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Uptake of Cadmium in Meals from the Digestive Tract of Young Non-smoking Japanese Female Volunteers

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Abstract: Uptake of Cadmium in Meals from the Digestive Tract of Young Non-smoking Japanese Female Volunteers: Yuriko KIKUCHI, *et al.* Department of Preventive Medicine and Public Health, School of Medicine, Keio University—Objectives—To estimate rates of cadmium (Cd) uptake from the digestive tract and changes in Cd in biological specimens after intake of Cd mainly in rice. Methods—Twenty-five young non-smoking Japanese female volunteers (20–23 in age) were recruited and a 20-d experimental study was conducted. With polished rice containing 0.004 ppm and 0.340 ppm of Cd, Meal L and Meal H were prepared. Approximately 12% of total Cd in Meal L and 92% of total Cd in Meal H originated in rice. The volunteers ate Meal L for 11 d to achieve a stable intake-output balance of Cd. Fifteen of the 25 volunteers ate Meal H on the 12th day (Group D1), and the remaining 10 ate Meal H on the 12th, 13th and 14th day (Group D3). All 25 subjects then resumed the consumption of Meal L to the end of the study (20th day). All meals, feces and urine were collected during the study, and Cd intake from the daily meals (Cd-I), Cd in feces (Cd-F) and Cd in urine (Cd-U) were determined. For measurement of Cd in blood (Cd-B), venous blood was collected from all volunteers on the day before the study and again on the 12th and 20th day; venous blood was also collected from 4–8 volunteers at additional time points. Results—Mean Cd-I was 4.51 μg/d (range: 1.85–6.93) or 48.48 μg/d (range: 27.98–56.27) when they ate Meal L or Meal H. Cd-F and Cd-B exhibited faster responses to the change in Cd-I than did Cd-U. The Cd_{uptake} rate, defined as (1-Cd-F_{excess} /Cd-I_{excess}) (Fig. 1), was 47.2% (range: –9.4–83.3%) in Group D1 and 36.6% (range: –9.2–73.5%) in Group D3, and the $Cd_{balance}$ rate, defined as (1-Cd-F_{output} /Cd-I_{intake}), was 23.9% (range: –4.0–37.7%) in Group D1 and 23.7% (range: –8.2–56.9%) in Group D3. Conclusions—Cd-F and Cd-B are better biological monitoring parameters for assessing change in Cd-I than Cd-U. The Cd_{uptake} and $Cd_{balance}$ rates appeared to be higher than those in previous papers when ingested Cd mainly originated in rice.
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Key words: Cadmium, Volunteer experiment, Rice, Dietary intake, Absorption, Biological monitoring

Toxicity due to cadmium (Cd) accumulation in the body is known to cause renal damage and resultant osteoporosis. Itai-Itai disease is one disastrous example of chronic Cd poisoning. Food is the main source of Cd intake for non-occupationally exposed people, and the average dietary Cd intake by the Japanese general population was recently calculated as 28 μg/d1), much higher than that in other countries; e.g., 9.9 in China2), 7.3 in Malaysia3), 14.5 in Finland4), 8.3 in Sweden5) and 9 to 10 in Germany6).

In Japan, rice and shellfish are the major sources of Cd intake, and other foods sold in Japanese markets contain a very wide range of Cd concentrations7). In order to determine a tolerable level of Cd intake from meals, we must establish a non-observed adverse effect level (NOAEL) based on the dose-response relationship between Cd in foods and adverse effects on the kidneys. At present, however, such data are not available, and in fact, a large-scale prospective study to establish the NOAEL is currently considered infeasible.

On the other hand, the dose-response relationships

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Table 1. Characteristics of volunteers

Items	units	mean	median	min	max	referent range[*]
Characteristics (n=25)						
Age	yr	20.8	21.0	20	23	
Height	cm	157.8	157.0	150	168.2	
Weight	kg	50.1	50.8	38.5	59.2	
BMI	kg/m^2	20.1	20.5	16.8	23	
Systolic blood pressure	mmHg	103.0	103.0	84	118	
Diastolic blood pressure	mmHg	70.0	69.0	58	82	
Excretion amount of feces and urine						
Feces, wet weight	g/d	113.5	95.0	4	425	
Feces, dry weight	g/d	23.9	21.0	1	112	
Urine	ml/d	1,530	1,530	342	3,269	
Number of walking steps	/d	15,700	15,192	1,002	41,150	
Sleeping time	h/d	6.2	6.2	4.7	7.6	
Urinalysis						
pH		6.3	5.8	5.5	7.5	4.5–8.0
B_2-Microglobulin	μg/l	81.8	72.0	<70	238	≤200
Creatinine	mg/dl	122.6	55.0	25.1	270.9	
Specific gravity		1.012	1.012	1.001	1.031	1.002–1.030
Hematological examination						
WBC	/μl	6,272	5,036	3,800	9,200	3,300–9,000
RBC	$10^4/\mu l$	450	406	361	548	380–500
Hemoglobin	g/dl	13.4	11.5	9.5	15.8	11.5–15.0
Hematocrit	%	42.9	37.2	31.5	50.3	34.8–45.0
Platelet count	$10^4/\mu l$	24.2	17.0	9.8	37.2	14.0–34.0
Serum biochemistry						
Total protein	g/dl	7.6	7.0	6.5	8.8	6.7– 8.3
Albumin	g/dl	4.8	4.5	4.2	5.3	3.8– 5.3
A/G		1.8	1.5	1.4	2.2	1.1– 2.0
Triglyceride	mg/dl	69.4	52	31	184	30– 149
Total cholesterol	mg/dl	187.8	159	131	237	120– 219
HDL	mg/dl	68.6	59	49	99	45– 75
TTT	U	2.8	2.1	0.8	5.9	≤4.0
ZTT	U	7.2	5.1	2.7	14.1	2.0–12.0
CK	IU/l	101.0	80	60	149	40–150
GOT	IU/l	17.6	15	13	25	10–40
GPT	IU/l	12.8	10	8	22	5–45
LDH	IU/l	186.7	165	143	247	120–240
ALP	IU/l	191.1	131	70	301	100–325
γ-GTP	IU/l	14.0	12	9	30	≤30
Creatinine	mg/dl	0.8	0.8	0.7	1	0.6–1.1
BUN	mg/dl	13.0	9.0	8	26	8–23
Na	mEq/l	141.6	139.8	138	146	137–147
K	mEq/l	4.3	4.1	3.8	5	3.5–5.0
Cl	mEq/l	101.0	99.0	97	106	98–108
Total bile acids	μmol/l	3.5	2.8	1.4	8.6	≤10.0
Total bilirubin	mg/dl	0.6	0.3	0.2	1.3	0.2–1.1
Direct bilirubin	mg/dl	0.3	0.1	0.1	0.7	0–0.5
β_2-Microglobulin	mg/l	1.7	1.4	1.2	2.1	1.2–2.2
Iron						
before study	μg/dl	77.4	75	18	144	40–180
12th day		57.5	50	25	126	
20th day		62.3	65	31	112	
Ferritin						
before study	ng/ml	33.0	25.8	5.9	109	4.0–64.2
12th day		30.2	28.5	4.2	100	
20th day		25.2	19.6	5.7	71.4	

[*] referent range for female adults

between renal effects and Cd levels in the urine, blood, and kidneys have been well studied. By using these data and the kinetics of Cd in the digestive tract, it should be possible to assess the tolerable Cd intake levels more accurately and scientifically.

Accordingly, the purpose of this study was to estimate changes in Cd in biological specimens after intake of Cd mainly in rice and to clarify the kinetics of Cd in the digestive tract by measuring dietary intake of Cd and excretion from feces and urine in human volunteers.

Subjects and Methods

This volunteer study was approved by the Ethical Review Committee of the School of Medicine, Keio University.

Subjects

We selected young non-smoking females as study subjects because the rate of absorption of Cd from the digestive tract is reported to be higher in females than in males[8)], the effects of Itai-Itai disease appear to be more severe in females than in males, and the body burden due to past Cd intake was expected to be small in a younger population.

Thirty-seven Japanese females, aged 20–23 yr, who were enrolled as students in the Department of Nutritional Science of Tokyo University of Agricultural were initially recruited for this study. All subjects gave their written consent after being fully informed about the toxicity of Cd, the purpose and significance of the volunteer study, the benefits of the study for human health, and their right to withdraw from the study at any time. Prior to the study, all subjects received a medical checkup that included hematological, serum biochemical, and urinary examinations. They were also requested to complete self-administered questionnaires concerning personal characteristics, such as age, smoking habits, dietary habits, past illness, current subjective symptoms, menstruation and evacuation habits. Among the 37 volunteers, 25 females who showed no problems in their medical checkups (Table 1), no past history of renal diseases or dysfunction, and good evacuation habits, and who agreed to participate in the 20-consecutive-day study, were enrolled as subjects. They were 20–23 yr in age, 150.0–168.2 cm in height, and 38.5–59.2 kg in body weight.

Preparation of meals

Based on the nutritional contents and Cd concentrations of various foods and beverages[7)], a registered dietitian made five daily menus for side dishes containing minimal levels of Cd. All foods except fresh tomatoes and green vegetables were cooked, weighed, packed in single-serving portions in boilable bags, and frozen at –30– –20 °C until consumption. The fresh tomatoes and green vegetables were grown by a hydroponic cultivation method and contained below trace levels of Cd. The chilled and frozen foods were also used after measuring Cd contents.

The nutritional composition of each meal was estimated by using the Standard Tables of Food Composition in Japan, 5th Editions[9)], and shown in Table 2. The levels of some nutrients exceeded twice the Recommended Dietary Allowance in Japan (RDA, 6th version) or above, but these excesses remained below the upper limits[10)]. Iron intake was approximately one-half of the RDA value[10)], mainly because we avoided foods containing substantial amounts of Cd, such as algae, fish and shellfish, meat offal, nuts and seeds, pulses, and spinach[7)], which are also rich sources of iron. Due to the uncertainty of interaction between supplemental iron intake and Cd absorption in the digestive tract, we decided not to use artificial products for the iron supplementation.

As a staple food, two lots of polished rice containing 0.004 ppm of Cd (Rice L) and 0.340 ppm of Cd (Rice H) were kindly supplied by the Ministry of Agriculture, Forestry and Fisheries of Japan. The Cd concentrations in both lots of rice were lower than the Japanese guideline level of Cd for rice, 0.4 ppm.

Combining the rice and the side dishes, two kinds of meals, namely, Meal L and Meal H, were prepared. Meal L consisted of Rice L and the side dishes, and Meal H consisted of Rice H and the same side dishes. Approximately 12% and 92% of the Cd in Meals L and H, respectively originated in the rice.

Study outline and Sample collection

The experiment was conducted at a university lodge in a mountainous resort area with a hot spa in September 2001. The volunteers stayed in Japanese-style rooms, with 4–5 volunteers to a room, and had no living constraints except for the strict restrictions of their food and beverage intake. They were encouraged to study, to exercise appropriately, to engage in the available recreational and athletic activities, and to attend *ad hoc* lectures provided by the authors. Staff members were always available to consult with participants regarding any worries or anxieties, physical complaints, or difficulty in sample collection.

Based on a regimen designed according to the results of a preliminary volunteer experiment[11)], all volunteers were requested to eat Meal L for 11 experiment days to achieve a short-term intake-output balance of Cd stable. Fifteen of the 25 volunteers ate Meal H on the 12th day, and then returned to eating Meal L from the 13th to the 20th day (Group D1). The remaining 10 volunteers ate Meal H from the 12th to the 14th day, and ate Meal L from the 15th to the 20th day (Group D3). Before breakfast on the 12th day, all participants took a capsule containing Carmine Red (The Merck Chemical), which is a red dye used for detecting the initial evacuation of unabsorbed

Table 2. Nutrition composition* of meals (/d) during the study

Nutrients		mean	(min	max)	RDA**	RDA (%)
Weight (total)	g	2,104.8	(1,829.7	2,255.1)	–	–
(steamed rice)	g	459.6	(253.0	602.0)	–	–
Energy	kcal	1,952.0	(1,704.0	2,175.5)	*1,600*	122.0
Water	g	1,413.2	(1,210.5	1,541.3)	–	–
Protein	g	72.5	(64.6	78.0)	*60*	120.8
Lipid	g	44.9	(36.2	53.6)	*40*	112.3
Carbonhydrate	g	308.2	(259.7	345.4)	*240*	128.4
Ash	mg	16.2	(14.8	17.4)	–	–
Sodium	mg	3,184.6	(3,065.5	3,268.7)	–	–
Potassium	mg	2,662.5	(2,376.4	2,913.6)	*2,000*	133.1
Calcium	mg	681.7	(471.6	856.2)	*600*	113.6
Magnesium	mg	224.9	(198.8	243.7)	*250*	90.0
Phosphorus	mg	1,159.4	(983.6	1,314.5)	*700*	165.6
Retinol	μg	148.4	(100.2	203.1)	–	–
Carotene	μg	7,405.7	(7,153.0	7,462.2)	–	–
Retinol equivalents	μg	1,384.7	(1,298.4	1,448.5)	*540*	256.4
Vitamin D	μg	8.6	(8.2	9.0)	*2.5*	342.8
Vitamin E	mg	7.6	(6.6	8.2)	*8*	95.5
Vitamin K	μg	374.5	(347.0	382.3)	*55*	680.9
Thiamin	mg	0.9	(0.8	1.0)	*0.7*	132.1
Riboflavin	mg	1.2	(0.9	1.5)	*0.8*	150.5
Niacin	mg	15.2	(14.5	16.0)	*10*	152.3
Vitamin B_6	mg	1.4	(1.4	1.5)	*1.4*	103.3
Vitamin B_{12}	mg	3.9	(3.3	4.3)	*2.4*	161.9
Folate	μg	417.6	(381.5	430.5)	*200*	208.8
Pantothenic acid	mg	7.4	(6.5	8.3)	*5.0*	148.1
Ascorbic acid	mg	171.8	(157.7	177.9)	*100*	171.8
Saturated fatty acids	g	17.2	(11.7	22.6)	–	–
Monounsaturated fatty acids	g	15.5	(13.4	17.6)	–	–
Polyunsaturated fatty acids	g	7.6	(7.2	7.9)	–	–
Cholesterol	mg	295.9	(272.6	326.2)	–	–
Water soluble dietary fibers	g	3.0	(2.9	3.1)	–	–
Water insoluble dietary fibers	g	12.1	(11.4	12.3)	–	–
Total dietary fibers	g	15.1	(14.3	15.3)	*16*	94.3
NaCl	g	8.1	(7.7	8.3)	*10*	80.5
Iron	mg	6.1	(5.7	6.3)	*12*	50.5
Zinc	mg	8.8	(7.7	9.5)	*9*	98.1
Copper	mg	1.2	(1.0	1.2)	*1.6*	72.3
Mn	mg	3.7	(2.9	4.0)	*3.0*	122.1
Cd (Meal L)	μg	4.51	(1.85	6.93)	–	–
Cd (Meal H)	μg	48.48	(27.98	56.27)	–	–

* :Nutrition composition of meals during experimental day were calculated by means of the Standard Tables of Food Composition in Japan, 5th Edition., **: RDA: Recommended Dietary Allowance in Japan (6th version)

foods taken on the 12th day. Subjects were permitted to consume any combination of water and/or beverages such as oolong tea, green tea, black tea, coffee, *Mugi-cha* (roasted barley tea), or fruit juice and the median total intake of beverages was 1,250 m*l*/d (range: 130–3250).

At each meal, the subjects recorded all food consumed, and these records were checked by dieticians the following morning. All duplicate meals were collected, sealed and stored at –20°C until Cd analysis for determining daily Cd intake (Cd-I).

During the study periods, all urine and feces excreted from 0 to 24 o'clock were collected in paper cartons or in biodegradable plastic bags and stored at 4°C until pretreatment. The times of excretion, the appearance of the feces, and the potential for contamination with menstrual blood were all recorded by the subjects.

Fasting blood samples were collected before breakfast on the day prior to the study and again on the 12th and 20th day by means of metal-free heparinized syringes and glass tubes, and were subjected to analysis for Cd in blood (Cd-B), as well as hematological and serum biochemical examinations. One m*l* of fasting blood was also collected from 5 to 6 systematically selected participants every 2 d until the 12th day in order to determine the trends in Cd-B. From the 13th to the 19th day, blood of 8 fixed participants, 4 from Group D1 and 4 from Group D3, was collected to trace the changes in Cd-B after Meal H intake.

Every morning before breakfast, body weight and blood pressure were measured, and body fat rate was measured with an impedance meter (Tanita cc, Tokyo). All participants wore a device to record their steps and the total number of walked steps during the day was recorded at night. For assessing mental stress during the experiment, a Profiles of Mood States (POMS)[12, 13] questionnaire was self-administered before the study, and on the 12th and 20th day of the experiments.

The methods used to measure Cd-I, Cd-F, Cd in urine (Cd-U) and Cd-B were described in detail elsewhere[11]. The quality of the Cd analysis was certified by the 28th Intercomparison Programme for Occupational-Medical and Environmental-Medical Toxicological Analyses in Biological Materials performed by the German Society for Occupational and Environmental Medicine.

Definition of Cd uptake and Cd intake-output balance

Cd in feces (Cd-F) is generally taken as the sum of the unabsorbed portion of Cd intake from the daily meals (Cd$_{unab}$) and the unabsorbed portion of Cd excreted into the digestive tract (Cd$_{excret}$). Cd$_{excret}$ includes Cd from either of two sources, namely, Cd in the bile,[14] which originates both in Cd currently absorbed and Cd moved from the organs storing Cd, such as the liver and kidneys, and Cd from peeled epithelial cells and other intrinsic sources (Fig. 1). The true amount of Cd absorption from meals was (Cd-I–Cd$_{unab}$), and the true Cd absorption rate (TAR) was (1–Cd$_{unab}$/Cd-I), but ethical consideration prevented our measuring either Cd$_{unab}$ or Cd$_{excret}$ in this study.

We therefore defined the Cd uptake as

$$\text{Cd}_{uptake}\ \text{rate}=1-\text{Cd-F}_{excess}/\text{Cd-I}_{excess}$$

where Cd-I$_{excess}$ is the sum of (daily Cd in Meal H–mean Cd of Meal L) during the day(s) when eating Meal H; Cd-F$_{excess}$ is the sum of (daily Cd-F–Cd-F$_{base}$) from the first day detecting when Carmine Red was stain detected in feces to the 20th d; and Cd-F$_{base}$ is the mean of Cd-F from the 6th to the 12th day (Fig. 1).

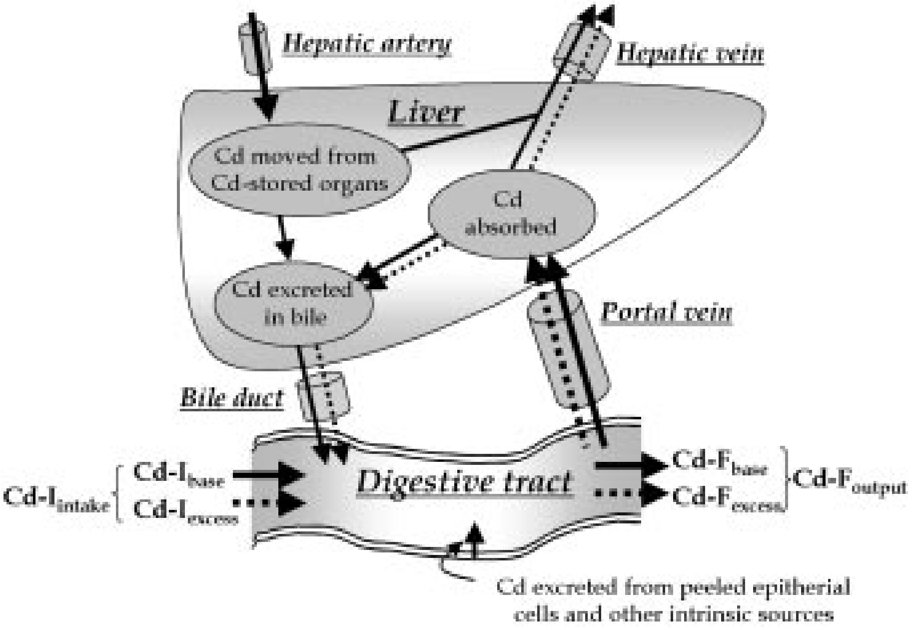

Fig. 1. Cd transportation in the digestive organs. Solid arrow: basic transportaion. Broken arrow: addtional transportation when excessive Cd in ingested.

Table 3. Cd-I and Cd in biological specimens during the experiment

Day	Cd-I (g/d)		Cd-F (μ g/d)		Cd-U (ng/d)		Cd-B (ng/dl)		
	All (n=25)		All (n=15–18)*		All (n=25)		All (n=25)		
B$^{\#}$							94 ± 27		
1	4.77 ± 0.27		13.61 ± 7.95		338 ±178		96 ± 21 **		
2	2.66 ± 0.24		23.10 ± 20.93		300 ±163		–		
3	6.07 ± 0.27		10.82 ± 12.37		212 ±114		74 ± 13 **		
4	5.03 ± 0.28		9.10 ± 6.03		189 ± 78		–		
5	3.95 ± 0.27		5.32 ± 3.02		127 ± 71		77 ± 30 **		
6	4.76 ± 0.22		4.88 ± 2.31		267 ± 88		–		
7	2.70 ± 0.25		5.53 ± 3.34		271 ±119		77 ± 20 **		
8	6.07 ± 0.27		5.97 ± 4.53		175 ± 81		–		
9	4.97 ± 0.25		6.35 ± 2.97		201 ± 87		73 ± 18 **		
10	3.96 ± 0.27		5.69 ± 2.16		183 ± 78		–		
11	4.69 ± 0.25		4.97 ± 3.07		180 ± 74		67 ± 22 **		
	Group D1 (n=15)	Group D3 (n=10)	Group D1 (n=9–12)*	Group D3 (n=5–6)*	Group D1 (n=15)	Group D3 (n=10)		Group D1 (n=4)	Group D3 (n=4)
12	46.53± 7.21	49.47 ± 3.41	6.62 ± 2.92	4.81 ± 2.48	147 ± 56	166 ± 59	63 ±16	58 ± 16	60 ± 7
13	6.00± 0.29	52.24 ± 0.68	8.14 ± 4.26	8.04 ± 5.26	234 ±119	246 ± 51	–	68 ± 15	68 ±15
14	4.96± 0.34	51.60 ± 0.48	14.81 ±11.74	20.74 ±15.37	216 ± 98	247 ± 76	–	75 ± 30	70 ±19
15	3.82± 0.35	4.03 ± 0.20	6.86 ± 3.09	22.86 ±20.23	173 ±105	188 ± 55	–	80 ± 21	83 ±29
16	4.67± 0.34	4.87 ± 0.16	6.42 ± 4.36	34.05 ±17.82	202 ± 60	219 ± 72	–	76 ± 19	77 ±29
17	2.69± 0.36	2.80 ± 0.17	6.41 ± 4.02	10.81 ± 7.60	253 ±126	239 ±135	–	66 ± 20	76 ±19
18	6.11± 0.31	6.14 ± 0.14	7.09 ± 4.38	23.89 ±21.79	233 ± 88	229 ± 54	–	63 ± 17	75 ±15
19	4.95± 0.44	5.22 ± 0.29	8.48 ± 3.81	10.44 ± 6.03	188 ± 75	246 ±120	–	59 ± 17	68 ±15
20	3.36± 0.88	3.66 ± 0.84	6.47 ± 2.98	8.39 ± 6.48	260 ±131	239 ±101	64 ±22	57 ± 17	63 ±18
A$^{\#}$							103 ±37***		

$^{\#}$: Before (B) and 9 months after (A) the study., *: Number was not steady because of constipation **: Based on data for selected 5 or 6 participants., ***: n=24.

We also defined the Cd intake-output balance rate as follows:

$$\mathrm{Cd}_{\mathrm{balance}}\ \mathrm{rate}=1-\mathrm{Cd}_{\mathrm{output}}/\mathrm{Cd}_{\mathrm{intake}}$$

where $\mathrm{Cd}_{\mathrm{output}}$ is the sum of Cd-F from the first day in which Carmine Red staining was detected in feces to the 20th d; and $\mathrm{Cd}_{\mathrm{intake}}$ is the sum of Cd-I from the 12th to the 19th d (Fig. 1).

Because the $\mathrm{Cd}_{\mathrm{uptake}}$ rate does not include the basic excretion of Cd into the digestive tract from Cd stored organs and other intrinsic sources (Fig. 1), it may be a better estimate of TAR than the $\mathrm{Cd}_{\mathrm{balance}}$ rate.

Statistical analysis

All data are summarized by using Excel and SPSS data bases. The mean, median, minimum and maximum values were calculated for each variable, and all other statistical analyses were carried out with the software package SPSS 10.0 J for Windows.

Results

Mental and physical condition, meal intake and evacuation

None of the participants withdraw from the study. Whereas the mean POMS fatigue and depression scores were unchanged throughout the study, the vigor score increased and the tension-anxiety score decreased compared to those before starting the study (data not shown). Medical checkups on the 12th and the 20th day did not reveal any health problems among the volunteers (data not shown). There were no significant changes in blood pressure, body weight, body fat rate, or menstrual cycle (i.e., irregularities).

Many participants enjoyed walking in the surrounding fields and forests for more than 1 h each d, and the median number of steps per day was 15192 (range: 1002–41150). These results indicated that the mental and physical condition of the volunteers was steady and good enough to continue the 20-d study.

The median wet and dry weight of feces and the urinary

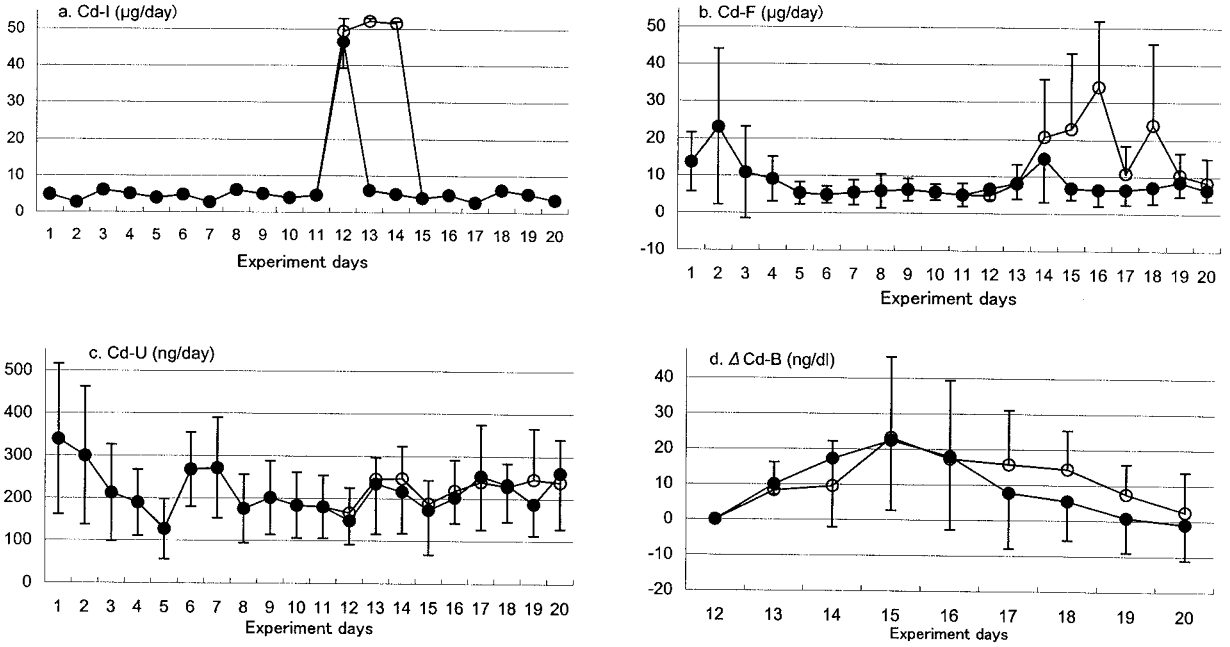

Fig. 2. 2a-2d. Cd-I, Cd-F, Cd-U and difference in Cd-B after eating Meal H. ● : Group D1. ○ : Group D3. Bar: SD

volume per day were 95 g (range: 4–425 g), 21 g (1–112 g) and 1,530 m*l* (342–3,269m*l*), respectively. Three of 15 volunteers in Group D1 and 4 of 10 volunteers in Group D3 were unable to evacuate their bowels for 5 d or longer, and their data were excluded from the statistical analysis for estimating Cd-F, $\mathrm{Cd}_{\mathrm{uptak}}$e and $\mathrm{Cd}_{\mathrm{balance}}$.

Change in Cd in biological specimens

Cd-I and changes in Cd in biological specimens are listed in Table 3 and shown in Fig. 2a–d.

During intake of Meal L, Cd-B decreased from 94 ± 27 ng/d*l* (mean ± SD) before starting the study to 63 ± 16 in the morning of the 12th day, about a 32% reduction (range 17–48). Fig. 2d illustrates the differences between Cd-B on the 12th day and that on the 13th to the 20th day after eating Meal H in 4 volunteers in Group D1 and 4 volunteers in Group D3. The decline in Cd-B in Group D3 was delayed compared to that of Group D1.

Cd-F gradually decreased from ca. 20 μg/d to ca. 5 μg/d on the 6th day during Meal L intake and seemed to be stable until the 12th day (Fig. 2b). In Group D1, after 15 volunteers ate Meal H on the 12th day, Cd-F sharply increased on the 14th day and decreased thereafter. In Group D3, after 10 volunteers ate Meal H on the 12th, 13th and 14th day, Cd-F clearly increased on the 14th day, reached a peak on the 16th day, and decreased thereafter.

Cd-U also decreased gradually until the 12th day, but its fluctuation was large (Fig. 2c). Change in Cd-U after eating Meal H was not clear in both Groups D1 and D3 compared to that in Cd-F and Cd-B.

Cd uptake and Cd balance

During 11-d intake of Meal L, intake-output balance of Cd seemed to become stable (Fig. 2a, 2b). The mean $\mathrm{Cd}_{\mathrm{uptake}}$ rate (%) was calculated to be 47.2% (range –9.4–83.3) in Group D1 and 36.6% (–9.2–73.5) in Group D3. The mean $\mathrm{Cd}_{\mathrm{balance}}$ rate (%) was 23.9% (range –4.0–37.7) in Group D1 and 23.7% (–8.2–56.9) in Group D3, respectively.

$\mathrm{Cd}_{\mathrm{uptake}}$ and serum iron or ferritin measured on the 12th day did not show a clear relationship (data not shown).

Discussion

In planning this study, we were concerned that stressors related to the study and to communal living in a lodge for 20 d might change the mental and physical status of the participants, and thereby alter the conditions of their appetite, digestion, and evacuation. Nevertheless, based on the results of items monitored for their mental and physical status, we believe that such stressors had no or only a minimal effect on the results of this experiment.

The changes in Cd in biological specimens before eating Meal H were similar to those observed in the previous volunteer experiment11). We thus concluded that 11 d was a sufficient period of Meal L intake to achieve a nearly stable Cd intake-output balance. After eating Meal H, the Cd-B and Cd-F values promptly reflected the change in Cd-I, but the Cd-U values did not. This

Table 4. Previous studies concerning Cd absorption from the digestive tracts

Ref. No.	Year	TS*	Subjects sex	n	age	Source of Cd	Cd-I	Absorption-related rate (%)**	Notes
22	1976	B	M	2	35, 37	Cd in natural foods for 30 d	48.18, 46.92 μg/d	25.44, 23.38	Cd-I by duplicate meal method and Cd-F were measured for 30 d. The percentages of Cd not recovered in feces were 25.44% and 23.38%.
16	1978	R	M	10	24 ± 1.1	$^{115m}CdCl_2$ once at breakfast	5μCi^{115m}Cd	2.6 ± 0.6	^{51}Cr was used as a marker of complete discharge of the ingested meal from the digestive tract. Radioactivity of ^{115m}Cd in the body was measured 1 week after ^{51}Cr disappeared from the feces.
			F	12	29 ± 3.2		25μg (22–29)	7.5 ± 1.8	
17	1978	R	M, F	14	21–61			4.6 ± 4.0	
18	1984	R	M	7	48 ± 11.7 (29-61)	^{115m}Cd containing crab meat once at lunch	24–166 μg	2.7 ± 0.9	Shrimp pellets were prepared by mixing shrimp meat with labeled $^{115m}CdCl_2$ and crabs were fed the pellets. Volunteers ate the crab meat and brown crab meat, and radioactivity of ^{115m}Cd in the volunteer's body was measured 26 d after the ingestion.
19	1984	B	M, F	23	70—85	Cd in natural foods for 5 d	8.6 μg/d	–15 (–188–32)	Cd-I by the duplicate meal method and Cd-F were measured for 5 d. Net absorption (%) was –15.
20 and 21	1994 and 1996	B	F	34	37 ± 7.4	Cd in natural foods for 4 d	11.1 ± 4.2 μg/d	2	Cd-I by the duplicate meal method and Cd-F were measured for 4 d. Average Cd-F was 98, 100 and 101% of Cd-I in 3 groups.
				23	36 ± 8.4		16.0 ± 7.1 μg/d	0	
				17	37 ± 7.9		27.8 ± 17.6 μg/d	–1	
2	2000	U	F	3	32, 46,51	^{106}Cd containing porridge once at breakfast	18.81, 17.84, 16.87 μg	42, 40, 45	Wheat was cultivated by the hydroponic cultivation technique so as to contain the stable isotope of ^{106}Cd, and porridge was made from the wheat. The total recovery of Cd-F over 5 d was 58, 60 and 55%.
24	2001	U	F	14	52 ± 13 (30–70)	^{113}Cd in sunflower butter once at breakfast	14.4 ± 5.8 μg	10.6 ± 4.4 (1.6–18.3)	The stable isotope of $^{113}CdCl_2$ was injected into the stem-head junction of sunflowers, and sunflower butter was made from the ^{113}Cd-labeled sunflower kernels. Cd-F was measured for the following 21 d, and the total amount of stable isotope in feces was calculated.

*: TS; Type of study. B; Intake-output balance study. R; Rate of remaining radioactivity of ^{115m}Cd in the body. U: Cd uptake was the amount of labeled Cd-F subtracted from the amount of labeled Cd-I. ** :Definition of absorptions-related rates were different among studies.

indicates that either Cd-B or Cd-F is a more appropriate indicator than Cd-U for monitoring the current Cd intake from meals in the general population, which is in agreement with the findings in our previous report(11).

Table 4 lists the past results of dietary Cd absorption-related papers. In the present study, the mean Cd_{uptake} rates were 47.2% in Group D1 and 36.6% in Group D3, and the mean $Cd_{balance}$ rates were 23.9% in Group D1 and 23.7% in Group D3. These rates seem fairly high in comparison with the absorption-related rates in Table 4.

This discrepancy may be mainly due to differences in the definition of the absorption-related rate among studies. Flanagan *et al.*(16), McLellan *et al.*(17), and Newton *et al.*(18) actually measured the remaining radioactivity of ^{115m}Cd in the body by mean of a whole-body scintillation counter after about 2 to 4 wk of $^{115m}CdCl_2$ ingestion (Table 4). These 3 studies clearly underestimate the TAR, because the remaining radioactive ^{115m}Cd in the body does not include the ^{115m}Cd excreted from the bile and urine prior to the day of the whole-body scanning.

Several investigators have measured both daily Cd-I by the duplicate meal method and Cd-F, and used these parameters to calculate the intake-output balance (Table 4). Bunker *et al.*[19] concluded that the mean of net Cd absorption was –15% (range: –188–32%) in elderly individuals when 8.6 μg/d of Cd (range: 2.1–22.5) was ingested for 5 d. Berglund *et al.*[20] and Vahter *et al.*[21] observed that the mean Cd intake-output balance reached near-equilibrium when 5.7–38 μg/d of Cd was consumed for 4 d. In our volunteer study, the Cd intake-output balance reached near-equilibrium after taking Meal L (4.51 ± 1.14 μg Cd/d) for ca. 1 wk. Suzuki and Lu[22] reported that approximately 25.4% and 23.4% of total Cd was not recovered in feces when the average Cd-I for 30 d was 41.18 and 46.92 μg/d, which was similar to the $Cd_{balance}$ rates of 23.9% or 23.7% in Groups D1 or D3 when 48.48 ± 6.81 μg Cd/d was taken for 1 or 3 d in the present study, respectively. These findings suggest that the absorption-related rate in an intake-output balance study is strongly dependent on the amount of Cd-I, and that a substantial but limited amount of Cd may be excreted into the digestive tract. Therefore, the Cd absorption-related rate calculated from the intake-output balance study is theoretically different from TAR.

The Cd absorption-related rates of Crews *et al.*[23] and Vanderpool and Reeves[24], and the Cd_{uptake} rate in this study may be comparable. Crews *et al.* and Vanderpool and Reeves avoided the effects of Cd moved from the Cd stored in the organs on the Cd absorption rate by using a stable isotope (Table 4) and we avoided them by subtracting the basic excretion of Cd in feces from Cd-F_{output}. Nevertheless, TAR may be higher than these rates, because re-excretion of Cd into the digestive tract though the enterohepatic circulation was not taken into account. The absorption-related rates of 40, 42 and 45% reported by Crews *et al.* were similar to those of 47.2% (range: –9.4–83.3) in Group D1 and 36.6% (range: –9.2–73.5) in Group D3 in our study. Vanderpool and Reeves, however, reported the rate as 10.6 ± 4.4% (range: 1.6–18.3), about a quarter of the value in the other two studies.

One possible reason for this discrepancy may be the difference in chemical formula and properties of Cd compounds. In our study, all Cd-I_{excess} originated in the Cd in Rice H, and in the study by Crews *et al.*, the stable isotope of ^{106}Cd originated in Cd in wheat; in both cases, the Cd was naturally transported and accumulated in crops as an intrinsic chemical form. In contrast, the ^{113}Cd used by Vanderpool and Reeves was artificially injected into the sunflower stem-head junction as $^{113}CdCl_2$, and the harvested kernels were used. The chemical form in the kernels is unlikely to be the natural form of Cd in the crops. In fact, the authors themselves did not state that the Cd was intrinsic. In animal experiments, for example, the intestinal absorption of Cd bound to metallothionein (Cd-Mt) has been shown to be lower than that of $CdCl_2$ in rats[25], and Cd concentrations in the liver and kidneys of rats and mice fed Cd-Mt or shellfish[26, 27, 28] were lower than those in rats and mice given similar concentrations of $CdCl_2$[29, 30]. Although no information is available on the chemical formula of Cd in rice or wheat, the absorption efficiency might be different from that of inorganic Cd salts such as $CdCl_2$. Further experimental studies will be needed to clarify the differences in Cd absorption between natural chemical compounds of Cd in crops and Cd ions such as $CdCl_2$.

Another reason for the high uptake rate in the present study may have been the characteristics of the volunteers. Our subjects were young females who probably had small body burdens of Cd and lower serum iron and ferritin level than males. Iron deficiency is known to increase Cd absorption[16, 24], although no clear relationship was found between Cd_{uptake} and either serum iron or ferritin levels in our volunteers, all of whose serum ferritin levels were within the normal range.

The results of this study suggest that the Cd uptake rate may have been higher than that reported in previous papers, though the definition of absorption-related rates in each paper was not always the same. For estimating TAR, the origin of the Cd excreted into the digestive tract is of great interest, but no information on this parameter is currently available. Further experiments that can evaluate Cd in bile and other intrinsic sources will be essential.

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