Absorption and Organ Content of Cadmium from the Kernels of Confectionery Sunflowers (*Helianthus annuus*) Fed to Male Rats†

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The purpose of this study was to determine the availability of cadmium from the kernels of sunflowers grown in soils containing a natural abundance of cadmium. Weanling male rats were used as the experimental model. Fresh sunflower kernels containing either 330 or 780 μg Cd/kg were ground and added to a purified rodent diet at 20%. Cadmium chloride was added to purified diets without kernels. After 10 weeks, a test meal of each diet, containing ^{109}Cd, was fed and whole-body counting techniques were used to estimate cadmium absorption. Cadmium absorption from all diets ranged from 0.39 to 0.55%. Absorption was 30% less ($P < 0.001$) from diets containing kernels than from those that did not. The concentrations of cadmium in various organs were proportional to the amounts in the diet but highest in the intestinal mucosa. Cadmium tended to be lower in organs of rats fed diets with sunflower kernels than in those of rats fed diets without sunflower kernels.

Keywords: *Cadmium; absorption; sunflower kernels; rats*

INTRODUCTION

Excessive intakes of cadmium can cause numerous health problems such as osteomalacia, emphysema, and kidney dysfunction in humans (Bernard and Lauwerys, 1986; Kjellstrom, 1986; Kido et al., 1988). Cadmium exposure in the general population comes primarily from the natural occurrence of cadmium in the environment. Most exposure to the general population of the United States is through the food supply, which provides an estimated normal intake of 140 μg cadmium/week (Gartrell et al., 1986). Each individual may react differently to cadmium exposure; thus, the World Health Organization (WHO) suggests that a small safety margin exists between the exposure to cadmium in the normal range of dietary intake and the exposure that might produce deleterious effects (FAO/WHO, 1989). They estimate that a sustained weekly intake of about 700 μg of cadmium over a long period could lead to about 2% of the population exceeding their individual critical concentrations. The provisional tolerable weekly intake for humans recommended by WHO is 7 μg/kg of body weight (BW). For a 70 kg person this intake would amount to approximately 490 μg/week.

Numerous plant species take up cadmium from the soil. The soils of the farm lands of the Red River Valley of North Dakota and Minnesota contain a higher natural abundance of cadmium than farm lands of other parts of the United States. Commodities such as confectionery sunflowers, durum wheat, and flax that are grown in this region take up cadmium, much of which can be found in the seeds of these plants. During the 1993 harvest season, the cadmium concentration of confectionery sunflower kernels from this area ranged from 260 to 740 μg/kg (mean ± SD, 480 ± 160). (During the 1993 harvest season, samples of sunflower kernels from 16 farms in the Red River Valley of North Dakota and Minnesota were collected and analyzed for cadmium in our laboratory.) Thus, individuals prone to consume relatively large amounts of sunflower kernels in the form of a snack item, or as additions to various food preparations, might approach the provisional tolerable intake of cadmium.

For food cadmium to cause ill effects it must be absorbed from the gut. Compared to other trace elements, the absorption of cadmium from the GI tract is relatively low: from 0.7 to 15% in humans (McLellan, 1978) and from 0.3 to 25% in animals (Kello and Kostial, 1977; Kostial et al., 1978). The rate of absorption depends greatly on the form of cadmium in the diet, the composition of the diet, and the age of the animal at the time of consumption.

Studies to determine cadmium availability from sunflower kernels are very limited. Stoewsand et al. (1986) fed Japanese quail diets containing the pomace of oil-extracted seeds of sunflowers grown on sludge-amended soil that contained high concentrations of cadmium. They found higher concentrations of cadmium in the organs of birds fed the seed-pomace diets from sunflowers grown on sludge than in those fed seed-pomace diets from sunflowers grown on control soils. However, absorption was not measured in this study. Because of the shortage of data in this field, we sought to examine the availability of cadmium in whole confectionery sunflower kernels to mammals and chose male rats as the model.

This study was approved by the Animal Use Committee of the USDA, ARS, 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 (National Research Council, 1985).

MATERIALS AND METHODS

Materials. Rats were purchased from Sasco, Inc., Omaha, NE. Two lots of raw whole kernels from sunflowers (*Helianthus annuus*) grown in the Red River Valley of North Dakota

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Table 1. Mineral Analysis of Sunflower Kernels[a]

	low-Cd kernels	high-Cd kernels
cadmium, μg/kg	330 ± 30	780 ± 50
copper, mg/kg	13.1 ± 0.9	21.3 ± 1.1
zinc, mg/kg	37.6 ± 3.1	50.0 ± 4.4
iron, mg/kg	40.0 ± 4.3	39.2 ± 4.1
manganese, mg/kg	21.8 ± 1.8	20.3 ± 0.8
molybdenum, mg/kg	0.9 ± 0.1	1.2 ± 0.1
calcium, mg/kg	865 ± 93	776 ± 79
phosphorus, mg/kg	5440 ± 510	6860 ± 640
magnesium, mg/kg	3280 ± 95	3650 ± 190
sodium,[b] mg/kg	<10	<10
potassium, mg/kg	4340 ± 290	4890 ± 220

[a] Values are means ± SD for eight replicates. Analytical procedures are described under Materials and Methods. [b] Because of extreme variability, an accurate analysis for sodium could not be obtained.

and Minnesota were provided by Agway, Inc., Grandin, ND. By analysis, one lot contained 330 ± 30 μg of cadmium/kg (LOCdSF) and the other, 780 ± 50 μg/kg (HICdSF). All chemicals and other materials used in the preparation of animal diets were of high quality and purchased from various suppliers (see footnotes, Table 2). Chemicals used for analyses were of the highest grade and purchased from Sigma Chemical Co., St. Louis, MO, unless otherwise stated.

Experimental Design. Raw sunflower kernels were ground and added to a purified diet base at 20% of the total diet. The mineral composition of sunflower kernels was estimated by direct analyses (Table 1). Specified amounts of purified ingredients were added to the diets so that those containing the kernels had the same kinds and concentrations of essential nutrients as the diets that did not contain sunflower kernels.

Upon arrival at the laboratory, 60 weanling male Sprague-Dawley rats were placed in stainless steel hanging cages (one rat per cage) and fed Laboratory Rodent Diet 5001 (Purina Mills, St. Louis, MO) for 24 h. The rats were randomly divided into six groups of 10 each. The mean weight ± SD of each group was 52 ± 5 g. Each of the groups was fed one of the six diets described in Table 2 for 17 weeks. Fresh deionized water was provided *ad libitum* at all times.

Diet Preparation. Fresh sunflower kernels were finely ground in a food processor, being careful not to create "sunbutter". The ground kernels were then mixed into the purified diet base at 20% (Table 2). Cadmium chloride was added to some diets to obtain the desired concentrations of cadmium. Each diet was then analyzed for cadmium concentration. The purified diet without sunflower kernels (BASAL-90) contained 90 μg of endogenous cadmium/kg. BASAL-270 contained additional cadmium to bring the total to 270 μg/kg. BASAL-880 contained additional cadmium to bring the total to 880 μg/kg. The LOCdSF-160 diet contained 20% low-cadmium sunflower kernels and a concentration of cadmium of 160 μg/kg. HICdSF-240 contained 20% high-cadmium sunflower kernels and a concentration of cadmium of 240 μg/kg. The LOCdSF-840 diet contained 20% low-cadmium sunflower kernels and additional cadmium to bring the total to 840 μg/kg.

Whole-Body Counting. After approximately 10 weeks of dietary exposure, the rats were fasted from 11:00 p.m. until 9:00 a.m. the next morning. Portions of each diet were thoroughly mixed with ^{109}Cd [$^{109}CdCl_2$, 1 Ci/mg of Cd (37 MBq/mg)] so that 2.0 g of diet contained 1.6 μCi (59.2 kBq) of ^{109}Cd. Four hours after the rats consumed 2.0 g of ^{109}Cd-containing diet, the amount of ^{109}Cd in each rat was determined by whole-body counting. The rats were placed inside a rat holder made of polycarbonate. The holder was placed inside the counting chamber of a small-animal whole-body gamma counter. The counter was custom-made and consisted of two 10.2 × 10.2 × 40.6 cm NaI (thallium) collectors, 10.2 cm apart. The whole apparatus was completely surrounded by 5 cm thick lead bricks. Signals from the crystals were collected with a ND-62 multichannel analyzer between 15 and 150 keV (Nuclear Data Instrumentation, Schaumburg, IL). The peak energy for ^{109}Cd is 88 keV.

After rats were assayed for radioactivity for the first time, diet cups were returned to the cages and the rats were allowed to consume their respective ^{109}Cd-free diets *ad libitum*. Thereafter, the amount of radioactivity in each rat was determined each day for the first 8 days of the study, then every 2–3 days for a total of 20 days.

To determine the counting efficiency of the machine, a phantom of approximately the same size as a rat was prepared by mixing ^{109}Cd in sucrose, placing the sucrose in a plastic bag, and placing the bag in a polypropylene bottle. Because ^{109}Cd emits a weak gamma, the efficiency of counting in our device was only about 2.3%. This resulted in a count rate of approximately 500 cpm above background during the last days of the counting period. Eight-minute counts were done during this period.

The amount of ^{109}Cd remaining in the body each day was represented as a fraction of the amount found in the body at day 0 (percent retained). The natural log of this value was plotted against days. The relative amount of ^{109}Cd absorbed was calculated by extrapolating the linear portion of the curve (days 8–20) to zero time. The antilog of this number represented the percentage of ^{109}Cd retained at zero time (Cotzias, 1961; Heth and Hoekstra, 1965). Curves were generated for each rat by a computer program designed for this purpose and the data expressed as the mean for each group plus or minus the estimated standard error of the mean (SEM). The biological half-life of cadmium was expressed as BHF = $(-\ln 2)/m$,

Table 2. Compositions of Experimental Diets

ingredient	BASAL-90	BASAL-270	BASAL-880	LOCdSF-160	HICdSF-240	LOCdSF-840
cornstarch[a]	393.95	383.95	343.95	339.05	349.05	295.75
ground sunflower kernels[b]				200.00	200.00	200.00
casein[c]	200.00	200.00	200.00	151.90	151.90	151.90
dextrinized starch[d]	100.00	100.00	100.00	100.00	100.00	100.00
sucrose	100.00	100.00	100.00	100.00	100.00	100.00
sunflower oil[e]	99.00	99.00	99.00			
fiber[f]	50.00	50.00	50.00	42.00	42.00	42.00
mineral mix[g]	35.00	35.00	35.00	35.00	35.00	35.00
cadmium premix[h]		10.00	50.00			43.30
vitamin mix[i]	10.00	10.00	10.00	10.00	10.00	10.00
α-linolenic acid[j]	6.50	6.50	6.50	6.50	6.50	6.50
L-cystine[k]	3.00	3.00	3.00	3.00	3.00	3.00
choline bitartrate[l]	2.50	2.50	2.50	2.50	2.50	2.50
TBHQ[m]	0.05	0.05	0.05	0.05	0.05	0.05
phytate content, mg/kg[n]	0.0	0.0	0.0	920	990	920

[a] Borden Food Service, Chatsworth, CA. [b] Agway, Inc., Grandin, ND. [c] Teklad, Madison, WI. [d] Dyetrose, Dyets Inc., Bethlehem, PA. [e] Hunt-Wesson, Fullerton, CA. [f] Cellulose, Teklad, WI. [g] See Table 3 for the composition of the mineral mixes. [h] 204 mg of $CdCl_2$ (20% water)/kg of powdered sugar. [i] See Table 4 for the composition of the vitamin mix. [j] Pfalz & Bauer, Inc., Waterbury, CT. [k] Sigma Chemical, St. Louis, MO. [l] Teklad, Madison, WI. [m] *tert*-Butylhydroquinone, Eastman Kodak, Rochester, NY. [n] Phytate analysis was done by Agway Inc., Ithaca, NY.

where m is the slope of the linear portion of the curve for each group.

Organ Collection. At the end of the experiment, each rat was anesthetized with an intraperitoneal injection of 50 mg of pentobarbital sodium/100 g of BW. The abdominal cavity was opened and blood withdrawn from the abdominal aorta into a Monovet syringe (Sarstedt, Newton, NC). The blood was allowed to clot at 22 °C for 1 h and then centrifuged for 20 min at 1000g at 4 °C.

Duodenal scrapings were collected for mineral and metallothionein (MT) analysis. Beginning at the pylorus, a 20 cm segment of intestine was excised, and the contents were washed out with ice cold 0.145 mol of NaCl/L. The segment was slit open and the mucosal lining scraped off with a glass slide. The scrapings were frozen at −20 °C until analyzed for MT, cadmium, zinc, and copper. Both kidneys and whole liver were removed and frozen until analyzed for MT, cadmium, zinc, and copper.

Mineral Analysis. Sunflower kernels were ground, and eight replicates of 1−3 g were digested in high-purity concentrated nitric acid (Fisher Scientific, St. Louis, MO). The residue was dissolved in 0.1 mol of nitric acid/L. Minerals were determined by inductive coupled argon plasma analysis (ICAP), except for cadmium which was determined by atomic absorption spectroscopy (AA). Table 1 gives values obtained for numerous mineral elements.

Three 2-g replicates of each diet were ashed in Pyrex beakers in a muffle furnace at 500 °C. Charred residue remaining after 24 h was dissolved in concentrated nitric acid and heated to dryness on a hot plate. These samples were returned to the oven and ashed until only a white residue remained. The residue was dissolved in 0.1 mol of nitric acid/L, and minerals were determined by ICAP (cadmium by AA).

Organs were weighed to the nearest milligram, and portions (anterior half of each kidney, the right lobe of the liver, and a portion of the intestinal scrapings) were ashed in fused silica crucibles at 450 °C. Charred residue remaining after 24 h was dissolved in concentrated nitric acid and heated to dryness on a hot plate. These samples were returned to the oven and heated until only a white residue remained. The residue was dissolved in 0.1 mol of nitric acid/L, and minerals were determined by AA. The accuracy of mineral determinations was monitored by analyzing certified diet and liver tissue standards (National Institute of Standards and Technology, Gaithersburg, MD) simultaneously with the experimental samples. Values for all minerals analyzed were within the range specified for the standards.

Metallothionein Assay. Organs were weighed to the nearest milligram, and the posterior half of each kidney, a portion of the left lobe of the liver, and a portion of the intestinal scrapings (1 part) were homogenized in buffer (2 parts) containing 50 mmol of Tris/L and 1 mmol of 2-mercaptoethanol/L, pH 7.4. One milliliter of the homogenate was heated for 10 min at 95 °C and centrifuged for 5 min at 10000g. Aliquots of the supernatant were analyzed for MT (Eaton and Cherian, 1991).

Statistical Analysis. A one-way analysis of variance (ANOVA) with single degree of freedom comparisons was used to determine differences between treatment means.

RESULTS

Weight Gain. Figure 1 shows the results of feeding diets with and without sunflower kernels on the growth of the rats. ANOVA with single degree of freedom contrasts showed no overall significant difference between growth of those rats fed diets with sunflower kernels and growth of rats that were not. However, when comparisons were made between LOCdSF-160 and HICdSF-240 + LOCdSF-840, there was a significantly ($P < 0.03$) lower weight gain in rats fed the diet with the lowest amount of cadmium. In the groups of rats that were not fed sunflower kernels in their diet, the group with the highest amount of cadmium tended to weigh more than those with the lower amounts of cadmium. However, the difference was not significant ($P > 0.05$). Body weights were shown to plateau at week 10 and then rise again at week 13 (Figure 1). This was probably caused by stress during the whole-body counting procedures.

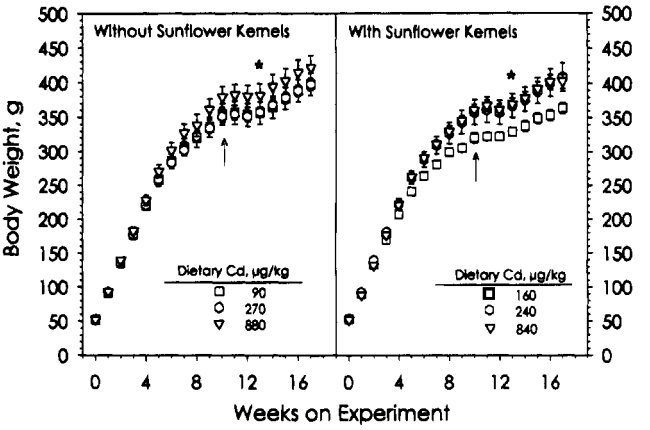

Figure 1. Change of body weight with time of rats fed purified diets containing various concentrations of cadmium in the form of cadmium salt or cadmium-containing sunflower kernels. Points of the graphs are means ± SEM of 10 replicates. Arrows represent the beginning of the whole-body counting period, and the asterisks represent the end.

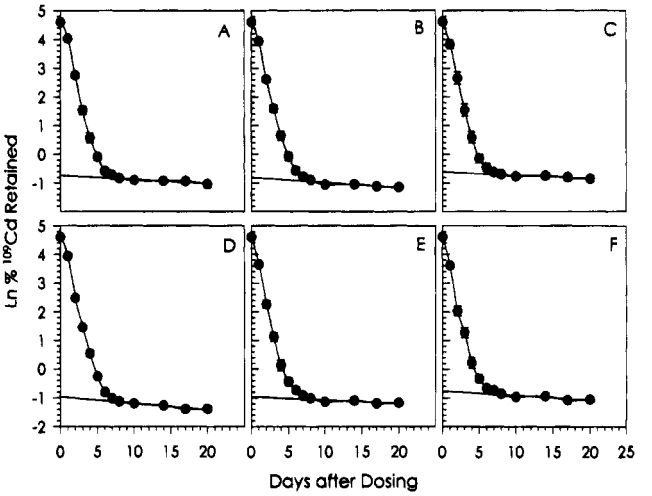

Figure 2. Whole-body retention curves of ^{109}Cd in rats fed purified diets containing various concentrations of cadmium in the form of cadmium salt or cadmium-containing sunflower kernels. Points of the graphs are means ± SEM of 10 replicates. The letter in the top right corner of each graph designates the group as follows: A, BASAL-90; B, BASAL-270; C, BASAL-880; D, LOCdSF-160; E, HICdSF-240; F, LOCdSF-840 (see text for description of diets). Curves represent the loss of ^{109}Cd from the body with time. Straight lines represent the slope of the retention curve calculated between days 8 and 20. The antilog of the y intercept of this curve for each group represents the estimate of cadmium absorption for that group.

Cadmium Absorption. Figure 2 shows whole-body cadmium retention during the 20-day counting period. The curves represent the disappearance of ^{109}Cd from the body with time. The rapid decline during the first 7 days probably represents transit through the GI tract. The slower linear portion of this curve represents the disappearance of absorbed ^{109}Cd from the body, i.e., turnover or biological half-life (BHF; Table 5). BHF ranged from 42 ± 7 days for LOCdSF-160 to 70 ± 13 days for the BASAL-880 group. No statistically significant differences in BHF were found.

Table 3. Composition of the Mineral Mixes for Experimental Diets

ingredient	BASAL diets	SF kernel diets
calcium carbonate ($CaCO_3$), g/kg of mix	448.65	380.60
calcium phosphate ($CaHPO_4$), g/kg of mix	36.14	113.70
potassium phosphate (KH_2PO_4), g/kg of mix	285.20	
potassium sulfate (K_2SO_4), g/kg of mix	46.62	16.62
potassium citrate ($K_3C_6H_5O_7$), g/kg of mix		116.00
sodium chloride (NaCl), g/kg of mix	74.00	74.00
sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$), g/kg of mix	7.25	7.25
ferrous sulfate ($FeSO_4 \cdot 7H_2O$), g/kg of mix	4.98	3.56
zinc carbonate ($ZnCO_3$), g/kg of mix	1.65	1.12
manganous carbonate ($MnCO_3$), g/kg of mix	0.63	0.15
cupric carbonate ($CuCO_3$), g/kg of mix	0.30	0.097
chromium potassium sulfate ($CrKS_2O_8 \cdot 12H_2O$), g/kg of mix	0.275	0.275
boric acid (H_3BO_3), g/kg of mix	0.0815	0.0815
sodium fluoride (NaF), g/kg of mix	0.0635	0.0635
nickel carbonate ($NiCO_3$), g/kg of mix	0.0635	0.0635
stannous oxide (SnO), g/kg of mix	0.0162	0.0162
ammonium vanadate (NH_4VO_3), g/kg of mix	0.0132	0.0132
ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), g/kg of mix	0.0106	0.0106
sodium selenate (Na_2SeO_4), g/kg of mix	0.01025	0.003
potassium iodide (KI), g/kg of mix	0.010	0.010
powdered sucrose, g/kg of mix	57.06925	256.3661

Table 4. Composition of the Vitamin Mix for Experimental Diets

ingredient	g/kg of mix	ingredient	g/kg of mix
nicotinic acid	3.00	vitamin B_{12} (cyanocobalamin) (0.1% in mannitol)	2.50
calcium pantothenate	1.00	vitamin E (*all-rac*-α-tocopheryl acetate) (500 IU/g)	15.00
pyridoxine hydrochloride	0.70	vitamin A (*all-trans*-retinyl palmitate) (5×10^5 IU/g)	0.80
thiamin hydrochloride	0.60	vitamin D_3 (cholecalciferol) (4×10^5 IU/g)	0.25
riboflavin	0.60	vitamin K (phylloquinone)	0.075
folic acid	0.20	powdered sucrose	974.655
D-biotin	0.02		

Table 5. Estimates of Absorption (AA), Total Absorbed per Day, and Biological Half-Life (BHF) of Cadmium in Rats Fed Purified Diets with and without 20% Ground Sunflower Kernels[a]

	BASAL-90	BASAL-270	BASAL-880	LOCdSF-160	HICdSF-240	LOCdSF-840
AA, %[b]	0.51 ± 0.05	0.47 ± 0.05	0.55 ± 0.04	0.39 ± 0.04	0.39 ± 0.03	0.47 ± 0.03
Cd absorbed,[c] ng/day	8 ± 1	19 ± 2	73 ± 6	9 ± 1	13 ± 1	62 ± 5
BHF, days	42 ± 7	44 ± 6	70 ± 13	36 ± 5	57 ± 12	45 ± 5

[a] Values are mean ± SEM of 10 replicates per group. [b] Single degree of freedom contrasts showed that the percentage absorption of cadmium in those rats fed diets containing sunflower kernels without added cadmium was significantly less ($P < 0.03$) than those without sunflower kernels in their diets. [c] The amount of cadmium absorbed per day was calculated by multiplying the total daily intake of cadmium by the fractional absorption. There was also a significant difference ($P < 0.03$) in the daily amount of cadmium absorbed between groups BASAL-270 and HICdSF-240. In addition, daily cadmium absorption was also significantly different ($P < 0.02$) between rats fed diets with and without sunflower kernels at the two highest levels of cadmium intake.

The straight line in each graph was generated by regression analysis of the linear portion of the curve between days 8 and 20. Extrapolating this line to zero provides an estimate of the amount of cadmium initially absorbed (Table 5). Overall, the amount of cadmium absorbed from the diets was 0.39–0.55% of that consumed. Single degree of freedom contrasts showed that rats fed the sunflower-kernel diets absorbed significantly less (about 30%; $P < 0.01$) cadmium than rats fed diets without sunflower kernels but with added cadmium. However, when cadmium chloride was added to LOCdSF-880, the amount of ^{109}Cd absorbed was not different from diets without sunflower kernels.

The amount of cadmium absorbed per day was calculated, and the data are presented in Table 5. Although there was only a 10% difference in cadmium concentration in the diet between BASAL-270 and HICdSF-240, the difference in the amount of cadmium absorbed per day was greater than 30% ($P < 0.03$), with less being absorbed by rats receiving diets containing sunflower kernels.

Organ Concentration of Cadmium. Table 6 shows the effects of feeding diets with cadmium on organ concentration of cadmium, copper, zinc, and MT. Of the three organs measured, intestinal mucosa had the highest concentration of cadmium, with kidney second and liver third. Regardless of the cadmium source, as the cadmium concentration in the diet increased, the amount in organs increased (Figure 3). However, at the higher concentrations of dietary cadmium, the amount of cadmium in the intestinal mucosa of rats fed diets containing sunflower kernels was lower ($P < 0.01$) than in rats fed diets without sunflower kernels.

To express the body burden of cadmium, we estimated the total amount of cadmium contained in the whole body by summing total liver and kidney cadmium and dividing by a factor of 0.87 (Table 7). This factor was derived from the work of Buhler et al. (1981), who showed that liver and kidney contributed 87% of the total body burden of cadmium in rats fed a cadmium-containing diet for 12 weeks. Although a substantial amount of cadmium was contained within the GI tract, this was considered as unabsorbed and not contributing to the body burden. ANOVA showed that the presence of sunflower kernels in the diet did not significantly affect the body burden of cadmium.

The cadmium data for liver and kidney were expressed as a ratio of total liver cadmium to total kidney cadmium and plotted against the dietary concentration

Table 6. Cadmium, Copper, Zinc, and Metallothionein (MT) Concentrations in Intestinal Mucosa, Liver, and Kidney of Rats Fed Purified Diets with and without 20% Ground Sunflower Kernels[a]

	BASAL-90	BASAL-270	BASAL-880	LOCdSF-160	HICdSF-240	LOCdSF-840
intestinal mucosa						
cadmium, ng/g	6 ± 6	307 ± 20	1342 ± 92	113 ± 9	229 ± 16	944 ± 74
copper, μg/g	1.3 ± 0.1	1.2 ± 0.04	1.3 ± 0.05	1.3 ± 0.03	1.3 ± 0.04	1.3 ± 0.04
zinc, μg/g	13.2 ± 0.5	13.4 ± 0.6	13.1 ± 0.6	12.4 ± 0.4	12.5 ± 0.5	12.9 ± 0.3
MT, μg/g	43.8 ± 3.9	46.5 ± 4.0	59.6 ± 3.9	45.1 ± 3.8	43.7 ± 2.7	55.8 ± 3.3
liver						
cadmium, ng/g	37 ± 3	61 ± 3	196 ± 35	43 ± 3	59 ± 3	162 ± 17
copper, μg/g	3.9 ± 0.2	4.5 ± 0.2	4.0 ± 0.1	4.3 ± 0.1	4.6 ± 0.3	4.0 ± 0.1
zinc, μg/g	30.7 ± 2.2	33.3 ± 1.1	31.8 ± 1.2	33.1 ± 1.1	29.8 ± 2.0	31.6 ± 1.0
MT, μg/g	35.8 ± 1.9	36.5 ± 2.3	38.2 ± 2.1	40.6 ± 2.2	41.3 ± 2.0	36.4 ± 2.2
kidney						
cadmium, ng/g	46 ± 4.7	127 ± 9	692 ± 81	68 ± 5	148 ± 9	561 ± 32
copper, μg/g	10.4 ± 0.5	9.3 ± 0.4	10.9 ± 0.5	11.5 ± 0.7	12.0 ± 0.8	13.2 ± 0.7
zinc, μg/g	22.6 ± 0.6	22.4 ± 0.4	22.3 ± 0.4	22.2 ± 0.5	22.1 ± 0.5	23.3 ± 0.7
MT, μg/g	76.4 ± 4.0	70.6 ± 4.5	77.0 ± 3.9	74.7 ± 4.9	77.9 ± 3.1	87.1 ± 2.9

[a] Values are means ± SEM of 10 replicates per group. Values are expressed on the basis of dry organ weight. ANOVA for organ cadmium concentrations showed highly significant differences ($P < 0.001$) between groups with differing concentrations of dietary cadmium. Single degree of freedom comparisons showed that among groups containing comparable amounts of dietary cadmium, there was a significant reduction in intestinal cadmium ($P < 0.001$) and kidney cadmium ($P < 0.04$) in rats fed sunflower kernels compared to rats not fed the kernels. This analysis also showed that intestinal MT was significantly higher ($P < 0.001$) in rats with the highest cadmium intake than in those with the lower intakes.

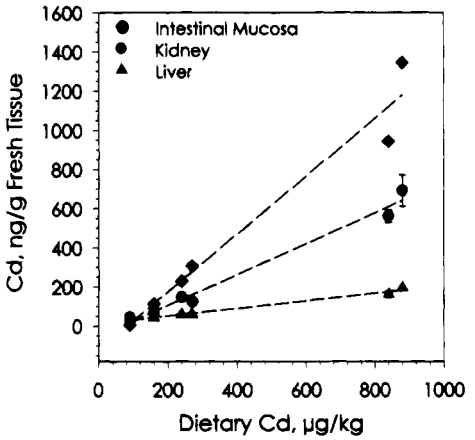

Figure 3. Cadmium concentrations in the organs of rats fed purified diets containing various concentrations of cadmium in the form of cadmium salt or cadmium-containing sunflower kernels. Points of the graphs are means ± SEM of 10 replicates. The amount of cadmium in each tissue was proportional to the amount of cadmium in the diet. For intestinal cadmium, $r^2 = 0.96$, $P < 0.001$; kidney cadmium, $r^2 = 0.98$, $P < 0.001$; liver cadmium, $r^2 = 0.98$, $P < 0.001$. Because of the large differences between high and low concentrations of dietary cadmium, the high r^2 values may reflect the calculation of a two-point regression.

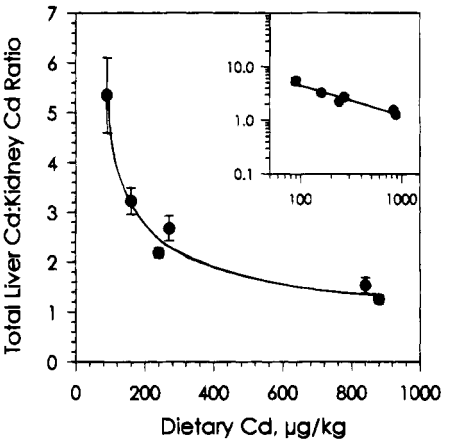

Figure 4. Change in the ratio of total liver cadmium:total kidney cadmium with a change in the dietary concentration of cadmium in rats fed purified diets containing various concentrations of cadmium in the form of cadmium salt or cadmium-containing sunflower kernels. Points of the graphs are means ± SEM of 10 replicates. As the concentration of dietary cadmium increased, there was a significant decrease ($P < 0.001$) in the liver:kidney ratio of cadmium. The inset represents the data expressed on a log–log scale.

of cadmium. Figure 4 shows that as dietary cadmium increased, the ratio of liver to kidney cadmium decreased. The ratio was 3.5 times greater at the low concentrations of dietary cadmium than at the high concentrations. When a log–log plot was constructed from the data, there was a linear relationship between the organ cadmium ratios and dietary cadmium concentration (Figure 4, inset).

Because cadmium induces the synthesis of MT, a correlation between the amount of MT and cadmium in the organs was expected. There was a slight increase in the concentration of MT in the kidney with an increase in cadmium, a greater increase in the intestinal mucosa ($r^2 = 0.99$, $P < 0.001$), and no effect in liver (Figure 5). The high r^2 value should be regarded with caution because of the large differences between high and low concentrations of dietary cadmium. Our calculations may be, in effect, a two-point regression. Although the kidney had the highest amount of MT, the amount of cadmium was only second highest of the organs measured.

Table 7. Total Cadmium in Liver and Kidney and Body Burden of Cadmium in Rats Fed Purified Diets with and without 20% Ground Sunflower Kernels[a]

	BASAL-90	BASAL-270	BASAL-880	LOCdSF-160	HICdSF-240	LOCdSF-840
total liver Cd, ng	515 ± 50	725 ± 36	2097 ± 254	470 ± 26	726 ± 64	1989 ± 228
total kidney Cd, ng	104 ± 11	284 ± 20	1680 ± 159	151 ± 10	334 ± 26	1307 ± 74
body burden,[b] ng	711 ± 59	1160 ± 47	4340 ± 449	713 ± 31	1218 ± 101	3788 ± 297

[a] Values are means ± SEM of 10 replicates per group. [b] Body burden of cadmium was estimated by summing total liver and kidney cadmium and dividing by a factor of 0.87.

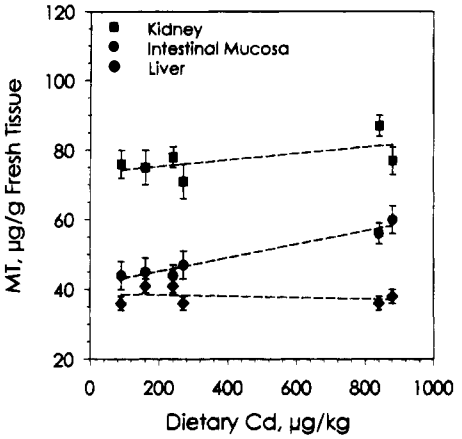

Figure 5. Metallothionein (MT) concentrations in the organs of rats fed purified diets containing various concentrations of cadmium in the form of cadmium salt or cadmium-containing sunflower kernels. Points of the graphs are means ± SEM of 10 replicates. The amount of MT in intestinal mucosa was proportional to the amount of cadmium in the diet ($r^2 = 0.99$, $P < 0.001$). Although there was a slight increase in kidney MT with an increase in dietary cadmium, it was not significant.

DISCUSSION

This study was designed to determine the availability of endogenous cadmium from sunflower kernels added to the diets of male rats. These results were compared to the availability of cadmium added to a purified diet. The rate of cadmium absorption was very small, 0.39–0.55% over the 17 weeks of consuming the diet, but absorption from the diets with sunflower kernels was about 30% less than from diets without the kernels. These values are comparable to those found by Kello and Kostial (1977) and Kostial et al. (1980) when purified diets containing cadmium were fed to rats. Using whole-body counting techniques, as in the present study, they found 0.5 and 0.3% of the dose absorbed at 6 and 52 weeks of feeding the diets, respectively.

When similar techniques for determining absorption of cadmium, but different sources of food cadmium, were used, the percentage absorption was greater than we found from sunflower kernels. Welch et al. (1978) used rats to determine the absorption of cadmium from lettuce leaves endogenously labeled with ^{109}Cd. Instead of feeding the leaves in the diet as we did with sunflower kernels, they gave a single meal of purified diet containing 40% dried labeled leaves. After monitoring the whole-body content of ^{109}Cd for 12 days, they found 3.4 ± 0.9% absorption. Kello and Kostial (1977) and Kostial et al. (1980) also showed that cadmium absorption in rats fed a diet containing milk was as much as 7%.

Whole-body counting techniques have also been used in human studies to determine cadmium absorption. McLellan et al. (1978) fed ^{115m}Cd-labeled meals consisting of rolled oats and powdered milk and found cadmium absorption to range between 0.7 and 16% (4.6 ± 4.0%) when subjects were monitored for up to 100 days. Shaikh and Smith (1980) fed subjects ^{115m}Cd-labeled beef kidney cortex followed by a glass of milk. They observed their subjects for up to 500 days and found that absorption averaged 2.5 ± 2.0%. However, another study (Newton et al., 1984) found 2.7 ± 2.3% absorption from labeled crab meat without milk.

These studies suggest that the amount of cadmium absorbed can be dramatically influenced not only by the dietary ingredients but also by the length of time the subjects are observed after dosing. Shaikh and Smith (1980) pointed out that the estimate of cadmium absorption by this method depends on the number of days the subjects are counted. The longer they are counted, the more components of the decay curve are revealed. Thus, the estimate of minimal absorption will become smaller and smaller and the estimate of BHF will become larger and larger.

Shaikh and Smith (1980) stated that the slow component of the whole-body retention curve was not well-defined until after 200 days and that the slow component accounted for 2.5% of the initial counts. They considered this an estimate of minimal absorption. While it is true that absorption had to be at least 2.5% if that much of the dose remained in the subjects at 200 days, the actual absorption was probably higher. At 200 days, 2.5% best described retention, not absorption. Absorption takes place when the meal is eaten, and the best estimate is to extrapolate back from the early, fast-turnover data that are obtained in the linear portion of the curve that occurs after GI transit ceases to dominate the whole-body retention curve. In the present study, an estimation of absorption was based on the extrapolation of the linear portion of the curve extending only 10 days beyond the point where GI transit of cadmium first reached a minimum.

Although in the present study the absorption of cadmium was low, there were measurable concentrations of cadmium in the organs. The concentrations of cadmium were directly proportional to the amounts in the diet regardless of whether cadmium was from sunflower kernels or added as the salt. However, the amount of cadmium in intestinal mucosa and kidneys of rats fed the sunflower kernel diets with the highest amount of cadmium was significantly lower than in those fed high-cadmium diets without sunflower kernels. This indicates that, overall, less cadmium was entering the body from the diets containing sunflower kernels.

In the whole-body counting technique used to determine cadmium absorption, we assumed that exogenously labeled cadmium equilibrated with the endogenous cadmium of the sunflower kernels. If this were true, then the reduction in cadmium absorption from diets containing sunflower kernels might have been caused by some factor in the kernel that complexed cadmium and prevented its absorption. One of the factors might be phytate (Wing, 1993). On a molar basis, there was approximately 900 times more phytate than cadmium in the sunflower diets. Although many sites on phytate may be taken up by calcium, zinc, and iron, enough cadmium could have been sequestered to cause the 30% reduction in absorption. An interesting phenomenon, however, was the observation that when exogenous cold cadmium was added to the sunflower diet containing low endogenous cadmium, the rate of absorption increased. Because the latter contained 5 times more cadmium than the former, all of the exogenous cadmium might not have been sequestered by phytate, leaving some available for absorption.

The diet was deliberately formulated to bias against cadmium absorption in that it contained a full complement of zinc and iron and more than an adequate amount of calcium. A reduced nutritional status of any one of these nutrients is known to enhance cadmium absorption (Flanagan et al., 1978; Foulkes, 1980), which implies that an excess would reduce absorption. However, with these factors maximized, liver and kidney cadmium still increased as dietary cadmium increased.

When we determined the ratios of total liver cadmium to total kidney cadmium and plotted them against the dietary concentration of cadmium, there was as much as 3.5 times more cadmium in the liver than in the kidney at the low dietary concentrations of cadmium compared to the high concentrations. This suggests that at very low cadmium intake the liver is able to sequester most of the body cadmium and retain it. However, as cadmium intake increases, the capacity of the liver to retain cadmium is exceeded and cadmium is redistributed to the kidneys. This agrees with the findings of Nordberg et al. (1985) and Groten et al. (1991).

Another interesting phenomenon was the reduction in the rate of gain in body weight of rats fed sunflower kernels with low cadmium content. When exogenous cadmium was added to this diet, the rate of weight gain was maximized and not different from that of rats fed the other diets containing higher concentrations of cadmium. The increased rate of gain paralleled the enhanced rate of cadmium absorption in these rats. Although Anke et al. (1987, 1990) have suggested that cadmium may be essential for ruminants (goats), it would be premature to suggest the same for monogastric animals. Ruminants depend on the microbial population of the rumen and intestinal tract to supply many of the required organic nutrients while the monogastrics have less dependency. The anaerobic prokaryotes of the gut may be able to utilize some of the ultratrace elements as components of metabolic factors and reactions more readily than aerobic cells of the body (Fraústo da Silva and Williams, 1991) and produce nutrients and other factors the animal needs. Other investigators have found relationships between the microfloral populations of the gut and mineral metabolism (Shinoda and Yoshida, 1989; Shurson et al., 1990). However, a recent study by von Zglinicki et al. (1992) showed that 100 pmol of cadmium/L of culture media, for three different mammalian cell types, caused a significant stimulation of cell growth and DNA synthesis when compared to cells with lower or higher concentrations of media cadmium. Whether a similar type of stimulation occurs at low cadmium concentrations in the whole animal remains to be determined.

In summary, the absorption of cadmium from sunflower kernels in a purified diet was only about 0.4% of the intake and 30% less than absorption of cadmium from purified diets alone. However, after 17 weeks of diets consumption, the body burden of cadmium, as estimated by total liver and kidney cadmium, was not different between rats fed the kernels and those that were not. Future studies may show that the rate of absorption of cadmium from sunflower kernels in humans is similar to that in rats. If so, a moderate consumption of sunflower kernels with cadmium concentrations as low as those used in this study would not add a significant amount of cadmium to the body burden that is already received from naturally occurring cadmium in the normal diet. This may not be the case, however, if the consumption of sunflower kernels is high or if the kernels contain high concentrations of cadmium, e.g., from sunflowers grown in high-cadmium, sludge-amended soils (Stoewsand et al., 1986).

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