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Organ content and fecal excretion of cadmium in male and female rats consuming variable amounts of naturally occurring cadmium in confectionery sunflower kernels (*Helianthus annuus L.*)

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*Sunflowers (*Helianthus annuus L.*) tend to remove cadmium (Cd) from the soil and deposit it in their seeds. The availability of Cd in sunflower kernels for absorption and deposition in animal tissues was studied using a 15-week feeding trial with both male and female rats begun at weanling age. Diets included (1) purified basal diet with no sunflower kernels (85 μg Cd/kg), (2) basal diet containing 20% ground low-Cd sunflower kernels (120 μg Cd/kg), (3) basal diet containing 20% ground high-Cd sunflower kernels (195 μg Cd/kg), and (4) basal diet containing 20% ground low-Cd sunflower kernels plus Cd chloride (175 μg Cd/kg). In a second experiment, adult rats were fed sunflower kernels that contained an endogenous or exogenous label of ^{109}Cd. Cd availability was assessed by measuring ^{109}Cd excretion in feces and by measuring the amount of label accumulated in liver and kidney. Results were as follows: (1) Although all diets were of similar nutrient composition, female rats that consumed diets containing 20% ground sunflower kernels gained significantly ($P < 0.02$) more weight than those without kernels in their diets. Increased weight was not the result of increased feed intake. (2) Increasing Cd intake twofold as a result of feeding sunflower kernels significantly ($P < 0.001$) increased the body burden (total liver plus kidney content) of Cd from 1.6 to 4.0 nmol in females and from 4.0 to 7.0 nmol in males. (3) The availability of Cd from sunflower kernels labeled endogenously with ^{109}Cd was not different ($P > 0.1$) than kernels labeled exogenously: 12% versus15%, respectively. Eight days after dosing, total liver ^{109}Cd was 1% of the initial dose; at 20 days it was only 0.3%. The corresponding amounts in kidney were 0.1% 8 days after dosing and 0.2% 20 days after dosing. The amount of label in liver and kidney was not affected by the method of labeling the kernels. This study clearly shows that Cd from sunflower kernels is available for absorption and accumulation in tissues of the rat, although in very small concentrations.* (J. Nutr. Biochem. 9:636–644, 1998) *Published by Elsevier Science Inc. 1998*

Keywords: cadmium; zinc; copper; sunflower kernels; liver; kidney; rats

Introduction

Excessive intake of cadmium (Cd) over a prolonged period may cause numerous health problems in humans including osteomalacia, emphysema, and kidney dysfunction.[1–3] The general population is exposed to Cd primarily through its natural occurrence in the environment. For people in the United States, the major portion of exposure is through the food supply, which provides an estimated weekly intake of 140 μg of Cd.[4,5] A small safety margin exists in the range of exposures between that obtained from normal intake and

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that which might produce harmful effects.[6] It has been estimated that a sustained weekly intake of approximately 700 μg of Cd over a lifetime could cause approximately 2% of the population to exceed the individual critical concentration and experience deleterious side effects. The provisional tolerable weekly intake (PTWI) for humans recommended by the World Health Organization is 7 μg/kg of body weight. For a 70 kg person the PTWI would be approximately 490 μg/week.

Some of the soils in the United States, including those in the Red River Valley region of North Dakota and Minnesota, contain a higher natural concentration of Cd than those of other parts of the country.[7,8] Certain plant species including confectionery sunflowers tend to take up Cd from the soil and deposit it in their seeds.[9] The Red River Valley region is a major producer of confectionery sunflowers with kernels that contain a higher natural concentration of Cd than might be found in kernels grown in soils with less Cd. For example, during the 1995 harvest season, the Cd concentration of sunflower kernels from 19 different lots from this region ranged from 330 to 670 μg/kg (mean ± SD, 480 ± 110 μg/kg). Thus, if individuals habitually consume relatively large amounts of these sunflower kernels in the form of snack items, or as additions to various food preparations, their intake might begin to approach the PTWI for Cd.

However, for food Cd to cause ill effects it must first be absorbed from the gut. Compared with other trace elements, Cd absorption from the gastrointestinal tract is somewhat low, ranging from 0.7 to 15% in humans[10] and from 0.3 to 25% in animals.[11–13] The amount of dietary Cd absorbed depends largely on the form of Cd in the diet, the composition of the diet, and the gender and age of the consumer.

The number of studies to determine the availability of Cd from sunflower kernels is small. Stoewsand et al.[14] found higher concentrations of Cd in the organs of Japanese quail fed diets containing the seed-pomace from sunflowers grown on Cd-laden sludge-amended soils than in those fed seed-pomace diets from sunflowers grown on control soils. However, Cd absorption was not measured in this study. In a previous study[13] we found that rats consuming diets containing 20% ground sunflower kernels absorbed 20% less Cd than rats not fed sunflower kernels. Absorption between the two groups was 0.4 and 0.5%, respectively. The study was designed to minimize Cd absorption; that is, the diet contained adequate calcium, iron, and zinc (Zn) concentrations, all known to retard Cd absorption. We examined whether Cd was available from the sunflower kernels under standard animal feeding practices. In spite of these constraints and low absorption rates, liver and kidney concentrations of Cd were significantly higher in rats as a result of their consuming the sunflower kernels.[13]

In the current article, we discuss experiments that were performed similarly to those above,[13] except we used both male and female rats and assessed the availability of Cd from sunflower kernels by using endogenous and/or exogenous ^{109}Cd-labeled kernels.

Table 1 Analysis of sunflower kernels (SFK) for selected minerals[1,2]

	Low Cd SFK	High CdSFK
Cadmium (Cd), μg/kg	230 ± 7–b	550 ± 28–a
Copper, mg/kg	14.3 ± 0.6–b	18.7 ± 0.5–a
Zinc, mg/kg	45.1 ± 1.9–b	53.7 ± 0.7–a
Iron, mg/kg	38.4 ± 1.0–b	43.0 ± 1.3–a
Manganese, mg/kg	26.7 ± 1.7–a	21.3 ± 0.6–b
Molybdenum, mg/kg	0.92 ± 0.06	0.91 ± 0.05
Calcium, mg/kg	920 ± 37–a	736 ± 41–b
Phosphorus, mg/kg	7,835 ± 316–b	9,260 ± 80–a
Magnesium, mg/kg	4,060 ± 204–b	4,610 ± 63–a
Sodium,[3] mg/kg	<10	<10
Potassium, mg/kg	6,770 ± 211	6,920 ± 44

[1]Values are means ± SD for four replicates. a and b indicate significant ($P < 0.003$) differences between groups by the Student's *t*-test.
[2]Analytical procedures are described in the Methods section.
[3]Because of extreme variability, an accurate analysis for sodium could not be obtained.

Materials and methods

This study was approved by the Animal Use Committee of the U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, and was in accordance with the guidelines of the National Institutes of Health on the experimental use of laboratory animals.[15]

Materials

Rats were purchased from Sasco, Inc. (Omaha, NE USA). Three lots of raw whole sunflower kernels (*Helianthus annuus L.*) were provided by Agway, Inc. (Grandin, ND USA). One lot contained 230 ± 7 μg Cd/kg, one contained 550 ± 28 μg Cd/kg, and another contained 750 ± 25 μg Cd/kg. All chemicals and other materials used in the preparation of animal diets were of high quality and were purchased from various suppliers. Chemicals used for tissue mineral analyses were of the highest grade and purchased from Sigma Chemical Co. (St. Louis, MO USA) unless otherwise noted.

Diet preparation

Experiment 1. Fresh sunflower kernels were analyzed for selected mineral content. These values are given in *Table 1*. The kernels were finely ground in a food processor and then mixed into the purified diet-base at 20% (*Table 2*). Concentrations of minerals, fat, and fiber in sunflower kernels were estimated from composition tables or by actual analysis. Each dietary component was adjusted so that similar amounts were contained in each diet whether the diet contained sunflower kernels or not. Cd chloride ($CdCl_2$) was added to one of the diets to obtain the desired concentration of Cd. Each diet was then analyzed for Cd concentration. The purified diet without sunflower kernels (basal) contained 85 ± 12 μg of endogenous Cd/kg. The low-Cd sunflower kernel (LoCdSFK) diet contained 20% low-Cd sunflower kernels and the total concentration of Cd was 120 ± 9 μg/kg. The high-Cd sunflower kernel (HiCdSFK) diet contained 20% high-Cd sunflower kernels and the total concentration of Cd was 195 ± 8 μg/kg. The LoCdSFK plus Cd diet contained 20% low-Cd sunflower kernels and additional $CdCl_2$ to bring the total Cd concentration up to that of the HiCdSFK diet; however, this diet by analysis contained only 175 ± 12 μg of Cd.

Experiment 2. The diet for this experiment was similar to the HiCdSFK diet of the first experiment except that sunflower kernels

Table 2 Compositions of experimental diets

Ingredients	Basal (g/kg diet)	LoCdSFK (g/kg diet)	HiCdSFK (g/kg diet)	LoCdSFK+Cd (g/kg diet)
Cornstarch[1]	393.95	349.05	349.05	339.05
Ground SFK[2]	—	200.00	200.00	200.00
Casein[3]	200.00	151.90	151.90	151.90
Dextrinized starch[4]	100.00	100.00	100.00	100.00
Sucrose	100.00	100.00	100.00	100.00
Sunflower oil[5]	99.00	—	—	—
Fiber[6]	50.00	42.00	42.00	42.00
Mineral mix[7]	35.00	35.00	35.00	35.00
Cadmium premix[8]	—	—	—	10.00
Vitamin mix[9]	10.00	10.00	10.00	10.00
α-Linolenic acid[10]	6.50	6.50	6.50	6.50
L-Cystine[11]	3.00	3.00	3.00	3.00
Choline bitartrate[12]	2.50	2.50	2.50	2.50
TBHQ[13]	0.05	0.05	0.05	0.05

[1]Borden Food Service, Chatsworth, CA USA.
[2]Sunflower kernels; Agway, Inc., Grandin, ND USA.
[3]Teklad, Madison, WI USA.
[4]Dyetrose, Dyets Inc., Bethlehem, PA USA.
[5]Hunt-Wesson, Fullerton, CA USA.
[6]Cellulose, Teklad, WI USA.
[7]See *Table 3* for the composition of the mineral mixes.
[8]12.23 mg $CdCl_2$/kg powdered sugar.
[9]See *Table 4* for the composition of the vitamin mix.
[10]Pfalz & Bauer, Inc., Waterbury, CT USA.
[11]Sigma Chemical, St. Louis, MO USA.
[12]Teklad, Madison, WI USA.
[13]*tert*-butyl-hydroquinone, Eastman Kodak, Rochester, NY USA.

with a higher Cd content were used. This diet contained 235 ± 10 μg Cd/kg.

Experimental design

Experiment 1. We randomly assigned 40 weanling male and 40 weanling female Sprague-Dawley rats to four subgroups of 10 rats each. The rats were placed in stainless steel hanging cages (one rat per cage). The initial mean weight ± SD of each group of male rats was 48 ± 2 g and for the female rats it was 46 ± 2 g. Each of the groups was offered one of the four diets described in *Table 1*. Each rat had free access to diet and fresh deionized water at all times. Rats were weighed at weekly intervals.

During the eleventh week, five rats were randomly selected from the basal and HiCdSFK diet groups, across sex, and food intake was measured for 4 consecutive days. The 4-day average food intake was expressed as grams per 100 g of average body weight across the 4 days.

After 15 weeks on their respective dietary regimens, the rats were anesthetized with sodium pentobarbital (50 mg/kg body weight) and blood was collected from the abdominal aorta. The blood was allowed to clot at room temperature and the serum separated for mineral analysis. Liver, kidneys, large intestine, and femurs were removed and frozen at –20°C until analyzed for mineral contents.

Table 4 Composition of the vitamin mix[1] for experimental diets

Ingredients	g/kg Mix
Nicotinic acid	3.00
Ca pantothenate	1.60
Pyridoxine-HCl	0.70
Thiamin-HCl	0.60
Riboflavin	0.60
Folic acid	0.20
D-Biotin	0.02
Vitamin B-12 (cyanocobalamin; 0.1% in Mannitol)	2.50
Vitamin E (all-*rac*-α-tocopheryl actetae) (500 IU/g)	15.00
Vitamin A (all-*trans*-retinyl palmitate) (5×10^5 IU/g)	0.80
Vitamin D-3 (cholecalciferol) (4×10^5 IU/g)	0.25
Vitamin K (phylloquinone)	0.075
Powdered sucrose	974.655

[1]Similar to the AIN-93G-VX vitamin mix.[23]

Table 3 Composition of the mineral mixes for experimental diets

Ingredients	Basal (g/kg mix)	LoCdSFK (g/kg mix)	HiCdSFK (g/kg mix)	LoCdSFK+Cd (g/kg mix)
Calcium carbonate ($CaCO_3$)	463.82	327.10	346.86	327.10
Calcium phosphate ($CaHPO_4$)	36.14	168.00	144.70	168.00
Potassium phosphate (KH_2PO_4)	285.20	—	—	—
Magnesium oxide (MgO)	40.70	—	—	—
Potassium sulfate (K_2SO_4)	46.62	46.62	46.62	46.62
Potassium citrate ($K_3C_6H_5O_7$)	—	116.00	116.00	116.00
Sodium chloride (NaCl)	74.00	74.00	74.00	74.00
Sodium metasilicate ($Na_2SiO_3 \cdot 9\ H_2O$)	7.25	7.25	7.25	7.25
Ferrous sulfate ($FeSO_4 \cdot 7\ H_2O$)	4.98	3.56	3.56	3.56
Zinc carbonate ($ZnCO_3$)	1.65	1.12	1.12	1.12
Manganous carbonate ($MnCO_3$)	0.63	0.15	0.15	0.15
Cupric carbonate ($CuCO_3$)	0.30	0.136	0.97	0.136
Chromium potassium sulfate ($CrKS_2O_8 \cdot 12\ H_2O$)	0.275	0.275	0.275	0.275
Boric acid (H_3BO_3)	0.0815	0.0815	0.0815	0.0815
Sodium fluoride (NaF)	0.0635	0.0635	0.0635	0.0635
Nickel carbonate ($NiCO_3$)	0.0635	0.0635	0.0635	0.0635
Stannous oxide (SnO)	0.0162	0.0162	0.0162	0.0162
Ammonium vanadate (NH_4VO_3)	0.0132	0.0132	0.0132	0.0132
Ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$)	0.0106	0.0106	0.0106	0.0106
Sodium selenate anhydrous, (Na_2SeO_4)	0.01025	0.003	0.003	0.003
Potassium iodide (KI)	0.010	0.010	0.010	0.010
Powdered sucrose	38.16625	255.5275	258.2335	255.5275

Experiment 2. Twenty male Sprague-Dawley rats weighing 185 ± 7 g were randomly assigned to two subgroups of 10 rats each and placed in stainless steel hanging cages (one rat per cage). Each of the groups was fed a diet similar to the HiCdSFK diet described in *Table 2*, except that the sunflower kernels used contained a higher amount of Cd (750 ± 25 μg/kg). After 4 days on this dietary regimen, each rat was fasted for 12 hours (overnight) and then given a test meal consisting of 2 g of finely ground sunflower kernel endogenously or exogenously labeled with ^{109}Cd and 0.1 mg of brilliant blue dye per gram. The labeling procedures are described below. After the test meal had been consumed, the rats were returned to their normal feeding regimen and placed into separate metabolic cages; feces and urine were collected each day for 8 days. Each rat had free access to diet and fresh deionized water at all times. Rats were weighed at the beginning and at the end of the study.

Each day's collection of feces was dried to constant weight and a portion assayed for the content of ^{109}Cd by gamma scintillation counting techniques (Cobra™ System, Packard Instrument Co., Meriden, CT USA). The concentration of dye in the feces was determined by extracting the dye into butyl alcohol and reading the color density at 630 nm. A summary of the procedure is as follows: 10 mg of freeze-dried feces were added to 2 mL of H_2O containing 50 μL of HCl and 0.4 g of $NaSO_4$. The mixture was extracted with 2 mL portions of warm (40°C) tertiary butyl alcohol until there was no more color remaining in the supernatant. Pooled extracts for each sample were centrifuged and a 10 mL volume removed, to which 0.3 mg Chloranil were added. After vortexing, the sample was heated to 80°C for 20 minutes, cooled, and the density of the color determined on a spectrometer at 630 nm. A blank reference was made from a butyl alcohol-extracted fecal sample from an animal that had consumed a similar diet but with no brilliant blue dye. A series of brilliant blue dye standards ranging from 50 ng to 2 μg were extracted similarly.

To assess the amount of Cd not absorbed by the rats, the cumulative total daily fecal excretion of ^{109}Cd was calculated and plotted against time. The best fit of the data was to a three parameter logistic equation of the form:

$$y = y_o/[1 + e^{(b1 - b2x)}].$$

Curve fitting was done by using the nonlinear regression component of SAS (SAS Institute, Cary, NC USA).

After 8 days on these regimens, four rats from each group were randomly selected and killed, and the livers and kidneys taken for analysis of ^{109}Cd. On day 20, the remaining six rats in each group were sacrificed and livers and kidneys again taken for analysis of ^{109}Cd.

Labeling of the sunflower kernels with ^{109}Cd

Endogenous labeling. Endogenous labeling of sunflower kernels with ^{109}Cd was done by incorporating the label into the growing plant. Sunflower seeds (Agway Royal Hybrid 2141, Lot #6418) were germinated and grown to maturity in 57 L polypropylene pots (46 × 36 cm) filled with a soil series called Fargo, a fine textured, silty clay known to cause an increase in Cd concentration of most varieties of sunflower seeds.[9] The plants were raised in a greenhouse during the months of May to September. Ten pots, thinned to one healthy plant each, were selected and fertilized with a slow release fertilizer (Osmocote™, 14-14-14, 100g/pot; Grace Sierra, Milipitas, CA USA) and micronutrients (Micromax™, 50 g/pot; Grace Sierra). Upon flowering, pollination was encouraged by transferring pollen from plant to plant with a feather brush. When the seeds began to form, each of the heads was injected with 122 kBq of ^{109}Cd once weekly for 3 consecutive weeks. Each series of injections was made in a total of 0.4 mL of buffer made of 23.3 mM citric acid and 53.4 mM sodium phosphate, dibasic, pH 5.2.[16] This volume was distributed among four injection sites at the back of the head of each plant; different sites were used at each week of injection. The heads were then allowed to mature to dryness. The seeds were harvested and shelled by hand, and the kernels were then dried to a constant weight. The incorporation of ^{109}Cd into the total amount of kernels harvested was 29% of that injected. The specific activity of the label was 6.5 kBq ^{109}Cd/μg of Cd.

The test meal containing endogenously labeled kernels was prepared by grinding 50 g of the kernels in a small stainless steel coffee mill. One milligram of brilliant blue dye per gram of kernel was added and the material was ground once again. Each animal received 2 g of labeled sunflower kernels.

Exogenous labeling. Exogenous labeling of the sunflower kernels was done by incorporating the label into ground fresh kernels. Being careful not to create sunflower butter, 50 g of sunflower kernels were ground in a small stainless-steel electric coffee mill; 0.1 mL of 0.1 M HCl containing the appropriate amount of ^{109}Cd (7.8 kBq/g) was added to match the amount of ^{109}Cd in the endogenously labeled kernels. One milligram of brilliant blue dye per gram of kernel was added and the material was ground once again. To determine if mixing was homogenous, six portions of 250 μg each were removed and assayed for the amount of label. The coefficient of variation among counts was 9%, indicating a fairly homogeneous mixture. The labeled kernels were used as the test meal for one group of rats.

Mineral analysis of sunflower kernels and rat organs

Sunflower kernels. Sunflower kernels contain approximately 49% oil, which makes them difficult to ash without the loss of Cd. The following procedure is used in our laboratory with 98 ± 2% recovery of Cd. Three grams of whole sunflower kernels are placed in a 150 mL Pyrex beaker, and 20 mL of concentrated HNO_3 are added. The beaker is covered with a watch glass and the mixture refluxed for 48 hours at just below the boiling point of nitric acid. The watch glass is then removed and the sample allowed to dry. The sample is then ashed in a muffle oven at 450°C for 24 hours. The sample is then dissolved in 10 mL of HNO_3 and 3 mL of 30% peroxide and refluxed at low temperature until it turns a clear to yellow color. The sample is allowed to dry with the watch glass removed, and then returned to the oven and heated at 450°C until nothing is left but the white ash (approximately 18 hours). The ash is dissolved in 1 mL of concentrated HNO_3 and 4 mL of deionized water. This solution is quantitatively transferred to a 10 mL volumetric flask, brought to volume with deionized water, and stored in a new, tightly capped, polypropylene or polystyrene tube until analyzed for Cd content by atomic absorption spectrometry. Accuracy of Cd determinations was monitored by analyzing sunflower kernel standards (National Sunflower Association, Bismarck, ND USA) simultaneously with the experimental samples. Values for analyzed Cd were within the range specified for the standards.

Rat organs. A portion of the liver (approximately 1 g), right kidney, and right femur were lyophilized to constant weight. Fecal pellets were extricated from the large intestine and residual material was washed from the lumen with cold saline. The whole tissue was then weighed and lyophilized to constant weight. All organ samples were dry ashed in covered Pyrex crucibles at 450°C for 48 hours. Each sample was then dissolved in 2 mL of *aqua regia* and slowly heated to dryness on a hotplate. Samples were returned to the oven and heated at 450°C until nothing was left but the white ash. The ash was dissolved in 5 mL of 1 N HNO_3 and analyzed for Cd, Zn, and copper (Cu) contents by inductively coupled argon plasma spectrometry. Accuracy of mineral determi-

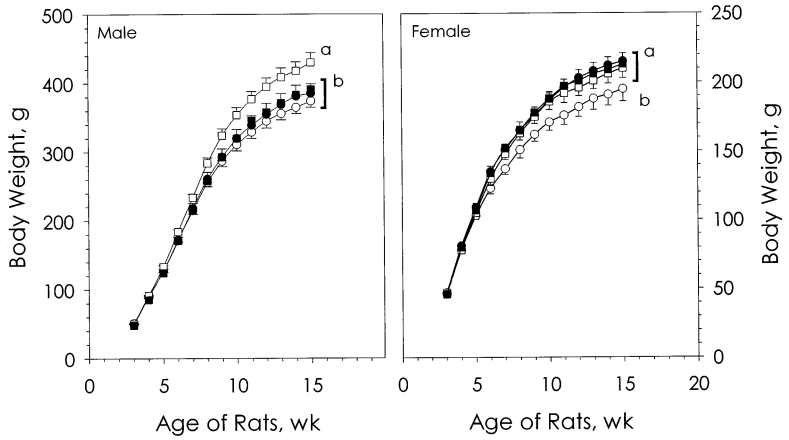

Figure 1 Change in body weight with time in male and female rats fed diets containing sunflower kernels with different amounts of cadmium for 15 weeks. Values are means ± SEM for 10 rats per group. Different letters at the end of the curves indicate significant ($P < 0.05$) differences between groups for only the last day of the experiment. Male rats consuming a diet with high-Cd sunflower kernels gained significantly more weight than all other groups. Female rats consuming a diet without sunflower kernels gained significantly less weight than all other groups. Group indicators for diet type are: ○ Basal (85 μg Cd/kg); ● LoCdSFK (120 μg Cd/kg); □ HiCdSFK (195 μg Cd/kg); ■ LoCdSFK plus Cd (175 μg Cd/kg).

nations was monitored by analyzing certified liver standards (National Institute of Standards and Technology, Gaithersburg, MD USA) simultaneously with the experimental samples. Values for all minerals analyzed were within the range specified for the standards.

^{109}Cd Analysis

Fecal material. The fecal pellets were collected each day from individual rats and frozen until analyzed for ^{109}Cd. The frozen feces were lyophilized to a constant weight and ground to a fine powder in a stainless steel coffee mill. Approximately 0.5 g of the dried feces was placed in a 12 x 75 mm plastic tube and the content of radioactive Cd determined by gamma counting.

Liver and kidney. Fresh liver and kidneys were weighed and prepared separately as a 1:5 homogenate (wt/vol) in water. One milliliter of the homogenate was assayed for the content of ^{109}Cd by gamma counting.

Statistical analysis

For Experiment 1, a two-way analysis of variance (ANOVA) with Tukey's[17] post-hoc test was used to determine differences between treatment means. In Experiment 2, Student's *t*-test was used to determine differences between treatment means.

Results

Experiment 1

Figure 1 shows the growth of the rats in this study. The growth of male rats was not affected by diets containing the low-Cd sunflower kernels but these rats had a significantly ($P < 0.01$) higher growth rate when high-Cd sunflower kernels were in their diet. On the other hand, female rats without sunflower kernels in their diet did not grow as well as those that had kernels in their diets. However, increasing the concentration of dietary Cd as a result of adding sunflower kernels to the diet did not significantly affect growth.

Part of the difference in body weight could have been caused by a change in food intake (*Table 5*). However, during the eleventh week when food intake was measured and expressed on the basis of grams per 100 g body weight there was not a significantly ($P > 0.05$) higher food intake in those rats receiving diets with sunflower kernels compared with those without sunflower kernels.

Table 6 shows the effects of feeding diets containing Cd from sunflower kernels on the concentration of Cd in liver, kidney, bone, and large intestine. Overall, there was no significant ($P > 0.1$) difference in liver Cd concentrations between male and female rats; however, there was a significant ($P < 0.001$) interaction between sex and diet. In male rats, there was significantly ($P < 0.001$) more liver Cd when either low- or high-Cd sunflower kernels were present in the diets compared with when there were no sunflower kernels in the diet (*Table 6*). Those rats receiving the low-Cd sunflower kernel diet with added Cd had significantly ($P < 0.007$) less liver Cd than those receiving diets with only low-Cd sunflowers. Liver Cd in female rats was significantly ($P < 0.001$) higher when they were fed the high-Cd sunflower kernels and low-Cd sunflower kernels plus added Cd than when fed low-Cd kernels alone, or with no kernels in the diet.

Overall, female rats had a higher ($P < 0.05$) concentration of Cd in the kidneys than males (*Table 6*). However, the kidney Cd response was similar to males when the females were exposed to different concentrations of Cd in the diet, although to a higher degree. Rats receiving diets with high-Cd sunflower kernels or low-Cd sunflower kernels plus Cd had significantly ($P < 0.01$) more Cd in the kidney than those receiving no sunflower kernels or low-Cd kernels

Table 5 Body weight and food intake in rats fed diets with and without 20% sunflower kernels[1]

	Male		Female	
	Basal	HiCdSFK	Basal	HiCdSFK
Body weight (BW) g[2]	369 ± 16	422 ± 16	178 ± 7	215 ± 13
Food intake, g/100g BW	3.8 ± 0.2	3.7 ± 0.2	5.4 ± 0.4	4.6 ± 0.2

[1]Values are mean ± SEM, five replicates per mean.
[2]An analysis of variance factorial analysis showed that body weights of males and females were significantly different ($P < 0.001$). There was also a difference in body weights between diets ($P < 0.004$). However, sex and diet did not significantly affect food intake when expressed on a body weight basis.

Table 6 Effect of dietary sunflower kernels (SFK) on the concentration of cadmium (Cd), zinc (Zn), and copper (Cu) in liver, kidney, femur and large intestine of male and female rats on experiment for 15 weeks[1,2]

	Male rats				Female rats						
Diet	Basal	LoCdSFK	HiCdSFK	LoCdSFK+Cd	Basal	LoCdSFK	HiCdSFK	LoCdSFK+Cd	ANOVA table *P* values		
Dietary Cd, μg/kg	85	120	195	175	85	120	195	175	Sex	Diet	S × D
Liver											
Cd, nmol/kg	318 ± 26–b	454 ± 17–a	493 ± 19–a	382 ± 19–b	260 ± 13–c	335 ± 23–c	534 ± 20–a	431 ± 24–b	NS	0.001	0.001
Zn, μmol/kg	404 ± 16	384 ± 8	367 ± 9	379 ± 14	353 ± 9	354 ± 7	372 ± 10	347 ± 11	0.001	NS	NS
Cu, μmol/kg	60.4 ± 1.8	57.2 ± 1.6	56.4 ± 1.3	62.8 ± 2.2	71.2 ± 3.0	72.0 ± 4.0	79.5 ± 2.2	74.1 ± 4.0	0.001	NS	NS
Kidney											
Cd, nmol/kg	274 ± 13–b	424 ± 40–b,c	760 ± 76–a	607 ± 40–a,c	183 ± 39–b	302 ± 34–b	932 ± 60–a	923 ± 53–a	0.050	0.001	0.001
Zn, μmol/kg	326 ± 5–a	290 ± 10–b	283 ± 6–b	287 ± 5–b	310 ± 10	318 ± 6	291 ± 5	323 ± 7	0.008	0.001	0.003
Cu, μmol/kg	151 ± 8	154 ± 8	147 ± 4	129 ± 5	172 ± 21	203 ± 20	137 ± 11	175 ± 22	0.001	0.006	NS
Femur											
Cd, nmol/kg	374 ± 6–b	386 ± 10–b	408 ± 9–a,b	446 ± 9–a	405 ± 22–b,c	467 ± 12–a	438 ± 12–a,c	421 ± 9–a,c	0.001	0.005	0.001
Zn, mmol/kg	2.53 ± 0.08	2.45 ± 0.05	2.21 ± 0.10	2.47 ± 0.05	3.36 ± 0.11	3.06 ± 0.07	3.07 ± 0.06	3.04 ± 0.08	0.001	0.003	NS
Cu, μmol/kg	31.2 ± 0.5	31.3 ± 0.7	30.2 ± 0.6	28.0 ± 0.4	36.2 ± 0.8–b	36.1 ± 0.6–b	40.0 ± 1.0–a	34.4 ± 1.2–b	0.001	0.001	0.008
Large intestine											
Cd, nmol/kg	239 ± 23	270 ± 49	220 ± 13	274 ± 30	275 ± 49	216 ± 26	262 ± 34	182 ± 33	NS	NS	NS
Zn, μmol/kg	236 ± 6–a	210 ± 6–b	201 ± 5–b	207 ± 6–b	222 ± 5	222 ± 5	230 ± 7	229 ± 6	0.002	NS	0.001
Cu, μmol/kg	18.8 ± 1.0	17.4 ± 1.0	16.5 ± 0.7	16.5 ± 0.05	16.5 ± 0.4	18.4 ± 0.6	18.5 ± 1.1	18.3 ± 1.0	NS	NS	NS

[1]Values are means ± SEM of 10 rats each.
[2]a, b, and c indicate significant ($P \leq 0.05$) differences among diets within sex.

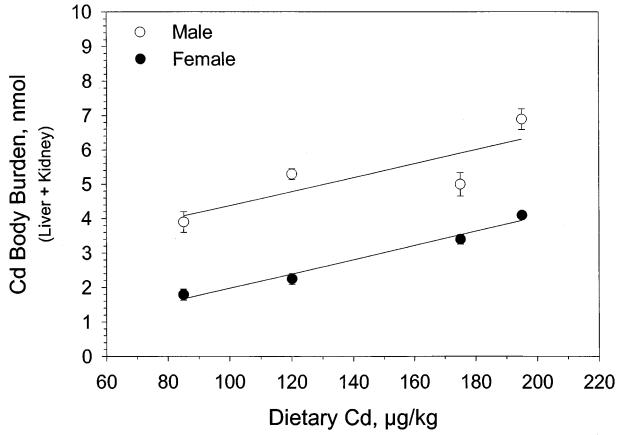

Figure 2 Body burden of cadmium (Cd) in male and female rats fed variable concentrations of dietary Cd in sunflower kernels for 15 weeks. Values are mean ± SEM of 10 rats per group. Body burden is expressed as the total amount of Cd found in the liver and kidney. There was a significant increase in body burden of Cd in both female and male rats as the amount of Cd in the diet increased (Pearson's correlation, $P < 0.001$).

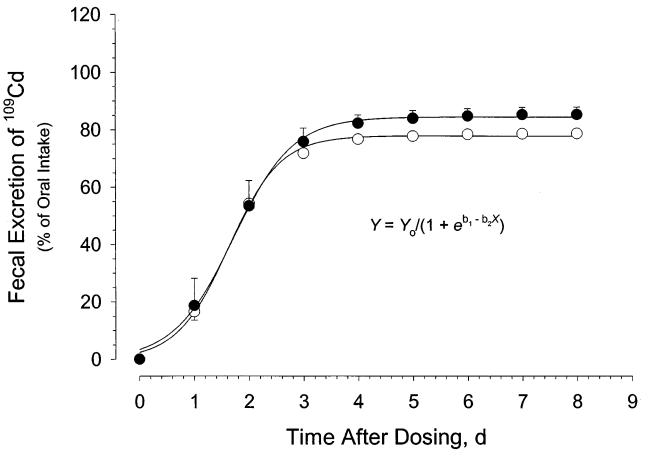

Figure 3 Cumulative fecal excretion (% of initial oral dose) of cadmium (Cd) in rats fed diets containing high-Cd sunflower kernels and labeled endogenously (○) or exogenously (●) with ^{109}Cd. Values are mean ± SEM of 10 rats per group and were fitted to a three-parameter logistical curve. There was not a significant difference between treatments.

alone. As a result of eating high-Cd sunflower kernels, females accumulated approximately 1.5 times as much kidney Cd as males.

Data in *Figure 2* represent the change in body burden as the dietary concentration of Cd increased, here expressed as the total amount of Cd in the body contributed only by liver and kidney. The results showed a significant ($P < 0.006$; Pearson's correlation) linear increase in Cd body burden of both male and female rats as dietary Cd increased. In addition, males had a significantly ($P < 0.001$) greater body burden than females.

Feeding sunflower kernels had no significant effect on femur Cd; however, female rats had significantly ($P < 0.001$) more femur Cd than male rats (*Table 6*). The concentration of Cd in the large intestine of rats was quite variable, with no overall significant differences among groups (*Table 6*).

Table 6 also shows the effect of dietary sunflower kernels on the concentrations of Zn and Cu in liver, kidney, femur, and large intestine. For liver, kidney, and femur Zn, there was a significant ($P < 0.01$) difference between male and female rats. Liver and kidney Zn concentrations were higher in males than females, but femur Zn was higher in females than males. In addition, there was a significantly ($P < 0.01$) higher concentration of copper in the liver and femur of females than males. Only Zn concentrations in the kidney and femur of male rats were significantly affected by dietary sunflower kernels. Rats receiving sunflower kernels in their diets had significantly ($P < 0.01$) less Zn in the kidneys and femur than those not receiving sunflower kernels.

Experiment 2

Figure 3 shows the cumulative excretion of ^{109}Cd in the feces of rats fed sunflower kernels labeled endogenously or exogenously with the radioisotope. Rats fed the endogenous ^{109}Cd tended to excrete less of the label than those fed exogenous ^{109}Cd, but the difference was not significant ($P > 0.1$). The turnover rate of the labeled Cd, indicated by the slope of the upper part of each curve, was extremely low and was similar for both types of label. The data are not shown, but after 3 days, only approximately 1% of the brilliant blue dye that was ingested remained in the feces. At 4 days and after, none of the dye could be detected.

Figure 4 shows the amount of ^{109}Cd remaining in the total liver and kidneys after 8 and 20 days of consuming the initial dose. The method of labeling did not significantly ($P > 0.1$) affect the accumulation of ^{109}Cd in these organs. At day 8, there was approximately 1% of the label retained in the liver of rats from both groups, but by day 20 the amount of label had decreased to only 0.3% of the original dose. Kidneys, on the other hand, had only approximately 0.1% of the dose on day 8, but by day 20, the amount had doubled.

Discussion

These studies were designed to assess the availability of endogenous Cd from the kernels of sunflowers fed to male and female rats at 20% of their diets. The values obtained were compared with those from similar rats fed diets without sunflower kernels. Previous work by our group[13] showed that male rats consuming diets with 20% sunflower kernels had a significantly higher weight gain than similar rats not receiving kernels in their diets. Similar results were found in the present study. In male rats, the highest rate of gain was in those rats that consumed diets with high-Cd content. Even though Anke et al.[18] suggested that small amounts of dietary Cd might be essential to some animal species, this does not seem to be the case here for two reasons: (1) Rats receiving low-Cd sunflower kernel spiked with a small amount of Cd did not gain any more than those receiving diets with no added Cd; and (2) in female rats, all diets containing kernels, whether with low or high Cd, caused a greater weight increase than diets with no sunflower kernels.

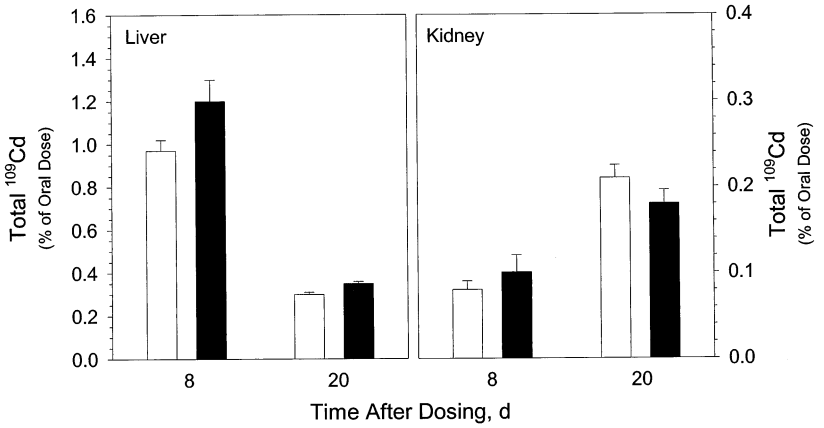

Figure 4 Change in total ^{109}Cd in liver and kidneys of rats fed diets containing high-cadmium (Cd) sunflower kernels and labeled endogenously (□) or exogenously (■) with ^{109}Cd. Values are mean ± SEM of four rats per group. The amount of label was significantly ($P < 0.001$) reduced in liver and increased ($P < 0.01$) in kidney with time after dosing. Assuming an exponential loss of liver Cd, the half-life was calculated to be approximately 7 days.

Part of the difference in body weight could have been caused by an increase in food intake. However, food intake measurements during the eleventh week of the study showed that the amount of food consumed by rats receiving diets with sunflower kernels was not significantly higher than those without sunflower kernels in their diet. This suggests that feed efficiency might have been improved in those rats receiving sunflower kernels. However, food efficiency cannot be measured accurately in rats that are not in the rapid growth phase, 5 to 7 g/day. At 11 weeks, all rats were in a maintenance phase and were gaining weight at a rate of only 0.5 g/day (female) to less than 2 g/day (male). It should be noted that all diets in this study were specifically designed to contain similar kinds and amounts of the major ingredients, including fat, and all nutrients known to be essential for the rat; therefore, some unknown growth factor in the sunflower kernels might have stimulated the faster growth.

Although the concentration of Cd in the diet was increased 1.4-fold (85–120 μg/kg) and 2.3-fold (85–195 μg/kg), by adding sunflower kernels to the diet, there were significant, but relatively small increases in Cd concentration in selected tissues. The concentration of liver Cd, for example, increased by 1.5-fold in male rats and approximately twofold in female rats. Cd in kidney, on the other hand, increased by approximately threefold (270–760 nmol/kg) in male rats and fivefold (180–930 nmol/kg) in female rats as a result of adding high-Cd sunflower kernels to the diet. This amount of accumulation was over a 15-week feeding period. Whether Cd would continue to accumulate with a longer feeding period is not known. Sex differences in tissue accumulation[19,20] and absorption[21] of Cd has been shown by others. There was no effect of dietary Cd on Cd concentrations in bone or large intestine.

Results for kidney and liver were similar to those reported by us in a previous paper.[13] There, we showed that male rats consuming 20% sunflower kernel diets with Cd concentrations similar to those in the present study had approximately a 1.5-fold increase in kidney Cd compared with those with no kernels in the diet. Although in the present study there was no effect of sunflower kernel Cd on Cd concentration in the large intestine, the previous study showed a 20-fold increase in the Cd concentration of the mucosa isolated from the upper small intestinal region in rats consuming Cd in sunflower kernels.

Results from Experiment 2, in which ^{109}Cd was fed as an endogenous label in sunflowers, showed that the label was entering the body. There was as much as 1% of the initial dose in the total liver after 8 days of consuming the diet. After 20 days, this amount had dropped to only 0.3%. Assuming exponential decay, the half-life of liver Cd was approximately 7 days. On the other hand, ^{109}Cd in kidney was rising from 0.1% to 0.2% of the initial dose in the same period. This experiment also showed that approximately 89% of the dose of Cd in endogenously labeled sunflower kernels was eliminated in the feces by day 8. These data indicate that at least 11% of the dose remained in the body. How much of this was absorbed into the body and how much remained in the gastrointestinal tract could not be detected by the methods used in this study. In addition, we did not determine to what extent ^{109}Cd was recycled by the practice of coprophagy, a well-known behavioral characteristic of rats.

Nonetheless, these data are not consistent with our previous experiments where similar methods were used.[13] In those studies, it was shown by whole body counting techniques that only approximately 0.3% of the initial dose remained in the body after 8 days. However, it should be noted that ^{109}Cd was retained in the gastrointestinal tract for a much longer period than in the present study: 8 days versus 3 days. The use of brilliant blue dye in the test meal also showed that the gut transit time was about the same length as it took for the major portion of ^{109}Cd to clear. The discrepancy between this and the previous study cannot be easily explained; however, although rats in both studies were fed diets with almost identical compositions, those in the previous study were fed the diets from weanling age to well beyond maturity (13 weeks) before they received ^{109}Cd. Those in the present study were near adult age (6 weeks) before they first received the diet, and then the diet was consumed for only 4 days before the rats were given an oral dose of ^{109}Cd. It is possible that long-term consumption of the sunflower diet could have initiated an adaptation response in the intestine and caused a change in the absorption rate of Cd. We are not aware of studies that

compare the effects of long-term versus short-term feeding regimens on mineral absorption.

It is obvious from these studies that over a 15-week period, the consumption of diets containing sunflower kernels that contribute twice the Cd concentration as diets with no sunflower kernels can increase the body burden of Cd approximately twofold. However, it should be noted that the kidney is more susceptible than other tissues measured. A twofold elevation in dietary Cd caused a fivefold increase in kidney Cd of female rats. Whether this has implications for human females who habitually consume large amounts of sunflower kernels is under investigation. In a recent report[22] we showed that adult humans, male and female, who reported consuming more than 1 oz of sunflower kernels per week over the previous 12 months had significantly higher Cd intakes than those who reported consuming less than 1 oz of kernels. Nevertheless, they did not have higher Cd concentrations in whole blood or urine. Because of the problems of obtaining reliable food consumption data from free-living human volunteers, controlled long-term feeding studies would have to be used to determine if the habitual consumption of large amounts of Cd containing sunflower kernels has the potential to increase the body burden of Cd.

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