure, respectively. This equation has been widely accepted to calculate Cd-RBA in contaminated soils.⁸⁻¹⁰ Recently, it was used to determine Cd-RBA in Cd-contaminated rice, wheat, and vegetables.⁷

Quality Assurance and Control and Data Processing. During Cd analyses, standard reference material for rice (GSB-21, Chinese Geological Reference Materials) was included. The accuracy of USEPA Method 3050B was acceptable, with a measured Cd concentration of 0.012 ± 0.002 mg kg⁻¹ in rice.
GSB-21 (0.012 ± 0.003 mg kg⁻¹). The detection limit of ICP-MS, calculated as three times the standard deviation of the blank values, was 0.005 μg Cd L⁻¹, sensitive enough to determine Cd concentration in the Cd-contaminated and control rice as well as mouse tissues. During analyses using ICP-MS, spikes and check samples (1–10 μg Cd L⁻¹) were included every 20 samples, producing recoveries of 102 ± 7.2% and 97 ± 7.1% (n = 30). All graphs were created using SigmaPlot (version 12.5, Systat Software Inc., San Jose, CA). One-way ANOVA was used to determine significant differences in rice Cd-RBA among nutrient supplement treatments using SAS (version 9.1.3 for Windows).

RESULTS AND DISCUSSION

Cadmium Dose Response and Cd Relative Bioavailability in Rice. To determine Cd-RBA in rice, an in vivo mouse bioassay was developed by establishing a linear relationship between Cd dose and Cd accumulation in the liver plus kidneys after 10 days of exposure. Following the administration of CdCl₂ to mice via Cd-amended rice containing 0.2, 0.5, and 1.0 mg kg⁻¹ Cd over a 10-day period, strong linear relationships were observed between Cd dose (23–129 μg Cd kg⁻¹ d⁻¹) and Cd accumulation in the liver (R² = 0.94) and kidneys (R² = 0.92) (Figure 1A); however, Cd accumulation was significantly higher in kidneys compared to the liver, consistent with previous studies.⁹,¹⁰ The ratio of Cd accumulation in kidneys versus liver was 1.4–3.9. This was related to different partitioning of Cd among different tissues following Cd absorption. At low dosages, Cd may be bound to metallothionein (MT) or other low-molecular-weight proteins, so it is preferentially distributed to the kidneys, whereas at higher dosages, Cd may be absorbed as free metal so it accumulates in the liver.¹⁶

To overcome the dose-dependent distribution of Cd in the liver and kidneys, we used the average Cd accumulation in the liver plus kidneys as the end point for Cd exposure, which has been used in previous studies.⁹,¹⁰ Similar to individual tissues, strong linear correlation (R² = 0.93) was observed between average Cd concentration in the liver plus kidneys and Cd dose, showing the suitability of the combined end point to determine Cd-RBA in rice at low Cd doses (23–129 μg Cd kg⁻¹ d⁻¹; Figure 1B). At higher Cd doses (80–800 μg Cd kg⁻¹ d⁻¹), Li et al.¹⁰ observed similar linear relationships (R² = 0.99) between Cd accumulation in mouse liver plus kidneys and the Cd dose. However, compared to Li et al.,¹⁰ the slope of the correlation of this study was significantly higher (Figure 1B), suggesting significantly higher (p < 0.01) Cd absorption by mice in this study. This was attributed to different Cd dosing approaches between the two studies. In this study, CdCl₂ was administered to mice via the control rice, which was low in mineral nutrients (17.8 mg kg⁻¹ Zn, 3.7 mg kg⁻¹ Fe, and 118 mg kg⁻¹ Ca), while CdCl₂ was incorporated into mouse feed in Li et al.,¹⁰ which was significantly higher in Zn, Fe, and Ca (42 mg kg⁻¹ Zn, 222 mg kg⁻¹ Fe, and 15687 mg kg⁻¹ Ca). These differences in Cd accumulation in mouse tissues suggested the influence of dietary nutrients on Cd absorption in mice and the importance of reinforcing matrix matching when in vivo RBA studies are undertaken.¹⁷

After the linear relationship between Cd accumulation in the liver plus kidneys and Cd dose was established, the mouse bioassay was applied to determine Cd-RBA in the Cd-contaminated rice (0.80 ± 0.01 mg kg⁻¹). The Cd-contaminated rice sample contained 19.1 mg kg⁻¹ Zn, 4.01 mg kg⁻¹ Fe, and 102 mg kg⁻¹ Ca (Table 1), similar to the nutrient status of the control rice that was used to establish Cd dose response (17.8, 3.7, and 118 mg kg⁻¹). For the Cd-contaminated rice without nutrient supplement, Cd-RBA was 43 ± 5.3%. The value was within our previous study, which varied from 17% to 57% in 10 rice samples.⁷

Impacts of Mineral Supplements on Rice Cd Relative Bioavailability. The impacts of different mineral supplements [Zn(NO₃)₂, Fe(NO₃)₂, and Ca(NO₃)₂] on Cd accumulation in mouse liver plus kidneys were assessed using Cd-RBA bioassay in mouse liver plus kidneys as the end point (eq 1). A Cd supplement administered as Ca(NO₃)₂ was the most effective in reducing rice Cd-RBA followed by Fe, while no significant reduction in Cd-RBA was observed following Zn amendment (Figure 2A–C). Compared to the rice without supplement (43 ± 5.3%), Cd-RBA increased to 49–57% at low Zn concentrations (<60 mg kg⁻¹), while Cd-RBA decreased slightly to 33–42% at higher Zn concentrations (200–1000 mg kg⁻¹) (Figure 2A). Similarly, lower Fe concentrations (6–40 mg kg⁻¹) had little effect on rice Cd-RBA (39–47%), while high Fe supplements (80–200 mg kg⁻¹) decreased Cd-RBA by 37% to 26–27%. Unlike Zn or Fe, Ca supplements (150–5000 mg kg⁻¹) decreased Cd-RBA by 31–80% to 8.5–29%, with increasing Ca concentration leading to lower Cd-RBA (p < 0.05).
Our study is the first to quantify the influence of different mineral nutrients (e.g., Zn, Ca, and Fe) on rice Cd-RBA, although studies have assessed their influences on Cd accumulation in animals.\textsuperscript{11,14} In rats, Fe supplement has been used to treat anemia by lowering hemoglobin and hematocrit impacts caused by Cd exposure.\textsuperscript{18} Evidence suggested that Cd is absorbed via the intestinal Fe transporter, divalent metal transporter-1 (DMT1), which is located in the apical
membrane of enterocytes. The amount of Cd retained in the body was 10-fold higher in rats fed with a Fe-deficient diet than in those fed with a Fe-sufficient diet, with the levels of DMT1 mRNA in the kidneys being 30% higher in rats fed with a Fe-deficient diet, suggesting that over-expression of the Fe transporter under Fe-deficiency situation contributed to elevated Cd absorption. Under sufficient Fe status, there is a competition between Fe and Ca for sites on the Fe transporters, thereby leading to decreased rice Cd-RBA with increasing dietary Fe levels (Figure 2B).

In addition to Fe transporters, Ca transporters are also associated with Cd absorption. Suzuki et al. determined Cd uptake in anemic mice, suggesting that DMT1 transporters are not the only transport proteins responsible for Cd absorption in the intestines. Instead, the low-molecular-weight Ca binding protein (CaBP) is responsible for both Ca and Cd absorption. The affinity of Cd to CaBP is almost as strong as that for Ca. Serum Cd concentrations in rats on low-Ca diets were 6-fold greater, while Cd accumulation in the liver, kidneys, and femoral bone increased 4-fold compared with those on normal dietary Ca. Larsson and Piscator showed that, under Cd exposure, rats fed on low-Ca diet accumulated ~60% higher Cd concentrations in the liver and kidneys than those fed on normal dietary Ca. Low dietary Ca intake can result in intensified synthesis of CaBP, thus increasing Cd absorption. Moreover, under Ca deficiency, Cd is more easily bound to CaBP due to lower competition from Ca, further contributing to increased Cd absorption. When Ca intake is adequate, it may inhibit Cd absorption by competition between Ca and Cd for binding sites in CaBP.

In this study, compared with Ca and Fe, Zn supplement was less effective in reducing rice Cd-RBA. This was possibly related to different response of Zn accumulation in mouse tissues to mineral supplements. For Zn, its accumulation in mouse liver and kidneys increased by 3.1- and 8.3-fold from 75.9 to 235 mg kg$^{-1}$ when Zn concentrations in the rice increased from 20 to 1000 mg kg$^{-1}$ (Figure 3A). However, following Fe supplements, Fe accumulation in mouse tissues showed a much lower increase of 0.94–1.4 fold (Figure 3B). Similarly, following Ca supplements, Ca accumulation in mouse liver showed no significant increase, while kidney Cd accumulation increased by 1.0–1.6 fold (Figure 3C). This suggested enhanced Zn but not Fe or Ca absorption following mineral supplementation.

Prior to bioavailability assessment, mice were fed with mouse feed, which contained 15687 mg kg$^{-1}$ Ca, 222 mg kg$^{-1}$ Fe, and 42 mg kg$^{-1}$ Zn. The mouse feed was rich in Ca and Fe, which was much higher than the supplement levels of Ca (150–5000 mg kg$^{-1}$) and Fe (6–200 mg kg$^{-1}$). However, the Zn concentration in mouse feed was close to the Zn supplement levels (30–100 mg kg$^{-1}$), being twice the Zn concentration in the contaminated rice (19.1 mg kg$^{-1}$). Therefore, prior to rice feeding, the mice might be low in Zn status compared with Fe and Ca. When higher Zn concentrations (30–1000 mg kg$^{-1}$) were added to rice to feed mice, transporters responsible for Zn absorption might be up-regulated to absorb Zn. The up-regulation of Zn transporters probably enhanced Cd absorption, countering the inhibition effect of increasing Zn intake on Cd absorption. This eventually resulted in an slight increase in rice Cd-RBA at low levels of Zn supplement (30–60 mg kg$^{-1}$) and no significant reduction in Cd-RBA at high levels of Zn supplement (200–1000 mg kg$^{-1}$) (Figure 2A). In contrast, because mice had been adapted to adequate Ca and Fe intake from mouse feed prior to rice exposure, Fe and Ca transporters might not be up-regulated following Ca and Fe supplement (150–5000 and 6–200 mg kg$^{-1}$), leading to decreased Cd absorption with increasing Ca and Fe concentrations in rice due to competition for transporters (Figure 2BC). Similarly, Ryu et al. showed that serum Fe concentrations of rats with marginal status of Fe increased following Fe supplement. Future study is needed to reveal the different responses of Zn, Fe, and Ca transporters corresponding to mineral supplements.

Impacts of Chloride on Rice Cd Relative Bioavailability. In contrast to Ca, Fe, and Zn, studies have shown that Cl may enhance Cd absorption and accumulation in plants. However, in vivo bioassays have not been conducted to assess its influences on Cd absorption in animals or Cd-RBA in rice, which is important because populations may differ significantly in their dietary salt intake. In this study, Ca supplements supplied as CaCl$_2$ and Ca(NO$_3$)$_2$ were compared. Unlike Ca(NO$_3$)$_2$, CaCl$_2$ at 150–2000 mg kg$^{-1}$ Ca was ineffective in reducing rice Cd-RBA (37–67%), with significant decrease in Cd-RBA (25%) only observed at the highest Cd concentration of 5000 mg kg$^{-1}$ (Figure 2D). Compared to rice supplemented with Ca(NO$_3$)$_2$, Cd-RBA values with CaCl$_2$ were significantly higher (25–67% versus 8.5–29%, Figure 2CD). For example, Cd-RBA increased from 43 ± 5.2% (rice without supplementation) to 54 ± 10% and 67 ± 9.4% at 150 and 300 mg kg$^{-1}$ Ca as CaCl$_2$, respectively. These differences in Cd-RBA between Ca supplements as CaCl$_2$ and Ca(NO$_3$)$_2$ suggested that Cl was effective in increasing Cd-RBA in rice.

It is possible that Cl$^-$ binds with Cd cations in the intestinal tract, forming Cd–chloro complexes, which could be directly absorbed. The direct uptake of Cd–chloro complexes by plants is responsible for increased Cd accumulation in plant with increasing Cl in soils. Based on Cd transport in human Caco-2 cells, Jumarie et al. determined that, at a low concentration of 43 nM Cd, initial Cd uptake rates by cells increased linearly with increasing concentration of Cd–chloro complexes from 0 to 250 nM, with Cd accumulation being 4-fold higher in the presence of 147 mM Cl than in low-CI medium. Overall, our study showed that Cl enhanced Cd-RBA in rice. Therefore, for populations susceptible to Cd-contaminated rice, avoiding a high-salt diet may reduce Cd exposure via rice consumption and the associated health risks.

Implications for Human Health. Previous studies showed that Cd accumulation was associated with human’s nutrient status; however, there is a lack of evidence showing decreased Cd-RBA in foods with increasing mineral status. For example, it is known that urinary Cd level is significantly higher for female than in male, presumably due to lower Fe storage in female. Lin et al. reported higher risk of Cd exposure in women with deficient dietary Zn, based on 5204 subjects aged 50 years old from the Third National Health and Nutrition Examination Survey, whereas Vaher et al. found no increase in blood Cd in young women who regularly consumed shellfish that added nearly three times as much Cd to their intake compared to women who did not consume shellfish. Perhaps the consumption of the Zn- and Fe-rich shellfish elevated their Zn and Fe status and possibly reduced their Cd absorption. Besides, the European and American inhabitants who consumed a variety of garden foods that accumulated Zn, Fe, and Cd along with Cd would have reduced Cd absorption.

Our study revealed a quantitative mechanism behind these negative relationships between Cd accumulation and nutrient
status in humans that is decreased dietary Cd-RBA with increasing dietary minerals.

Overall, our research provided strong evidence that increased dietary levels of Fe and Ca effectively reduced Cd exposure by decreasing Cd-RBA in rice, thereby reducing Cd accumulation in humans via rice intake. Also, consumers who subsist on rice-based diets might suffer a greater Cd absorption than individuals with other staple diets because rice is often poor in mineral nutrients. People with poor nutrition and high-salt diets are at higher risk of Cd exposure than those with adequate nutrition and low-salt diets. Nutrient supplement strategies may be used to alleviate human Cd exposure from rice consumption and associated health risks. For the validation of the effectiveness of nutrient supplement strategies to decrease dietary Cd exposure, a long-term population-based intervention study needs to be conducted to assess their effects on human Cd body burden.

**ASSOCIATED CONTENT**

5 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02993.

Figures showing detailed information on mouse body weight before and after exposure and Cd accumulation in mouse liver and kidneys following rice consumption with different nutrient treatments. (PDF)

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**Notes**

The authors declare no competing financial interest.

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