Mineral Status of Female Rats Affects the Absorption and Organ Distribution of Dietary Cadmium Derived from Edible Sunflower Kernels (Helianthus annuus L.)

Philip G. Reeves* and Rufus L. Chaney†

*USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58203; and †USDA, ARS, Environmental Chemistry Laboratory, Beltsville, Maryland 20705

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The intake of food cadmium (Cd) in μg/day over time can increase the body burden of this element. Some human populations that consume subsistence rice-based diets low in calcium (Ca), iron (Fe), and zinc (Zn) are more susceptible to Cd poisoning than populations that consume more nutritious diets. This study determined the effects of marginal deficiencies of these essential elements on the absorption and organ retention of Cd from a natural food that contains Cd, edible sunflower kernels (Helianthus annuus L.; SFK). Weanling female rats were fed diets containing 20% SFK in a 2 × 2 factorial design with marginal and adequate amounts of Ca, Zn, and Fe. Marginal Zn (11 mg/kg) and Fe (13 mg/kg), and Cd (0.18 mg/kg) were derived solely from 20% SFK. These amounts of Fe and Zn represented 39 and 90% of the NRC requirement for the rat, respectively. The marginal dietary Ca concentration (2.5 g/kg) was one-half the NRC requirement. After 5 weeks on the experiment, rats were fed 1 g of their respective diets containing SFK extrinsically labeled with 37 kBq 109Cd, and absorption was determined by whole-body counting techniques. Rats were then killed and organs collected for 109Cd analysis.

INTRODUCTION

Over time, dietary intakes of cadmium (Cd) greater than the provisional tolerable weekly intake (7 μg/kg body weight) established by the World Health Organization (WHO, 1989) can increase the body burden of this toxic element to the point of causing renal proximal tubular dysfunction. In some human populations that consume subsistence rice-based diets, low nutritional states in calcium (Ca), iron (Fe), and zinc (Zn) occur. As a result, these populations seem to be more susceptible to adverse health effects of Cd than those who consume more nutritious diets, but with similar intakes of Cd (McKenzie-Parnell et al., 1988; Tachechi, 1978).

The concentrations of Ca, Fe, and Zn in the diet are known to influence the absorption of Cd and its distribution in organs and tissues. If the
concentrations of these minerals are low, Cd absorption increases, and if they are high, Cd absorption decreases (Brzoska and Moniuszko-Jakoniuk, 1998; Evans et al., 1970; Flanagan et al., 1978; Fox et al., 1979; Kello et al., 1979; Koo et al., 1978; Reeves and Vanderpool, 1998). Thus, the mineral composition of the diet could be an important factor that controls the extent of food Cd absorption and tissue accumulation. However, this factor generally has not been taken into account when interpreting the potential risk of food-Cd to humans.

Most of the animal studies done in the past have used much higher amounts of Cd than those found in natural food sources, and the concentrations of the competing minerals used were unnaturally low or high, depending on the effect to be measured. In addition, the concentration ratios of Cd to Ca or Cd to Zn in many tests were not considered in the experimental designs. Furthermore, the diets used were formulated mostly with semipurified ingredients in which Cd and the other competing minerals were added as salts (Reeves and Rossow, 1996). These types of experimental designs limit the interpretation of the interactions between dietary sources of Cd and other minerals in the natural food itself. This limitation complicates the extrapolation of findings to humans who consume a variety of foods containing Cd and other elements from natural sources.

To test the hypothesis that low intakes of Fe, Zn, and Ca enhance the absorption and tissue distribution of Cd from edible sunflower kernels (Helianthus annuus L.; SFK), we used a factorial design in which adequate or marginal concentrations of the three minerals were supplied before and during the feeding period. Also, is the risk of Cd from foods, such as SFK, reduced by supplementing the diet with essential Fe, Zn, or Ca at concentrations similar to the required level but not excessive?

SFK were chosen because they contain a higher concentration of Cd than most edible seeds and they are well tolerated as a food source by small rodents; thus, they can be mixed into the diet at high concentrations without being rejected by the animal. Consequently, reasonably high concentrations of dietary Cd from a natural source can be obtained without resorting to adding Cd salts. In addition, even though the amount of Cd absorbed by the animals is small, it is high enough to accurately measure tissue accumulation of Cd after only a few weeks of exposure.

METHODS AND MATERIALS

The experimental design was a $2 \times 2 \times 2$ factorial. The three factors were dietary Ca, Zn, and Fe at two different concentrations each. Because females tend to absorb Cd more readily than males, the experimental model was the female rat [Strain: SAS:VAF (SD), Charles River/Sasco, Wilmington, MA] beginning at 3 weeks of age. There were eight groups of seven rats each. Another group of seven rats was fed the AIN-93G-EGG rodent diet (Reeves, 1996) with no added Cd to obtain baseline values.

Processing of SFK. Shelled SFK were obtained from Heartland Mills, Inc., Marienthal, Kansas. The kernels contained 0.82 mg Cd/kg, 52 mg Zn/kg, 65 mg Fe/kg, and 1300 mg Ca/kg of edible kernel. Before incorporating SFK into the diet, they were oven-roasted in shallow pans, 2.5 cm deep, at 350°F for 45 min and then kept at −20°C until used. The frozen roasted kernels were ground in a food processor until they reached the consistency of coarse powder and then incorporated into the animal diets.

Diet. The diets were formulated according to Reeves et al. (1998) and were similar to the AIN-93G-EGG diet (Reeves, 1996), except that 15% of the carbohydrate source (starch) and 5% of the protein source were replaced with roasted ground SFK. The remainder of the protein source was egg white solids (Harlan Teklad, Madison, WI). The dietary source of fat was supplied solely by SFK. The mineral supplements to the basal diet were balanced so that none of the minerals in question would be far less than or far in excess of the dietary requirements for the laboratory rat (National Research Council, 1995). The amounts of Zn and Fe in the marginal diets were supplied solely by SFK. However, the Ca concentration in SFK was not sufficient to obtain a marginal concentration; therefore, extra Ca was added as calcium carbonate to bring it to the marginal amount. In the adequate diet, the concentrations of Ca, Zn, and Fe were adjusted by the addition of calcium carbonate, zinc carbonate, and ferric citrate, respectively. Chemical analysis showed that the marginal diets contained (mean ± SD) 10.8 ± 0.9 mg Zn/kg, 13.9 ± 2.3 mg Fe/kg, and 2540 ± 81 mg Ca/kg. The adequate diets contained 32.0 ± 1.0, 39.1 ± 3.7, and 4710 ± 290 mg of Zn, Fe, and Ca/kg, respectively. The marginal values represented approximately 90, 39, and 51% of the requirement of Zn, Fe, and Ca, respectively, for the growing rat (National Research Council, 1995). Zn concentration in the adequate diets was higher than the requirement because this is the value for the standard rodent diet, AIN-93G (Reeves et al., 1993a). Chemical analysis showed that the diets contained 0.18 ± 0.02 mg Cd/kg, and 90% of this was supplied by SFK.
Rats were fed their respective diets for 5 weeks; then four rats from each group were randomly selected and fasted between 700 and 1700 h. The fasted rats were given a 1-g sample of their respective diets that contained 0.2 g of SFK extrinsically labeled with 185 kBq \(^{109}\)Cd/g. Labeling was accomplished by blending the SFK to a slurry in deionized water to which a specified amount of the radioactive Cd was added. The slurry was then lyophilized and 0.2 g of the dried material per g of basal diet was added.

The rats had completely consumed their labeled meal after about 2 h. Then they were placed individually into a small animal whole-body counter to determine the amount of \(^{109}\)Cd ingested (Reeves et al., 1994). These values were used as the initial doses. Afterward, animals were allowed to resume normal food consumption, and \(^{109}\)Cd activity was measured at 12, 24, and 36 h after dosing. Activity was then measured at 24-h intervals until day 7 and then at 48-h intervals until day 16. The activity of each animal at each counting period was expressed as a percentage of the initial dose (Fig. 1, top) and the data were plotted as the ln (percentage dose) against time (Fig. 1, bottom). An attempt was made to fit the data to exponential models, but they would not converge. The alternative was to estimate the percentage retention of \(^{109}\)Cd by taking the average of values between days 11 and 16 and dividing by the initial dose. This is a modification of the original method of Heth and Hoekstra (1965).

After completing the collection of \(^{109}\)Cd activity data, each rat was anesthetized and blood was withdrawn from the abdominal aorta until the rat expired. Whole liver, both kidneys, one femur, and 20 cm of small intestine were collected for \(^{109}\)Cd activity determination and Cd analysis. Serum was separated from whole blood for Cd determination. Tissue samples were lyophilized to a constant weight and samples weighing 0.2 to 0.5 g were transferred to Pyrex beakers and covered with a watch glass. Ten milliliters of high-purity concentrated H\(_2\)SO\(_4\) was added to each sample; samples were heated on a hot plate at subboiling temperature overnight. The nitric acid was evaporated and then dry-ashed at 475°C for 12 h. Samples were cooled before 10 mL of concentrated H\(_2\)SO\(_4\) was added. Five milliliters of 30% hydrogen peroxide was added slowly and the mixture was allowed to reflux for 12 h. After the acid-peroxide mixture was allowed to evaporate, the samples were placed back into the 475°C oven and ashed overnight. The mineral residue was dissolved in a constant volume of 1% high-purity HCl and analyzed for Zn, Fe, Ca, and Cu by inductively coupled argon plasma techniques. Cd was determined by graphite furnace atomic absorption spectrometry with Zeeman background correction (GFAA). Quality control samples of bovine liver (National Institutes of Standards and Technology, Gaithersburg, MD) with certified concentrations of minerals were analyzed with each batch of tissues, and all values were within the acceptable range. Cd was analyzed in whole, untreated serum by GFAA. There is no certified standard for serum Cd; therefore, internal Cd standards were used to assure accuracy of measurement.

The data were statistically analyzed by ANOVA for a full factorial design by using StatView or SAS computer programs (SAS Institute, Inc., Cary, NC). Differences between treatment means were assumed to be significant if \( P \leq 0.05 \).
RESULTS

Weight gain. The data clearly showed that feeding female rats diets containing Ca and Fe at lower than the estimated requirements, and Zn at a concentration lower than the standard rodent diet, had no significant effect on weight gain over the 7-week feeding period (Table 1). The average gain over the course of the experiment was about 2.5 g/day, which is normal for the female rat.

Cadmium absorption. The reduced intake of Fe and/or Ca significantly \((P < 0.001 \text{ and } P < 0.004, \text{ respectively})\) elevated \(^{109}\text{Cd}\) absorption and significantly \((P < 0.001)\) increased the percentage of radiolabeled SFK Cd remaining in liver-plus-kidney 16 days after dosing (Table 1). The consumption diets marginally deficient in Zn did not affect \(^{109}\text{Cd}\) absorption or tissue \(^{109}\text{Cd}\) accumulation in female rats (Table 1).

Organ cadmium. There was a significant \((P < 0.011)\) interaction between Fe and Ca that affected the accumulation of Cd in the intestine, serum, liver, and kidney, but not in femur (Fig. 2). In all cases, the interaction showed that marginal dietary Ca had a greater effect on Cd concentration when dietary Fe was marginal than when it was adequate. Cd was higher in the intestine \((P < 0.001)\) when dietary Fe was marginal than when it was adequate. Opposite to the effect of Fe and Ca, marginal dietary Zn caused a significant \((P < 0.014)\) reduction of intestinal Cd concentration. Also, there was a complex three-way interaction between Zn, Fe, and Ca for kidney Cd (Fig. 2). This showed that when dietary Zn and Fe were marginal, and dietary Ca was adequate, kidney Cd was elevated by nearly three-fold, compared with the effect when dietary Zn and Ca were adequate and Fe was marginal.

Organ zinc. There were significant interactions between Zn and Ca for Zn concentration in intestine, serum, liver, and kidney (Table 2). In all cases, there was a significant reduction of organ Zn when Zn was marginal and dietary Ca was adequate but not when Ca was marginal. There was a complex three-way interaction among Zn, Fe, and Ca for Zn concentration in femur. This showed that when dietary Zn was adequate and Fe was marginal, marginal Ca elevated femur Zn. On the other hand, when dietary Zn was marginal, marginal dietary Ca maintained femur Zn at a normal concentration, regardless of the dietary Fe concentration. In other words, femur Zn concentration was reduced only in rats fed diets containing marginal Zn and adequate Ca.

Organ iron. There were significant interactions between Fe and Ca for Fe concentrations in intestine (Table 2). Marginal dietary Ca elevated tissue Fe when dietary Fe was adequate but had no effect when dietary Fe was marginal. Overall, except for serum, there was less Fe in tissues of rats fed marginal Fe than in those fed adequate Fe. There was a complex three-way interaction among Zn, Fe, and Ca for Fe concentration in liver and kidney. This showed that when rats were fed diets adequate in Zn and Fe, marginal dietary Ca elevated kidney Fe by almost two-fold over that in rats receiving adequate Ca. However, when dietary Zn was marginal and Fe was adequate, dietary Ca had no effect. Only main effects were calculated for femur Fe. There was less iron in the femur of rats fed marginal dietary Fe than in those fed adequate Fe. Conversely, there was more iron in the femurs of rats fed either marginal Zn or Ca than in rats fed an adequate supply of these minerals.

Organ calcium. The concentration of Ca in the intestine was significantly lower when the rats were fed marginal Ca diets than when fed adequate diets (Table 2). Although there were very small differences among groups, there was a significant interaction between Fe and Ca for Ca concentration in serum. This showed that when dietary Fe was adequate, Ca was slightly elevated when dietary Ca was marginal, but when dietary Fe was marginal, serum Ca was slightly reduced when dietary Ca was marginal. There was also a significant interaction between Fe and Ca for liver Ca. This showed that when dietary iron was marginal, marginal dietary Ca lowered liver Ca more than when dietary Fe was adequate. Marginal dietary Fe significantly elevated kidney Ca. Also, there was an interaction between Zn and Ca, which showed that when dietary Zn was marginal, the amount of Ca in the kidney was higher when Ca was marginal than when Ca was adequate. When dietary Zn was adequate, there was no Ca effect. There was a Zn x Fe interaction for Ca in femur, but the differences were so small that they probably were not physiologically significant.

Organ copper. Although copper was not a dietary variable, it was included in the analysis because there are known interactions between it and Fe or Zn. There was a significant interaction between Zn and Fe for intestinal, liver, and kidney Cu (Table 2). In all cases, marginal dietary Fe lowered tissue Cu in rats fed adequate Zn but elevated it in rats fed marginal Zn. The differences were greater for liver than for intestine. Marginal dietary Fe alone reduced serum Cu. Also, there was a Fe x Ca
### TABLE 1
The Effects of Marginal and Adequate Dietary Zn, Fe, and Ca on Body Weight Gain and on the Absorption and Liver + Kidney Accumulation of Cd from Sunflower Kernels Fed to Female Rats at 20% of the Diet

<table>
<thead>
<tr>
<th>Dietary variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
<th>ANOVA table P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Zn, Fe, Zn x Fe, Ca, Zn x Ca, Fe x Ca, Zn x Fe x Ca</td>
</tr>
<tr>
<td>Fe</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Body weight gain, g/day\(^a\)

\[
\begin{align*}
\text{2.49} & \pm 0.25 \\
\text{2.36} & \pm 0.21 \\
\text{2.72} & \pm 0.23 \\
\text{2.64} & \pm 0.38 \\
\text{2.63} & \pm 0.44 \\
\text{2.56} & \pm 0.50 \\
\text{2.59} & \pm 0.29 \\
\text{2.63} & \pm 0.40
\end{align*}
\]

\(^{108}\)Cd absorption, % of initial dose\(^a\)

\[
\begin{align*}
0.37(0.28-0.47) & \\
0.39(0.27-0.57) & \\
1.02(0.65-1.22) & \\
1.46(0.88-2.45) & \\
0.38(0.31-0.45) & \\
0.67(0.48-0.93) & \\
0.87(0.80-0.95) & \\
1.35(0.93-1.96) & \\
\end{align*}
\]

\(^{104}\)Cd in liver + kidney, % of initial dose\(^a\)

\[
\begin{align*}
0.24(0.22-0.26) & \\
0.22(0.19-0.26) & \\
0.48(0.33-0.69) & \\
0.98(0.56-1.71) & \\
0.19(0.15-0.23) & \\
0.35(0.23-0.54) & \\
0.44(0.32-0.66) & \\
0.72(0.50-1.03) & \\
\end{align*}
\]

\(^a\) Adequate and marginal amounts of test elements (± Zn; 32/10.8 mg/kg diet); (± Fe; 39.1/13.9 mg/kg diet); (± Ca; 4710/2540 mg/kg diet). The diets contained 0.18 ± 0.02 mg Cd/kg.

\(^b\) Values represent the mean ± SD for seven replicates.

\(^c\) Four rats were randomly selected from each of the original groups of seven rats and fed a 1-g sample of their respective diets that contained 0.2 g SFK radioactively labeled with \(^{108}\)Cd. After complete consumption of the diet, they were placed individually in a small animal whole-body counter and the amount of \(^{108}\)Cd was measured. Activity was measured for 16 days. The activity was expressed as the percentage of the original dose and the data were plotted as the ln (percentage dose) against time. Percentage retention was estimated by averaging values between days 11 and 16 and dividing by the initial dose. This value was used as an estimate of absorption.

\(^d\) Because the data for these parameters did not follow a normal distribution, a natural logarithm transformation was performed before the ANOVA was run. These values represent the back transformed means plus 1 SD range for four replicates selected at random from each of the original groups containing seven rats each.

\(^e\) On day 16 after dosing, total cpm \(^{104}\)Cd in liver and kidney were summed and divided by cpm in the original dose × 100.

\(^f\) Dashes indicate no significant difference.
interaction for Cu in femur, which showed effects similar to that for the Zn × Fe interaction in other tissue, namely that marginal dietary Ca lowered tissue Cu in rats fed adequate Fe but elevated it in rats fed marginal Fe.

DISCUSSION

The main thrust of this study was to determine whether marginal intakes of essential dietary minerals, such as Zn, Fe, and Ca, known to modulate the metabolism of Cd, would affect the absorption and organ concentration of Cd when the sole contribution of Cd to the diet was from a natural food source.

Grains differ in Zn and Fe concentration and/or bioavailability that could cause crops to differ in Cd bioavailability. The results clearly show that marginal dietary intake of Ca and Fe but not Zn increased the absorption and tissue accumulation of SFK Cd.

The effect of marginal Fe was much greater than marginal Ca. Although there were no overt signs of mineral deficiency, the amounts of Fe in the small intestine, liver, and kidney were reduced considerably when marginal Fe diets were used. At the same time, however, the reduced intake of Fe significantly elevated Cd absorption and significantly increased the percentage of radio-labeled SFK Cd remaining in
<table>
<thead>
<tr>
<th>Dietary variable</th>
<th>Group 1</th>
<th>Group 2</th>
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<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
<th>ANOVA Table P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (mmol/kg)</td>
<td>1.10 ± 0.02</td>
<td>1.09 ± 0.08</td>
<td>1.03 ± 0.03</td>
<td>1.05 ± 0.08</td>
<td>0.91 ± 0.03</td>
<td>1.11 ± 0.03</td>
<td>0.95 ± 0.02</td>
<td>1.04 ± 0.01</td>
<td>0.049</td>
</tr>
<tr>
<td>Fe (µmol/L)</td>
<td>23.4 ± 0.7</td>
<td>23.9 ± 0.7</td>
<td>26.0 ± 1.5</td>
<td>24.5 ± 0.9</td>
<td>19.1 ± 1.0</td>
<td>26.5 ± 1.0</td>
<td>21.0 ± 0.7</td>
<td>26.0 ± 1.1</td>
<td>-</td>
</tr>
<tr>
<td>Liver (mmol/kg)</td>
<td>1.69 ± 0.04</td>
<td>1.67 ± 0.07</td>
<td>1.66 ± 0.11</td>
<td>1.61 ± 0.04</td>
<td>1.47 ± 0.07</td>
<td>1.65 ± 0.05</td>
<td>1.46 ± 0.05</td>
<td>1.72 ± 0.03</td>
<td>0.064</td>
</tr>
<tr>
<td>Kidney (mmol/kg)</td>
<td>1.50 ± 0.08</td>
<td>1.57 ± 0.04</td>
<td>1.50 ± 0.08</td>
<td>1.57 ± 0.08</td>
<td>1.32 ± 0.02</td>
<td>1.47 ± 0.02</td>
<td>1.32 ± 0.03</td>
<td>1.48 ± 0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Femur (mmol/kg)</td>
<td>3.65 ± 0.15</td>
<td>3.95 ± 0.09</td>
<td>3.74 ± 0.08</td>
<td>5.01 ± 0.39</td>
<td>2.33 ± 0.08</td>
<td>4.09 ± 0.13</td>
<td>2.22 ± 0.08</td>
<td>4.14 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Iron (mmol/kg)</td>
<td>1.93 ± 0.13</td>
<td>2.47 ± 0.16</td>
<td>0.92 ± 0.04</td>
<td>1.09 ± 0.10</td>
<td>2.01 ± 0.12</td>
<td>2.91 ± 0.21</td>
<td>1.17 ± 0.04</td>
<td>1.15 ± 0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum (µmol/L)</td>
<td>56.6 ± 2.0</td>
<td>64.8 ± 6.9</td>
<td>80.0 ± 4.4</td>
<td>78.6 ± 4.3</td>
<td>54.3 ± 5.6</td>
<td>60.2 ± 5.4</td>
<td>59.0 ± 4.7</td>
<td>63.9 ± 3.1</td>
<td>-</td>
</tr>
<tr>
<td>Liver (mmol/kg)</td>
<td>17.7 ± 1.0</td>
<td>28.4 ± 1.9</td>
<td>9.0 ± 0.7</td>
<td>8.7 ± 0.7</td>
<td>24.6 ± 1.7</td>
<td>25.2 ± 1.6</td>
<td>8.8 ± 0.8</td>
<td>10.3 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>Kidney (mmol/kg)</td>
<td>2.49 ± 0.22</td>
<td>4.21 ± 0.16</td>
<td>2.03 ± 0.06</td>
<td>2.17 ± 0.09</td>
<td>3.17 ± 0.19</td>
<td>3.61 ± 0.23</td>
<td>2.07 ± 0.15</td>
<td>2.41 ± 0.13</td>
<td>-</td>
</tr>
<tr>
<td>Femur (mmol/kg)</td>
<td>1.16 ± 0.08</td>
<td>1.28 ± 0.08</td>
<td>0.72 ± 0.04</td>
<td>1.07 ± 0.04</td>
<td>1.51 ± 0.04</td>
<td>1.49 ± 0.13</td>
<td>0.91 ± 0.07</td>
<td>1.02 ± 0.06</td>
<td>0.006</td>
</tr>
<tr>
<td>Calcium (mmol/kg)</td>
<td>7.13 ± 0.65</td>
<td>5.55 ± 0.37</td>
<td>6.89 ± 0.48</td>
<td>5.31 ± 0.43</td>
<td>6.01 ± 0.37</td>
<td>5.89 ± 0.21</td>
<td>6.52 ± 0.45</td>
<td>5.40 ± 0.10</td>
<td>-</td>
</tr>
<tr>
<td>Serum (mmol/L)</td>
<td>2.75 ± 0.05</td>
<td>2.80 ± 0.05</td>
<td>2.94 ± 0.08</td>
<td>2.75 ± 0.05</td>
<td>2.65 ± 0.06</td>
<td>2.83 ± 0.07</td>
<td>2.77 ± 0.06</td>
<td>2.76 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>Liver (mmol/kg)</td>
<td>2.68 ± 0.09</td>
<td>2.73 ± 0.15</td>
<td>2.68 ± 0.16</td>
<td>2.28 ± 0.05</td>
<td>2.59 ± 0.11</td>
<td>2.59 ± 0.11</td>
<td>2.74 ± 0.17</td>
<td>2.51 ± 0.09</td>
<td>-</td>
</tr>
<tr>
<td>Kidney (mol/kg)</td>
<td>6.14(6.4-8.1)</td>
<td>7.7(5.4-10.9)</td>
<td>10.5(4.9-22.1)</td>
<td>6.8(4.7-9.8)</td>
<td>6.1(4.9-7.5)</td>
<td>12.8(5.7-28.7)</td>
<td>7.8(5.8-10.3)</td>
<td>27.1(8.7-84.5)</td>
<td>0.018</td>
</tr>
<tr>
<td>Femur (mol/kg)</td>
<td>4.66 ± 0.25</td>
<td>4.66 ± 0.04</td>
<td>4.97 ± 0.05</td>
<td>5.15 ± 0.11</td>
<td>5.70 ± 0.08</td>
<td>5.35 ± 0.14</td>
<td>5.44 ± 0.08</td>
<td>5.39 ± 0.10</td>
<td>0.001</td>
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<tr>
<td>Copper (µmol/kg)</td>
<td>96.7 ± 3.7</td>
<td>97.7 ± 2.1</td>
<td>87.4 ± 2.3</td>
<td>90.1 ± 1.0</td>
<td>85.4 ± 2.2</td>
<td>95.9 ± 2.0</td>
<td>95.7 ± 3.6</td>
<td>93.7 ± 2.3</td>
<td>-</td>
</tr>
<tr>
<td>Serum (µmol/L)</td>
<td>24.6 ± 0.8</td>
<td>25.8 ± 1.0</td>
<td>23.2 ± 0.5</td>
<td>23.2 ± 1.3</td>
<td>23.7 ± 1.1</td>
<td>26.3 ± 1.7</td>
<td>22.9 ± 0.8</td>
<td>24.1 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td>Liver (µmol/kg)</td>
<td>226.9 ± 9.9</td>
<td>247.4 ± 5.9</td>
<td>242.1 ± 11.5</td>
<td>234.6 ± 9.1</td>
<td>236.7 ± 11.3</td>
<td>236.5 ± 10.6</td>
<td>362.2 ± 39.1</td>
<td>311.0 ± 24.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Kidney (µmol/kg)</td>
<td>334 ± 21</td>
<td>320 ± 18</td>
<td>329 ± 35</td>
<td>344 ± 25</td>
<td>302 ± 8</td>
<td>290 ± 12</td>
<td>411 ± 30</td>
<td>355 ± 11</td>
<td>-</td>
</tr>
<tr>
<td>Femur (µmol/kg)</td>
<td>10.8 ± 0.4</td>
<td>10.8 ± 0.6</td>
<td>10.2 ± 1.6</td>
<td>14.9 ± 5.5</td>
<td>11.9 ± 0.7</td>
<td>9.0 ± 0.5</td>
<td>12.2 ± 0.6</td>
<td>10.2 ± 0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

-a Each value is the mean ± SE for seven replicates, expressed on a dry weight basis for the organs and on a liquid volume basis for the serum.
-b Adequate and marginal amounts of test elements (± Zn; 32/10.8 mg/kg diet); (± Fe; 39.1/13.9 mg/kg diet); (± Ca; 4710/2540 mg/kg diet). The diet contained 0.18 mg Cd/kg.
-c Dashes indicate no significant difference.
-d Because the data for these parameters did not follow a normal distribution, a natural logarithm transformation was performed before the ANOVA was run. These values represent the back transformed means plus 1 SD range for seven replicates.
the liver plus kidney. Although there were no signs of Ca deficiency such as low serum Ca, marginal dietary Ca increased \(^{109}\)Cd absorption and accumulation in liver plus kidney. Because there were no significant interactions affecting Cd absorption, marginal deficiencies of Ca and Fe apparently were acting independently of each other.

There was a small reduction in intestinal Ca and a large increase in kidney Ca in rats fed the marginal Ca diets. Although the overall dietary concentration of Ca was lower that the requirement, the Ca:P molar ratio was about 0.5. It has been shown that female rats have a tendency to produce deposits of calcium phosphate in their kidneys when the dietary Ca:P molar ratio is far below 1.3 (Reeves et al., 1993b).

The absorption of Cd in the control diet, made of 20% SFK and adequate in all nutrients, was strikingly similar to that in another study in which diets with similar amounts of SFK were fed (Reeves et al., 1994). In that study, the rate of absorption was \(0.39 \pm 0.15\%\) compared with 0.38 \(\pm 0.09\%\) in the present study. However, it should be pointed out that the determination of Cd absorption by the whole-body counting technique is not highly accurate. This is primarily because Cd is not absorbed well and most of the ingested Cd is excreted in the feces in a very short time. Much of the remaining Cd is in a turnover pool in the intestinal cells, which leaves the body very slowly; part of the turnover pool moves to the liver and kidney, the long-term storage locations for body Cd. In addition, although an attempt was made to fit the raw data to a double exponential decay equation, they would not converge; thus, an estimation of absorption could not be obtained by this method. The alternative was to take the average percentage retention over the last 3 days of the counting period and use that as an estimate of absorption. It is likely that this procedure overestimates the amount of Cd absorbed because much of the labeled Cd is bound up by metallothionein (MT) in the intestinal mucosa and might never enter the body. Still, it is accounted for in the whole-body count, and, presently, there is no easy way to estimate its contribution and to subtract it.

It is well known that Zn interferes with the absorption and metabolism of Cd (Jacobs et al., 1978); therefore, it was somewhat surprising to find that marginal dietary Zn did not increase Cd absorption in this study. However, the findings might be reasonable if we consider the fact that the estimated dietary requirement of Zn for the rat is only 12 mg/kg diet (National Research Council, 1995). Because of the inherently high concentration of Zn in SFK (52 mg/kg), feeding the kernels at 20% of the diet alone contributed nearly 90% of the Zn requirement for the rat. In the present study, the adequate concentration of dietary Zn (30 mg/kg) was chosen because this is the amount recommended for the standard rodent diet, AIN-93G (Reeves et al., 1993a). If we could have obtained SFK with lower amounts of Zn, we probably would have observed an effect of marginal Zn deficiency on Cd absorption. Thus, the data suggest that SFK contains enough Zn to significantly retard Cd absorption without having to resort to the addition of supplemental Zn.

The intestinal mucosa is the first line of defense against the entry of toxic substances into the body. In the case of heavy metals, such as Cd, the cells produce the metal binding protein, metallothionein (Masters et al., 1994). This protein binds the element and prevents it from entering the body. It was observed in this study that when dietary Ca and Fe were limiting, more Cd accumulated in the small intestine than when the minerals were adequate. This suggests that Cd was sequestered by MT in the mucosal cells. However, because MT also binds Zn, the intestinal Zn concentration should have been elevated, but it was not. Therefore, it is doubtful that marginal Fe and Ca influence intestinal Cd through MT. In addition, not all of the Cd was tightly bound, because it was also shown that more Cd entered the serum and internal organs when dietary Ca and Fe were marginal than when they were adequate. It seems that marginal Ca and Fe status in the gut might have altered the binding capacity of MT for Cd and allowed more Cd to pass out of the mucosal cell and into the body.

Compared with other organs, the kidney data were somewhat of an anomaly. The lower amount of dietary Zn seems to have lowered the amount of nonradioactive Cd in kidney, especially when dietary Fe and Ca were also marginal. Studies have shown that Zn inhibits Cd uptake in isolated kidney cells (Endo et al., 1997; Endo and Shaikh 1993); therefore, the elevation in kidney Cd in rats fed the higher amount of Zn might be regarded as an artifact of the mineral analysis procedures and should be further investigated. However, \(^{109}\)Cd uptake into the kidney was only about 60% of that in the low-Fe, low-Ca, normal Zn group. If this finding is real, it might be related to the fact that Zn induces MT in the kidney, which could bind Cd. In animals consuming marginal Zn diets, the MT concentration would be reduced and perhaps depress the amount of Cd bound.

Based on previous investigations, we expected low dietary Fe and/or Ca to increase Cd absorption, but
because the concentrations in the present study were marginal, the magnitudes of the effects were surprisingly large. Other investigations showing an enhancing effect of dietary Fe on Cd absorption and tissue distribution were done in animals so severely deficient that a reduction in growth was observed (Flanagan et al., 1978; Ragan, 1977). In the present study, however, although tissue Fe was reduced by 50% in rats receiving the low-Fe diets, the deficiency was marginal at best, because there were no effects on growth or serum Fe concentrations during 7 weeks of consuming the diet. Nonetheless, Cd absorption was enhanced 1.6-fold over that in rats fed an adequate Fe diet. This suggests that an iron status of low magnitude in an individual could be an important factor in Cd absorption and that an overt deficiency is not required to obtain an increase in Cd absorption and tissue distribution. It has been shown in some human studies that a negative correlation exists between serum ferritin concentrations and Cd absorption (Flanagan et al., 1978) or blood Cd concentration (Berglund et al., 1994). We did not measure serum ferritin in this study.

Dietary intake of Cd greater than the provisional tolerable weekly intake (7 μg/kg body weight), as established by the World Health Organization (WHO, 1989), over time can increase the body burden of this toxic element. Although the Fe, Zn, or Ca nutritional status of the consumer has long been recognized to affect Cd absorption and retention, this factor generally is not entered into the assessment of the potential risk of food Cd to humans. Recently, Chaney et al. (1996) described a new paradigm for understanding the human health effects of Cd from foods. This paradigm recognized that not all crops accumulate equal amounts of bioavailable Fe, Zn, or Ca when soils become contaminated with Cd and Zn. We now can identify several distinct differences in ways that humans are injured by food Cd. For example, subsistence rice consumers in Japan and China were found to experience a high prevalence of renal tubular dysfunction when rice soils became contaminated with mine or smelter wastes that contain both Cd and Zn (Nogawa, 1984; Tsuchiya, 1978). Such wastes contained Cd at the normal geological ratio of about 5 mg Cd/g Zn. Because of the chemistry of flooded rice soils and the physiology of rice, rice grain had up to a 200-fold increase in Cd compared to rice grown on uncontaminated soils, whereas rice Zn was not increased above background levels (Fukushima et al., 1973). These soils contained 2–10 mg Cd/kg, whereas Zn was 400–1200 mg/kg soil. Rice excluded soil Zn from its grain, which allowed increased Cd exposures without any counteracting increase in food Zn.

Because of concerns about possible health effects of soil Cd, epidemiological studies were conducted at locations in Western countries where soils had become very highly contaminated because of dispersal of mine wastes and smelter emissions. In three cases where human studies were conducted, soils contained approximately 100 mg Cd and 10,000 mg Zn/kg—remarkably contaminated compared to the Japanese and Chinese soils that caused human Cd disease. Only by keeping soil pH high to reduce Zn uptake and prevent Zn phytotoxicity could the owners produce normal garden crops for consumption. At Shipham, United Kingdom (Strehlow and Barltrap, 1988), Stolberg, Germany (Ewers et al., 1985, 1993), and Palmerton, Pennsylvania (Sarasua et al., 1995), where gardening was common, researchers found no evidence of proximal tubular dysfunction in older long-term residents—the fraction of the population in which Cd disease would be found if it were present.

These unexpected findings regarding populations who lived for most of their lives in communities where soils were highly contaminated with Cd and Zn, and some of whom consumed garden foods grown on the contaminated soils, stand in remarkable contrast with the subsistence rice farmers in Japan and China. Another population in Bluff, New Zealand, which consumed large quantities of Cd from oysters during the harvest season, was studied by Sharma et al. (1983a) in more detail. This oyster contains about 5 mg Cd/kg fresh weight, which is higher than that in most other oysters described in the literature. Upon examination of blood and urine Cd, they found that smoking cigarettes caused a much larger increase in blood Cd than did the consumption of large amounts of oyster Cd at daily Cd intakes high enough to cause Cd disease in subsistence rice farmers in Japan and China. In a further study of the oyster-consuming population, Mckenzie-Parnell and Eynon (1987) found no evidence of kidney dysfunction in oyster eaters based on β2-microglobulin excretion in the urine. Further examination of kidney Cd in deceased individuals from this community over time found that kidney Cd was not unusually high after taking into consideration the effect of smoking on body Cd burden (Mckenzie-Parnell and Eynon, 1987).

After it was recognized that there was a large difference in the effects of ingested Cd in these populations (rice consumers compared to oyster consumers), and it was recognized that rice excluded soil Zn from grain compared with the relative
exclusion of Cd by wheat, soybean, lettuce, and other crops grown on nonflooded soils in the West, we sought information to explain the lesser risk of grain Cd in the West, where rice is not the dominant food enriched in Cd by soil contamination. Others had shown that rice was deficient in Zn and Fe for humans (Pedersen and Eggum, 1983) and that the Fe remaining in polished rice had very low bioavailability compared with Fe in other foods (Layrisse and Martinez-Torres, 1971). National surveys in the United States measured the composition of major foods grown on soils where the bulk of the U.S. supply of those foods is grown; the median concentrations of Fe and Zn in rice, corn, wheat, and soybean in the survey results of Wolnik et al. (1983, 1985) were 3.6, 17, 32, and 65 mg Fe/kg wet weight, respectively, and 13.5, 18.5, 26.5, and 41 mg Zn/kg wet weight, respectively. This was for whole grain or brown rice, and it is known that milling removes most of the Fe, Zn, and Ca present in the brown rice (Pedersen and Eggum, 1983).

Because staple grains consumed by different societies can differ so greatly in concentrations and bioavailability of Fe, Zn, and Ca, we hypothesized that the nutritional status of rice consumers, resulting from an inadequate supply of Fe, Zn and Ca from rice, could significantly contribute to their apparent susceptibility to soil Cd contamination (Chaney et al., 1996). The results of the present study lend credence to this hypothesis. It is apparent that agronomy and nutrition played significant roles in the fundamental risk to humans consuming foods from soil contaminated with Cd and Zn. Such contaminations have occurred at many locations, both from geological sources and from industrial activities. It is our feeling that the appropriate public response to soil contamination by Cd plus Zn should be based on the actual risk from these sources rather than presuming that all crops are equal to rice or that Cd without Zn comprises a risk equal to that of Cd with 100-fold higher Zn, which occurs with most geological Cd plus Zn sources.

Although the concentration of Cd in a food source is an important factor in determining Cd exposure and eventual organ accumulation, it is not the only factor. This study clearly shows that a marginal nutrient status of the experimental subject, relative to certain dietary minerals, plays a major role in determining the rate of absorption and tissue accumulation of Cd. Rice subsistence diets favor Cd absorption, whereas SFK, even though relatively high in Cd, contains Zn and Fe that reduces Cd absorption by preventing deficiency—consuming the source provides protection against risk. The present data strongly support previous findings by others suggesting that populations exposed to dietary sources of Cd and subsisting on marginal mineral intakes could be at greater risk than populations well nourished with respect to the mineral nutrients. Thus, crops have unequal Cd risk at equal Cd concentration.

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REFERENCES


