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Dietary exposure to cadmium at close to the current provisional tolerable weekly intake does not affect renal function among female Japanese farmers ☆

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Abstract

Dietary cadmium (Cd) exposure and renal tubular function were investigated in 1381 female farmers from five districts in Japan (Japanese Multi-centered Environmental Toxicant Study project; JMETS). Dietary Cd exposure of the five populations was assessed from the individual Cd concentrations of the rice consumed by the study participants and the quantities of rice consumed daily. The populations showed a sequential difference in dietary Cd exposure, ranging from a level as low as that of the general Japanese population to one close to the current provisional tolerable weekly intake (PTWI). The levels of urinary Cd excretion, an indicator of Cd accumulation in the kidneys, increased along the same sequential pattern as dietary Cd exposure. However, no differences were observed among the populations in levels of urinary α_1-microglobulin and β_2-microglobulin excretion, which are indicators of renal tubular function. These results indicate that the current PTWI is sufficient to prevent Cd-induced renal dysfunction among the general population.

Keywords: Cadmium; Kidney; PTWI; α_1-microglobulin; β_2-microglobulin

1. Introduction

Long-term exposure to cadmium (Cd), a ubiquitous environmental contaminant, impairs renal tubular function, as evidenced by low-molecular-weight proteinuria, such as excretion of α_1-microglobulin (α1-MG) and β_2-microglobulin (β2-MG), because of the long biological half-life of Cd in humans (Friberg et al., 1986; WHO, 1992). Because industrial and agricultural activity has dispersed Cd into the environment, the effect of Cd pollution on the health of the general population is an important public health issue. One of the major routes of environmental Cd exposure is inhalation from the air, including occupational exposure, emissions from smelters, and tobacco smoking (Oberdorster, 1992). The other major route is dietary intake of Cd-contaminated food, for example, shellfish, animal livers, mushrooms, or cereals such as rice and wheat, in which environmental Cd can accumulate from polluted water and soil due to biological magnification. For nonsmokers in the general population, the dietary exposure route contributes much more to Cd accumulation in the body than does inhalation. Thus, it is important to establish the safe level of dietary Cd intake.

The current provisional tolerable weekly intake (PTWI) of Cd, the dietary exposure level that can be ingested weekly over a lifetime without appreciable

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health risk, was set at 7 μg/kg of body weight in 1989 by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA), a scientific advisory body of the Codex Committee on Food Additives and Contaminants (CCFAC), and has been maintained since then (WHO, 1989, 2000). But this PTWI was originally calculated from "the normal and critical Cd levels" in human renal cortex, 50 and 200 mg/kg wet weight respectively, obtained empirically from autopsies of Cd-exposed and nonexposed people in various countries (WHO, 1972). The JECFA documents say, "In order that levels of Cd do not exceed 50 mg/kg in renal cortex, assuming an absorption rate of 5% and a daily excretion of 0.005% of body burden, total intake should not exceed about 1 μg/kg body weight per day continuously for 50 yrs."

This calculation is based on some uncertain estimates. First, the critical Cd level in renal cortex, 200 mg/kg, was derived from studies on workers occupationally exposed to Cd via inhalation, not the general population who ingested Cd-contaminated food for a lifetime. In other words, the safety level of occupational Cd inhalation has been substituted for the intake safety level in the general population. Second, the reported values of renal Cd levels are distributed over such a wide range that it is impossible to draw a definite line between normal and abnormal Cd concentrations in renal cortex according to renal dysfunction (Nomiyama, 1977). Third, the dietary Cd absorption rate is not necessarily fixed at 5% but can differ with sex, age, iron storage status, and other factors (Flanagan et al., 1978). This less-than-ideal method of setting PTWI was probably chosen because insufficient information was available on the dose–response relationship between dietary exposure and renal dysfunction in the general population. In one example with extreme oversimplification, the values of Cd concentration in the kidneys (mg/kg) were regarded as those of intake in food (μg/day), although they should be unrelated to each other, to justify recommending that the current PTWI should be lowered (Järup et al., 1998).

In addition to the PTWI established by the JECFA, the Japanese government has been using a safety standard for Cd concentration in rice grains (Cd-R). Because rice is a staple food in Japan and is more contaminated with Cd in Japan than in other countries (Rivai et al., 1990; Watanabe et al., 1996), rice has been the major contributor to Cd body burden for the Japanese population. As a result, the amount of Cd accumulation among the Japanese is relatively high, and there have even been cases of severe chronic Cd toxicity among female farmers in particularly Cd-polluted areas, famous as Itai–itai disease, which is characterized by renal tubular dysfunction, multiple bone fractures due to osteomalacia (Yamagata and Shigematsu, 1970), and renal anemia (Horiguchi et al., 1994). In 1970, the Japanese Ministry of Health and Welfare established the critical limit of Cd-R (unpolished rice) as 1 μg/g, based on the results of studies conducted in some Cd-polluted areas of Japan that indicated that "rice containing less than 1 μg/g Cd cannot be judged as harmful for human health." However, rice with a Cd concentrations of 0.4 μg/g or more has not actually been allowed to come onto the market out of consideration for "the anxiety of consumers for their health and the balance between demand and supply of rice" (Japanese Ministry of Health and Welfare, 1970). Thus, the current Japanese safety standard for Cd-R, 0.4 μg/g, is not necessarily derived from science-based observation. Furthermore, it is higher than the draft maximum Cd level in polished rice proposed by CCFAC, 0.2 μg/g, which is derived from the current PTWI (CCFAC, 2002).

Thus, it is necessary to find the threshold of Cd-induced renal dysfunction in the dose–response relationship obtained from the actual observation of a population with various levels of continuous dietary Cd exposure in order to establish a reliable safety level. For this purpose, we investigated five populations from various areas in Japan, including four Cd-polluted areas and one control area (Japanese Multi-centered Environmental Toxicant Study project; JMETS). The populations consisted of female farmers who had consumed rice and vegetables grown in their own fields and locally produced foods ever since their birth. Although Cd values in rice are monitored before market release in Japan, Japanese farmers usually consume their own rice without monitoring, so they are assumed to have been exposed on a sustained basis to the level of Cd in that rice. These conditions enabled us to assess total dietary Cd exposure of the individual farmers from the Cd-R and the amounts of rice consumed. We then made a group comparison of the relation between the Cd exposure levels and the renal function among the populations. We discuss the validity of the current safety levels for Cd in rice and dietary intake of Cd.

2. Materials and methods

2.1. Study populations and health examinations for sample collection

Using the database on Cd-R monitored by the Japan Ministry of Agriculture, Forestry, and Fishery during 1980–1999, we selected four districts as Cd-polluted, in which rice with relatively high Cd contamination, sometimes more than 0.4 μg/g, has often been detected (districts B, C, D, and E), and one district where highly Cd-contaminated rice has never been detected (district A) as a control. The five districts are scattered in the Japanese archipelago, including the Kyushu, Kinki, Kanto, and Tohoku areas. They consist of rural

agricultural communities with inhabitants immobile even after marriage. During the winters of 2000 and 2001, female farmers in each district were asked through the local Agricultural Cooperative to participate in the group health examinations we organized for the study on the adverse health effects caused by dietary Cd exposure. One week prior to the examinations, we held group orientations for the study participants, where we explained the study purposes and protocol and obtained written informed consent from each participant. At the same time, we instructed them on how to fill out two kinds of questionnaires and asked to bring them to the examinations. One questionnaire was on general information about each participant's health status, including birth date, lifestyle, and present and past medical history. The other was a self-administered diet history questionnaire (DHQ), which has been described and well validated (Sasaki et al., 1998, 2000). Briefly, the questions were designed to determine food and nutrient intake levels in the previous month with regard to the quantity and semiquantitative frequency of consumption of 110 food items commonly consumed in Japan. By this DHQ, we determined each participant's usual intake levels of rice and miso (traditional Japanese bean paste made from rice and soybeans), which are the most common foods that Japanese people eat almost every day. In addition to the questionnaires, we also asked the participants to bring small amounts of polished rice and miso that they had made themselves for determination of Cd content of the foods. At the health examinations, we measured the participants' weight and height, took peripheral blood and urine samples, and collected rice and miso samples as well as the questionnaires, which were double-checked by nurses and nutritionists. There were 202 participants in district A, 202 in B, 204 in C, 204 in D, and 569 in E—1381 in total. In district C, one participant only provided rice and miso, so the number of subjects used for exposure assessment of Cd was 203. In district E, in response to our request, 330 subjects out of 569 submitted two samples of rice, one newly harvested in 2001 and one stocked from 2000, which we used to examine year-to-year variation in Cd-R. For these subjects, we used the average value of the 2 yrs as each individual's Cd-R. Therefore, we used data from 1381 subjects for the comparison of Cd-R in the five districts and data from 1380 subjects for dietary Cd exposure assessment. For the analysis of the relation between internal Cd accumulation and renal dysfunction, however, we selected 1310 subjects comprising 187 in district A, 194 in B, 194 in C, 197 in D, and 538 in E. We excluded 70 subjects from the original 1381 for the following reasons: past or current smoking, 52 (3 and 4 with more than 20 cigarettes, smoked per day in the past and currently, respectively); chronic renal failure treated with hemodialysis, 1; history of collagen diseases such as rheumatoid arthritis, systemic lupus erythematosus, or sarcoidosis, 13; extremely high serum β2-MG concentrations leading to suspicion of an inflammatory disease, 1; insufficient urine sample volume, 3; data for rice and miso Cd concentration only, 1. In consideration of the importance of risk assessment among subgroups vulnerable to Cd toxicity, we included diabetic individuals, defined as those with fasting blood glucose concentrations higher than 140 mg/dL (2.8% of total participants), and anemics, defined as those with hemoglobin concentration lower than 11.5 g/dL (6.8% of total participants), as well as individuals with hypertension, malignancies, or past urinary abnormalities, which may impair renal function in advanced stages of the illnesses. There were no significant differences in the prevalence of the subjects with these diseases between five districts. The rate of the non-child-bearing subjects did not show any significant difference either (3.3% of total participants), which would affect Cd absorption through gastrointestinal tract.

2.2. Measurements of α1-MG, β2-MG, and creatinine (Cr) in urine

Second morning urine samples were collected from the participants at the site of the health examinations. Since β2-MG in urine can be very unstable at low pH (Donaldson et al., 1989), one drop of sodium hydrogen carbonate solution was added to each urine sample just after collection in a plastic tube. These samples were kept at −80°C until they were assayed. The concentrations of α1-MG and β2-MG in urine were determined by a latex agglutination method (Eiken Chemical). Cr in urine was measured by the Jaffé reaction method (DIA-IATRON) and used for the normalization of these two proteins.

2.3. Determinations of blood Cd (Cd-B), urinary Cd (Cd-U), Cd-R, and miso Cd

Peripheral blood samples taken from a participant's arm and urine samples with one drop of nitric acid added to stabilize the Cd content were stored at −80°C, and rice and miso samples were kept at 4°C until analysis. Cd determinations in all samples were conducted by Metocean Environment Inc. (Shizuoka, Japan). The samples of blood and rice were decomposed with nitric acid by a microwave device, MDS-2000 (CEM), and miso samples were decomposed with nitric acid and hydrogen peroxide on hot plates, after which the Cd concentrations were measured using HP 4500 series ICP-MS (Yokokawa Analytical Systems). Urine samples were mixed with nitric acid and held for 24 h, and the Cd concentrations were measured by flameless atomic absorption spectrometry, SIMAA 6000 (Perkin–Elmer). Indium (In) and thallium (TI) were added to all samples as internal standards prior to treatment. The

standard solutions for Cd, In, and TI were purchased from Wako Pure Chemicals. The influence of the sample matrix on Cd measurement was checked using standard substances, including CRM195 (Institute for Reference Materials and Measurements) and AMI B1701 (National Institute of Occupational Health of Denmark) for peripheral blood, 69071 Level 1 (human urine) (Bio Rad) for urine and NIES No. 10 (the National Institute of Environmental Science in Japan) for rice. Results for all of these standard substances were within reference ranges. All items that would be in contact with the samples, including plastic bottles, tubes, and syringes, had no detectable Cd contamination.

2.4. Statistical analysis

We used two cutoff values of Cd-R, 0.2 and 0.4 μg/g, for the polished rice that the study participants submitted; these are the current proposed maximum levels set by the CCFAC and the Japanese government, respectively, although the latter would actually correspond to approximately 0.44 μg/g in unpolished rice in consideration of the loss of up to 10% of Cd that occurs during polishing (Masironi et al., 1977). The urinary concentrations of Cd and proteins were normalized by Cr. Values of not detected (ND), less than the minimal measurable limit, were replaced with half the value of the limit for the statistical calculation. The average and variance values of the data, which were considered to follow a normal distribution from their normal probability plots, were presented by arithmetic mean (AM) and arithmetic standard deviation (ASD). Geometric means (GM) and geometric standard deviations (GSD) were used for data with lognormal distributions, such as Cd-R, Cd-B, Cd-U/Cr, α1-MG/Cr, β2-MG/Cr, and estimates of Cd exposures from foods, which were also converted into base-10 logarithms before the following statistical analyses. The significance of differences among the five populations was examined by one-way ANOVA, followed by comparison between district A as a control and each Cd-contaminated area using Bonferroni's multiple comparison procedure. The difference in the distributions of Cd-R and the participants with renal dysfunction among the five populations was analyzed using the χ^2 test, followed by Bonferroni's multiple comparison procedure. Multiple regression models were used to examine the relationships between α1-MG/Cr and β2-MG/Cr and age, Cd-B and Cd-U/Cr.

3. Results

3.1. Regional differences in Cd pollution in rice

First, we evaluated the reliability of Cd-R as an indicator of individual Cd exposure level using the 2-yr data for Cd-R in district E. The scatter diagram of individual data showed a wide range of variation between years with a low correlation coefficient ($R = 0.309$); for example, Cd-R values in the 1-yr stocked and newly harvested rice provided by one participant were 1.36 and 0.22 μg/g, respectively (Fig. 1), This result suggests that Cd-R shows such a major yearly change even when harvested from the same paddy fields that a spot Cd-R from a particular year cannot be considered representative of the individual's Cd exposure level for a lifetime. This is why we performed group rather than individual analyses to assess exposure to Cd through rice and its effects on health among the female farmers who had been eating their own crops in the same location ever since their birth.

Then we looked into the proportional differences in Cd pollution of rice among the five districts using the two cutoffs of Cd-R, 0.2 and 0.4 μg/g (Fig. 2). As expected, no rice samples exceeded 0.2 μg/g of Cd-R in district A, and the percentage of Cd-R at 0.2 μg/g and higher gradually increased in the order of B, C, D, and E. In district E, 7.4% of rice samples exceeded 0.4 μg/g of Cd-R, including two with more than 0.9 μg/g, corresponding to 1.0 μg/g in unpolished rice, indicating that this area was the most Cd-polluted. On the other hand, the GMs of Cd-R in the five districts showed a slightly different sequence, A, C, B, D, and E (0.022–0.156 μg/g) (Table 1). These results indicate that the gradient of Cd pollution in the five districts was A<B=C<D<E, which enabled us to make a sequential group comparison among the districts, using district A as a control.

3.2. Assessment of total dietary Cd exposure

Next we assessed the regional difference in dietary Cd exposure of the participants as well as the proportion of Cd-polluted rice in these districts (Table 1). We first calculated the daily Cd intake from rice and miso by multiplication of each individual participant's

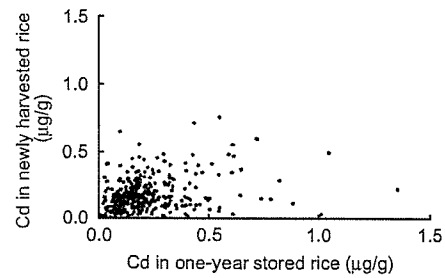

Fig. 1. The correlation between cadmium (Cd) concentration in rice samples newly harvested in 2001 and those harvested in 2000 and stored by each participant, collected from the study participants in district E. Total subject number is 330.

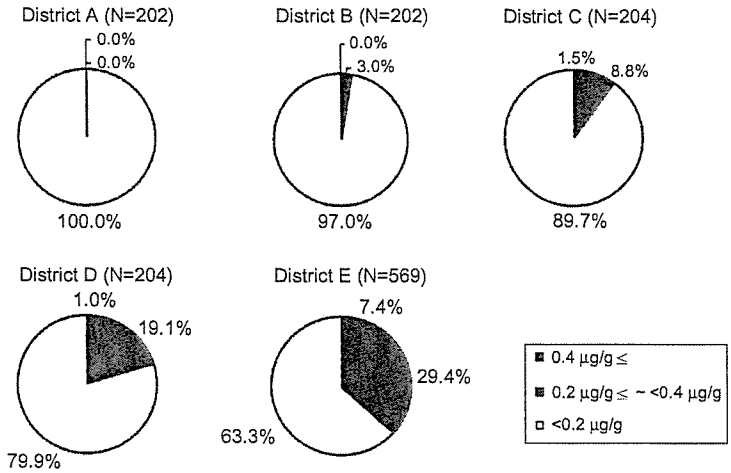

Fig. 2. Distribution of cadmium contamination in rice (cutoffs were 0.2 and 0.4 μg/g) collected from study participants in the five districts.

Table 1
Exposure assessment of Cd by individual food comsumption data and Cd contamination in their rice and "miso," and two kinds of estimates of Cd intake calculated based on the data of daily Cd intake from rice and the average Japanese values in the percentage contribution from rice of total dietary Cd intake (50%) and the amount of Cd intake from the food other than rice (15.0 μg/day) cited from the total diet survey in Japan conducted by National Institute of Health Sciences in 2001

Parameter	Unit	District					
		A	B	C	D	E	All
Number of study participants		202	202	203	204	569	1380
Body weight (AM±ASD)	kg	57.8±9.1	54.8±8.1**	55.0±7.9**	54.3±7.5**	54.8±8.5**	55.2±8.3
Cd concentration in rice (GM (GSD))	μg/g	0.022 (2.29)	0.061 (2.07)**	0.053 (2.66)**	0.114 (1.96)**	0.156 (2.01)**	0.083 (2.81)
Daily rice intake (AM±ASD)	g/day	166.1±50.2	168.8±61.0	173.1±40.1	179.0±56.1*	179.1±61.2**	174.8±56.4
Daily Cd intake from rice (GM (GSD))[a]	μg/day	3.50 (2.56)	9.57 (2.30)**	8.83 (2.88)**	19.40 (2.18)**	26.00 (2.25)**	13.68 (3.07)
Cd concentration in "miso" (GM (GSD))	μg/g	0.019 (2.08)	0.025 (2.28)**	0.030 (1.94)**	0.039 (1.86)**	0.053 (2.16)**	0.036 (2.30)
Daily "miso" intake (GM(GSD))	g/day	8.6 (2.3)	6.8 (1.9)**	13.8 (1.9)**	7.9 (2.1)	11.6 (2.4)**	10.0 (2.3)
Daily Cd intake from "miso" (GM (GSD))	μg/day	0.16 (3.05)	0.17 (2.96)	0.41 (2.61)**	0.31 (2.81)**	0.62 (3.27)**	0.36 (3.44)
Cd intake from "miso"/Cd intake from rice (GM (GSD))		0.05 (3.51)	0.02 (3.46)**	0.05 (3.54)	0.02 (2.87)**	0.02 (3.63)**	0.03 (3.68)
Assumption A (50% of percentage contribution from rice is constant)							
Total daily Cd intake (GM (GSD))[b]	μg/day	6.99 (2.56)	19.14 (2.30)**	17.65 (2.88)**	38.81 (2.18)**	51.99 (2.25)**	27.36 (3.07)
Weekly Cd intake (GM (GSD))[c]	μg/kg/week	0.86 (2.60)	2.47 (2.33)**	2.27 (2.85)**	5.05 (2.19)**	6.72 (2.30)**	3.51 (3.14)
Assumption B (15.0 μg/day of Cd intake from the other food is constant)							
Total daily Cd intake (GM (GSD))[d]	μg/day	19.88 (1.27)	26.46 (1.37)**	26.75 (1.50)**	36.90 (1.51)**	44.07 (1.60)**	32.95 (1.65)
Weekly Cd intake (GM (GSD))[c]	μg/kg/week	2.43 (1.33)	3.41 (1.41)**	3.44 (1.50)**	4.80 (1.53)**	5.70 (1.66)**	4.23 (1.70)

Note: AM, arithmetic mean; ASD, arithmetic standard deviation; GM, geometric mean; GSD, geometric standard deviation.
*$P<0.05$.
**$P<0.01$ (compared to the value in District A).
[a] Daily Cd intake from rice or "miso" = Cd concentration in rice or "miso" × daily rice or "miso" intake.
[b] Total daily Cd intake by assumption A = Daily Cd intake from rice + 0.5.
[c] Weekly Cd intake = Total daily Cd intake × 7 + Body weight.
[d] Total daily Cd intake by assumption B = Daily Cd intake from rice + 15.0.

consumption data from the DHQ by the Cd concentrations in the foods and then calculated the total daily Cd intake based on the average Japanese Cd intake levels cited in the total diet study conducted by the National Institute of Health Sciences in 2001 (National Institute of Health Sciences of Japan, 2002). According to this, the average Japanese total Cd intake in 2001 was 29.3 μg/day, about half of which was from rice, without significant changes for the past 10 yr. Therefore, we assessed total Cd intake by two methods, (1) doubling the Cd intake from rice (assumption A) and (2) adding half the value of the average Japanese total Cd intake (assumption B), assume that all foods other than rice might be contaminated by Cd at the same percentage contribution as rice (50%) and at a constant level (15.0 μg/g), respectively. Actual total Cd intake should fall between values derived from the two assumptions, since the amounts of Cd intake from miso, the representative food other than rice, increased, but the ratio of Cd intakes from miso to those from rice decreased, almost in accordance with the regional sequence. Furthermore, we calculated the weekly Cd intakes per body weight from the total daily Cd intakes and individual body weight values under each assumption.

Using this method, we obtained the same regional sequence in dietary Cd exposure as that for Cd pollution in rice, A < B = C < D < E, in the five districts, evidenced by both the average values of weekly Cd intakes (Table 1) and the proportion of participants exposed to Cd at 7 μg/kg or more weekly (Fig. 3). The average weekly Cd intake in district E was 5.7–6.7 μg/kg, very close to the current PTWI of 7 μg/kg, meaning that we would be able to evaluate the actual effects of dietary exposure to Cd at around the PTWI in the general population. It is noteworthy that as many as 33–51% of participants in district E, and even one or two in the control district A, which was the least Cd-contaminated, had dietary Cd intake in excess of the current PTWI.

3.3. Study population and normalization by age

In the following analyses, we used the study populations, 1310 in total, from which the subjects who had specific conditions that could affect Cd accumulation or renal function had been eliminated (see Materials and Methods). We divided these populations into three subpopulations aged 40–49, 50–59, and 60–69 yr, respectively, since the averages of age in districts C and D were lower than those in A, B, and E, and this difference might affect Cd accumulation or renal function (Table 2). Subjects who were less than 40 or more than 69 yr of age were eliminated from the analyses in the subpopulations divided by district and age.

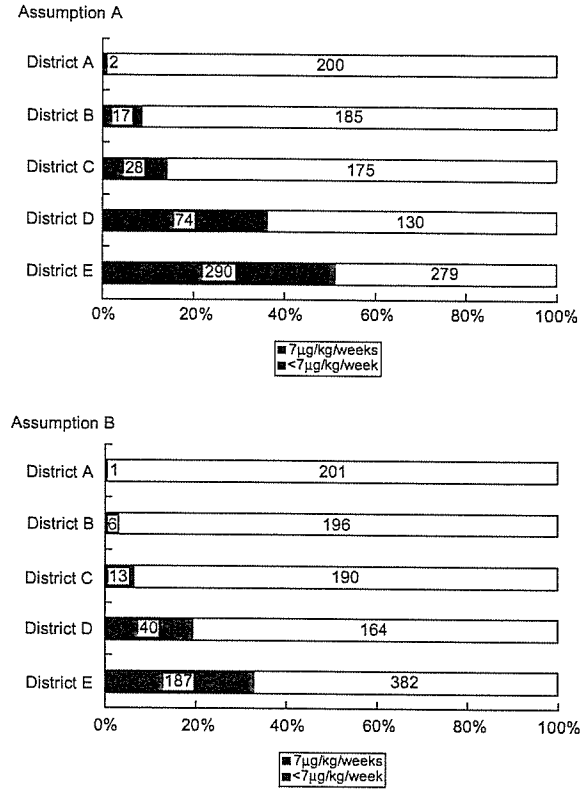

Fig. 3. Distribution of the study participants exposed to cadmium intakes above or below the PTWI (7 μg/kg/week) in five districts, estimated by two methods (assumptions A and B). The number of participants in each category are presented on the bars.

3.4. Difference of Cd body burden in the five districts

We checked the actual Cd exposure levels in the populations by Cd-B and Cd-U, which reflect recent exposure to Cd and the total amount of Cd accumulated in the kidneys, respectively (Table 3). District E, the most Cd-contaminated, showed significantly higher average values of Cd-B than district A in every age group, but there were no clear trends of area-dependent increases on the whole. On the other hand, the comparison by Cd-U revealed that the pattern of area-dependent increase was consistent with the regional sequences of Cd pollution in rice and the dietary Cd exposure in all age groups (A < B = C < D < E). In addition, clear age-dependent increases in Cd-U were observed in all districts in spite of the cross-sectional design of the study, indicating that the women we investigated had been exposed to presumably similar levels of Cd in each district. These results strongly indicate that the dietary Cd exposure levels in all districts had been assessed appropriately.

Table 2
The age distribution of study population in the five districts

Age classes	District					
	A	B	C	D	E	All
All ages						
N	187	194	194	197	538	1310
AM±ASD	57.2±9.2	58.0±9.2	55.8±9.5	51.4±7.8**	57.4±9.5	56.3±9.4
Max	75	77	76	71	78	78
Min	33	35	30	34	30	30
40–49 yr						
N	39	34	57	80	88	298
AM±ASD	45.8±2.5	45.8±2.7	45.4±2.7	45.1±2.8	45.3±3.0	45.4±2.8
50–59 yr						
N	58	75	52	78	175	438
AM±ASD	53.3±2.5	54.3±2.8*	53.3±2.8	54.1±2.8	54.6±2.9**	54.1±2.8
60–69 yr						
N	71	59	70	29	221	450
AM±ASD	65.4±2.8	65.5±2.6*	64.4±2.8	63.2±3.0*	64.5±2.8	64.5±2.8

N, number; AM, arithmetic mean; ASD, arithmetic standard deviation.
*$P<0.05$.
**$P<0.01$ (compared to the value in District A).

Table 3
Cd concentration in peripheral blood (μg/L) and urine (μg/g cr.) in the five districts

Age classes	District					
	A	B	C	D	E	All
Peripheral blood Cd						
All ages	2.00 (1.58) (range ND–6.81)	1.91 (1.73) (ND–6.23)	2.56 (1.52)** (0.82–7.70)	1.65 (2.35)** (ND–8.69)	3.61 (1.63)** (0.55–13.07)	2.56 (1.89)
40–49 yr	1.82 (1.73)	2.04 (1.68)	2.32 (1.55)	1.79 (2.34)	3.45 (1.61)**	2.32 (1.94)
50–59 yr	2.07 (1.59)	1.74 (1.80)	2.54 (1.46)	1.61 (2.25)**	3.43 (1.58)**	2.41 (1.89)
60–69 yr	2.07 (1.50)	2.00 (1.64)	2.82 (1.51)**	1.60 (2.70)*	3.93 (1.57)**	2.92 (1.81)
Urinary Cd						
All ages	2.63 (1.74) (range ND–7.93)	3.47 (1.70)** (0.70–10.82)	3.16 (1.71)** (ND–13.05)	3.16 (1.77)** (0.29–9.81)	4.08 (1.74)** (0.51–27.26)	3.46 (1.77)
40–49 yr	2.14 (1.59)	2.72 (1.78)	2.16 (1.70)	2.69 (1.82)*	3.59 (1.75)**	2.73 (1.80)
50–59 yr	2.53 (1.86)	3.20 (1.65)**	3.54 (1.53)**	3.53 (1.54)**	4.01 (1.68)**	3.50 (1.69)
60–69 yr	3.10 (1.65)	4.15 (1.61)**	3.95 (1.59)**	4.27 (1.65)**	4.50 (1.72)**	4.10 (1.69)

Data are presented by geometric mean (geometric standard deviation). The number of subjects in each subpopulation is the same as that in Table 2. ND, not detected (blood Cd: less than 0.4 μg/L, urinary Cd: less than 0.3 μg/L).
*$P<0.05$.
**$P<0.01$ (compared to the value in District A).

3.5. *Prevalence of renal dysfunction in five districts*

We next made the same regional comparison of levels of urinary α1-MG and β2-MG excretion, indicators of renal tubular function, among the five districts in order to examine the dose–response relationship between Cd exposure at levels close to the PTWI and the occurrence of renal dysfunction (Table 4). There were neither significant differences among the five districts nor area-dependent increases corresponding to Cd exposure or Cd body burden in either of the renal proteins. The proportions of subjects with renal dysfunction, diagnosed by urinary β2-MG level (300 μg/g cr. or higher), did not show any regional or sequential difference (Fig. 4). These results indicate that renal tubular dysfunction would not be induced by dietary exposure to Cd at the level of the current PTWI. It is noteworthy that there was no excess fraction of the study population with renal dysfunction in spite of dietary Cd intakes over the current PTWI. On the other hand,

Table 4
Urinary α_1-microglobulin (mg/g cr.) and β_2-microglobulin (μg/g cr.) in the five districts

Age classes	District					
	A	B	C	D	E	All
α_1-microglobulin						
All ages	4.94 (2.00) (range ND–37.33)	5.00 (1.90) (1.03–35.56)	4.32 (1.87) (1.01–16.01)	4.19 (1.94)* (ND–51.39)	4.75 (2.08) (ND–56.04)	4.66 (1.99)
40–49 yr	3.25 (1.86)	3.68 (1.85)	3.17 (1.71)	3.39 (1.81)	2.95 (1.79)	3.22 (1.80)
50–59 yr	4.88 (1.87)	4.49 (1.88)	4.10 (1.85)	4.95 (1.92)	4.64 (2.03)	4.63 (1.94)
60–69 yr	5.88 (1.95)	6.03 (1.71)	5.38 (1.82)	4.95 (1.80)	5.79 (1.94)	5.71 (1.88)
β_2-microglobulin						
All ages	148 (2.41) (range ND–9352)	147 (2.48) (ND–5911)	127 (2.00) (ND–1274)	127 (2.04) (ND–5797)	163 (2.32) (ND–5689)	147 (2.28)
40–49 yr	94 (1.96)	119 (2.00)	103 (1.75)	111 (1.86)	111 (1.88)	108 (1.87)
50–59 yr	147 (2.05)	121 (2.26)	114 (1.99)	144 (2.13)	164 (2.17)	144 (2.16)
60–69 yr	169 (2.57)	169 (2.59)	160 (1.95)	133 (1.97)	178 (2.27)	169 (2.29)

Data are presented by geometric mean (geometric standard deviation). The number of subjects in each subpopulation is the same as that in Table 2. ND, not detected (α_1-microgloblin: less than 0.9 mg/L, β_2-microglobulin: less than 70 μg/L).
*$P<0.05$ (compared to the value in District A).

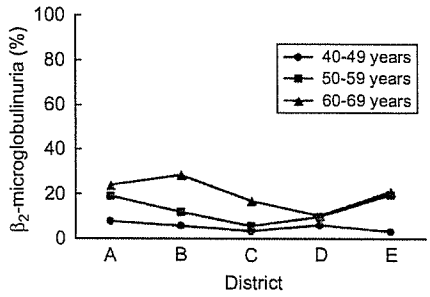

Fig. 4. Prevalence of study participants with β_2-microglobulinuria (300 μg/g cr. and more) in five districts in three age groups, 40–49, 50–59, and 60–69 yr old. The number of subjects in each subpopulation is the same as that in Table 2.

age-dependent increases were observed in both the levels of renal protein excretion and the proportion of renal dysfunction in almost all districts, suggesting that renal tubular function might be much more affected by aging than by Cd exposure at the levels observed in this study.

3.6. Relationship between urinary proteins and Cd exposure

In order to further confirm the contribution of aging to renal dysfunction, we analyzed the relationship between the levels of urinary α1-MG and β2-MG and age or Cd exposure levels in all subjects using the four kinds of multiple regression models, taking each of the urinary proteins as a dependent variable and age, Cd-B, and Cd-U as independent variables (Table 5). Cd-B, and Cd-U were not selected simultaneously in the models out of consideration for their collinearity. All of the models showed much bigger standard partial regression coefficients (SPRCs) for age than for Cd-B or Cd-U. Although the SPRCs of Cd-B, and Cd-U were statistically significant ($P<0.05$) in models B, C, and D due to the high degrees of freedom, their actual correlations with renal function were not considered to be significant since their partial correlation coefficients (PCCs) were very low. These results indicate that age is a much more important contributor to impairment of renal tubular function than Cd exposure at the levels observed in our study.

4. Discussion

We demonstrated that dietary exposure to Cd at levels close to the current PTWI did not have any evident adverse effects on renal tubular function in Japanese female farmers in five districts with varying Cd exposure levels. This result suggests that the current PTWI is sufficient to prevent Cd-induced renal dysfunction among the general population, although there has recently been a report suggesting that the current PTWI is too high (Järup et al., 1998). On the contrary, one-third to one-half of the study population in district E, and one or two even in district A, have been exposed to Cd over the PTWI without any apparent adverse effects on renal function, suggesting that the current PTWI, 7 μg/kg/week, may be lower than necessary.

We adopted urinary α1-MG and β2-MG, which have been commonly used in recent human epidemiological studies, as the indicators for Cd-induced renal dysfunction in this study. They are so sensitive in detecting the early renal dysfunction induced by Cd exposure that the threshold is often discussed with them, although adjustment of aging effect is necessary. Other than these proteins, urinary metallothionein (MT) is sometimes used to find the threshold independent of age in animal

Table 5
The effects of age, blood Cd, and urinary Cd on urinary protein levels among Japanese female farmers analyzed by the four kinds of multiple regression models (the subject number is 1310)

Model	Dependent variables	Independent variables	SPRC	*P*-value	PCC	*R'*
A	log α1 MG/Cr	Age	0.412	0.000	0.405	0.411
		log Cd-B	0.001	0.979	0.001	
B	log β2 MG/Cr	Age	0.299	0.000	0.295	0.313
		log Cd-B	0.057	0.033	0.059	
C	log α1 MG/Cr	Age	0.376	0.000	0.367	0.424
		log Cd-U/Cr	0.111	0.000	0.116	
D	log β2 MG/Cr	Age	0.272	0.000	0.263	0.328
		log Cd-U/Cr	0.119	0.000	0.119	

SPRC, standard partial regression coefficient; PCC, partial correlation coefficient; *R*, multiple correlation coefficient adjusted for the degrees of freedom; α1 MG, α_1-microglobulin; β2 MG, β_2-microglobulin; Cd-B, blood Cd level; Cd-U, urinary Cd level; Cr, creatinine.

experiments or epidemiological studies for highly Cd-polluted populations. When cellular Cd exceeds the capability of MT production, renal tubular cells are injured by the increment of intracellular non-MT-binding Cd (WHO, 1992). In the human population study exposed to low doses of Cd, however, urinary α1-MG and β2-MG are still much more useful indicators than others.

The strongest point of our current study is that we have derived this result from the actual observation of human populations with dietary exposure to presumably similar levels of Cd from birth, whereas previous reports on the safety of Cd exposure levels were based primarily on extrapolation from nondietary Cd exposure. The use of nondietary data was unavoidable since it is very difficult to know the exact dietary Cd exposure level in a human population. In particular, individual Cd-R, even cropped from the same paddy field or one close by, can differ too widely from year to year, depending on the weather and water and soil conditions in the fields, to be used as a representative indicator for individual dietary Cd exposure (Izuno et al., 2000; Masui et al., 1971; Watanabe et al., 1993). In fact, our study showed a very low correlation between the individual Cd-Rs for two successive years in district E (Fig. 1). Therefore, it is more appropriate to perform group, rather than individual, analysis in studies of dietary Cd exposure.

In addition to Cd-R, measurement of individual rice consumption is also important for the precise assessment of dietary Cd exposure. In this study, we used a self-administered DHQ method, which is designed to obtain information about dietary habits for the previous month. Direct measurement of dietary Cd exposure in meal duplicates has often been used, but this method is difficult to apply to large populations, and the data, which usually reflect only 1–2 days of intake, tend to show a wide range, just as Cd-R does. Average dietary Cd exposure from rice in our study was 27.4–33.0 μg/day (assumptions A, B), which is similar to the level in the total diet study in Japan (29.3 μg/50 kg body weight/day) and close to that in Korea (21.1 μg/day) (Moon et al., 1995), indicating accuracy in our assessment of dietary Cd exposure.

Still, it is much more difficult to estimate total lifetime dietary Cd exposure than current exposure because of scanty information on past exposure. The five populations we chose for this study, however, are assumed to have had similar Cd exposure levels in the past for several reasons. First, the database for Cd-R in the same districts from Japan's Food Agency in the 1990s revealed that the sequencing differences in incidence and degree of Cd-contaminated rice in these districts were similar to those in the current study (GMs of Cd-R are 0.000, 0.073, 0.060, 0.150, and 0.171 μg/g, and proportions of Cd-R exceeding 0.4 μg/g are 0.0%, 1.3%, 0.6%, 6.7%, and 10.1% in districts A, B, C, D, and E, respectively). Second, most Japanese farmers have continued to follow traditional Japanese dietary patterns, consuming their own crops, such as rice and vegetables, for decades. This is supported by our study result that the actual Cd amounts accumulated in the kidneys, as indicated by Cd-U, increased constantly with aging despite the cross-sectional study design (Table 3). Third, the sequential difference of Cd-U perfectly corresponded to that of the distributions of Cd-polluted rice and dietary Cd exposure levels (Tables 1 and 3, Fig. 2). The GM of Cd-U in our control district (2.63 μg/g cr.) is very close to the values in other non-Cd-contaminated areas in Japan, such as 2.3 μg/g cr. (Ikeda et al., 1995), making our study much more reliable.

In addition, none of the districts in this study currently have industrial operations such as mining or smelting; even district E has been free of industry for approximately 30 yr. The humid and wet climates of the study areas and rice fields prevent airborne exposure to

Cd in the dust and soil. This is why routes of Cd exposure other than oral ingestion can be assumed to be negligible in this study population. The soil contamination with Cd reflects geological distribution of Cd in the fields and/or mining and smelting in the distant past. These background conditions indicate that the selected five districts are very suitable locations for assessment of the effects of dietary Cd exposure at levels up to and around the PTWI.

One possible problem in this study may be the lack of information on the variation of data from year to year due to the cross-sectional study design. In fact, we detected yearly fluctuations of average values and distributions of Cd-R from the 2-yr rice samples in district E (GM values in the stocked and newly harvested rice were 0.171 and 0.136 μg/g, respectively, and the percentages of Cd-R at 0.2 μg/g and more were 42.7% and 31.2%, respectively). The actual differences are not considered to be particularly meaningful, however, since Cd concentration in rice is gradually elevated by long storage due to desiccation. It would be necessary to follow up these populations, collecting fresh rice samples continuously in the future to observe the actual yearly changes of Cd-R.

We were not able to find the real observation-based threshold of dietary Cd exposure to induce renal dysfunction because even the district E population, which was exposed to Cd at levels close to the PTWI, did not show any increase in the proportion of subjects with renal dysfunction. However, it would be possible to estimate it from the previously reported critical level of Cd-U. Since it has generally been accepted that renal tubular dysfunction can begin to occur when Cd-U exceeds about 10 μg/g cr. (Bernard et al., 1979), which is 2.5 times higher than the value of 4 μg/g cr. in district E, the threshold of dietary Cd exposure can be roughly estimated as 14.3–16.8 μg/kg/week (from assumptions B and A, respectively), multiplying 5.7–6.7 μg/kg/week in district E (Table 1) by 2.5. Furthermore, these dietary Cd exposure levels can be transformed into the values of Cd-R, 0.38–0.51 μg/g, from the linear regression lines of assumptions A and B (Fig. 5). This estimate indicates that the current safety standard of Cd-R adopted by the Japanese government, 0.4 μg/g, is reasonable as a "maximum allowable level."

In contrast with the results from our study, there have recently been reports indicating that renal tubular damage could develop after Cd exposure at much lower levels, such as 2.5 μg/g cr. (Järup et al., 1998), including two major epidemiological studies conducted in Europe, the CadmiBel study (Buchet et al., 1990; Hotz et al., 1999; Lauwerys et al., 1990) and the OSCAR study (Alfvén et al., 2002; Järup et al., 2000). The discrepancy might reflect differences in the study populations, such as differences between the lifestyles of Japanese farmers and Western populations (Leblanc et al., 2000; Ysart et al., 2000) or the routes of exposure to Cd (airborne plus diet vs diet only), but some major problems common to these studies cannot be ignored. The most important is that the Cd exposure levels in the study populations were too low for them to be considered "Cd-polluted." The GM values of Cd-U in the CadmiBel and OSCAR studies were 0.84 μg/24 h (almost the same value expressed by μg/g cr.) and 0.66 μg/g cr., respectively, much lower than that of even our control population (2.63 μg/g cr.). Second, those studies ignored the aging effect, which is an important factor affecting renal function, as we have demonstrated (Table 5). Third, they may have overinterpreted findings of statistical significance in their correlation analyses, ignoring the fact that high degrees of freedom can produce "false significance" or no clinical significance (Armitage and Berry, 1994). In our multiple regression model B using as many as 1310 subjects' data, the *P*-value of SPRC of log Cd-B was statistically significant (0.033) despite the low value of PCC (0.059), which should be judged as no correlation (Table 5).

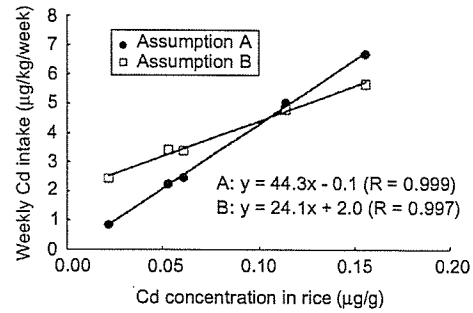

Fig. 5. Simple regression lines between cadmium (Cd) concentration in rice (*X*-axis) and weekly Cd intake (*Y*-axis) in five districts derived from assumptions A and B, along with the respective regression equations and correlation coefficients (*R*).

There have been quite a few reports from one Japanese research group, too, suggesting that the current Japanese safety standard of Cd-R, 0.4 μg/g, should be lowered to 0.1–0.2 μg/g (Nakashima et al., 1997; Osawa et al., 2001; Watanabe et al., 2002). In contrast to the studies conducted in Europe mentioned above, the populations investigated were from heavily Cd-polluted areas, including the Kakehashi River and Jinzu River basins, in Japan. One of the major problems in these studies, however, is their definition of "maximum allowable exposure level." The researchers first made several kinds of regression models from the dose–response relationship observed in those Cd-polluted populations and then calculated the "maximum allowable level" of Cd-R by substituting the response level in a non-Cd-polluted population into each regression equation. The "maximum allowable level" is, however, actually nothing more than a "general

exposure level." That is why the various "maximum allowable levels" that they have proposed, including those for total Cd intake (Nogawa et al., 1989) and Cd-U (Hayano et al., 1996; Ishizaki et al., 1989; Nogawa et al., 1979) as well as Cd-R, are unreasonably low. In addition, they also ignored the effects of aging on the development of renal tubular dysfunction or Cd accumulation in the body (Park, 1991).

In conclusion, female Japanese farmers who had been exposed to Cd at a level close to the current PTWI via foods did not show any excess development of renal tubular dysfunction compared to other groups with less Cd exposure. This result will provide useful information to help establish a reasonable PTWI.

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Comprehensive study of the effects of age, iron deficiency, diabetes mellitus, and cadmium burden on dietary cadmium absorption in cadmium-exposed female Japanese farmers

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Abstract

The absorption rate of dietary cadmium (Cd) was investigated among 38 female farmers who had been exposed to Cd at levels close to the current provisional tolerable weekly intake (PTWI); these levels were much higher than those examined in previous studies. The study group composed of 7 diabetics and their 13 age-matched controls and 6 anemic subjects and their 12 controls. With their informed consent, the study participants were confined in an inn for 7 nights and 8 days to collect all feces and urine and duplicates of all food consumed. The dietary Cd absorption rate was calculated for each subject from her total Cd intake and fecal excretion. The means and 95% confidence intervals (CI) of the diabetic group and the anemic group did not differ significantly from those of their respective controls. By individual analysis using all 38 subjects, however, significant Pearson's correlation coefficients were observed between Cd absorption rate and age, serum ferritin, serum iron, and blood and urine Cd levels. Among these, multiple regression analysis revealed that only age was a significant factor contributing to Cd absorption rate. The actual Cd absorption rate in the youngest age group (20–39 years) was 44.0%, which was highly accelerated compared with the rate in the total subject group of 6.5%, while zero to negative balance was observed in the older subjects. These results demonstrate that age, rather than iron deficiency, diabetes mellitus (DM), or Cd burden, is the only independent factor affecting the Cd absorption rate, suggesting that young women are always at high risk.

Keywords: Cadmium; Absorption; Human; Female; Farmer

Introduction

Cadmium (Cd) is one of the heavy metals commonly found in the general environment. Human beings are normally exposed to only a very small amount of environmental Cd, leading to gradual Cd accumulation in their bodies, especially in the liver and kidneys, with aging due to the long biological half-life of Cd (Friberg et al., 1986; WHO, 1992). The sentinel sign of Cd's adverse effect is renal tubular dysfunction, which is characterized by low-molecular-weight proteinuria (Friberg, 1950; Nogawa et al., 1984), and can occur in concert with anemia (Horiguchi et al., 1994, 1996) or bone mineral loss (Aoshima et al., 2003; Goyer et al., 1994) if the exposure level is high. The most severe form of Cd toxicity, known as Itai-itai disease, is characterized by severe general pains due to osteomalacia or osteoporosis ("itai" is a Japanese word for "ouch"); it is found in a heavily Cd-polluted area in Toyama prefecture, Japan (Kasuya et al., 1992).

The main routes of Cd exposure from the environment are inhalation and ingestion. Although the efficiency of Cd absorption through inhalation (25–50%) is much higher than that through ingestion (1–10%) (Friberg et al., 1986; WHO, 1992), concerns about airborne exposure are limited to special populations, including smokers, people living near smelters, and metal-processing workers. In contrast, dietary Cd intake is an important public health issue for the

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general world population despite the lower bioavailability of Cd through the gastrointestinal tract (GIT). The current provisional tolerable weekly intake (PTWI) for Cd has been set at 7 μg/kg of body weight on the assumption of a 5% absorption rate for dietary Cd and a daily excretion rate of 0.005% of body burden (WHO, 1989).

The actual absorption of Cd through the GIT, however, can be affected by several factors, such as age, sex, nutritional status, and preceding Cd burden. Among these, young age, iron deficiency, and being female are reported to accelerate the absorption of Cd through the GIT in both humans and animals (Berglund et al., 1994; Flanagan et al., 1978; Hamilton and Valberg, 1974; Kowel, 1988; Taguchi and Suzuki, 1981). Thus, there is reason for concern about higher susceptibility to Cd in the subpopulation with iron deficiency anemia, a condition common among young women, because of accelerated Cd uptake from the GIT. Diabetes mellitus (DM), also very common and increasing worldwide, is another focus of concern because we currently have no reliable information about Cd bioavailability through the GIT in humans with DM despite the potential aggravating effect of Cd on diabetic glomerulonephropathy (Bernard et al., 1991; Buchet et al., 1990).

The Japanese have higher Cd accumulation in their kidneys compared to people in other areas (Friberg et al., 1986) because their staple food is rice, which is inclined to absorb Cd from the soil (Muramoto et al., 1990). In addition, several areas in Japan are polluted with Cd due to past mining and smelting. The residents of these polluted areas, especially farmers who eat their own crops, tend to have even higher Cd burdens than the general Japanese public. The Cd-exposed population might absorb Cd through the GIT more efficiently than the nonexposed group, as suggested by a report that pre- or posttreatment with Cd prolongs the biological half-life of Cd in mice (Engstrom and Nordberg, 1979), although there are no reliable data about human beings exposed to Cd pollution. These circumstances allow us to speculate that the Japanese, especially female farmers in Cd-polluted areas with iron deficiency anemia or DM, would be at higher risk from environmental Cd because of accelerated Cd absorption through the GIT. The meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2000 called for information on the toxicokinetics of Cd in high-risk groups, such as people with iron deficiency or DM, and on the bioavailability of Cd from specific foods and factors that influence it, such as age and health status (WHO, 2000).

In this study, we investigated weekly Cd intake and excretion in 38 female farmers of a wide range of ages from one of the Cd-polluted areas of Japan, who had been exposed to Cd at levels that were near the PTWI (Horiguchi et al., in press) and much higher than those examined in previous studies, including subjects with either iron deficiency anemia or DM, then calculated the actual rates of dietary absorption for Cd among this population. We first compared Cd absorption rates between the groups with DM or iron deficiency anemia and their respective controls, then further conducted comprehensive individual analyses of the effects of age, iron storage level, blood glucose level, Cd burden, and other factors on dietary Cd absorption to detect the most important contributors to Cd absorption.

Methods

Study participants. This research protocol was approved in advance by the Committee on Medical Ethics of Jichi Medical School. In the winter of 2002, we conducted health checkups on approximately 600 female farmers in a rural area of Japan with Cd exposure near the PTWI; the geometric mean of creatinine-adjusted urinary Cd level in all participants was about 4.0 μg/g cr. (Japanese Multicentered Environmental Toxicant Study; JMETS) (Horiguchi et al., in press). At the checkups, peripheral blood and urine samples were collected for determination of red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), serum iron, serum ferritin, HbA_{1C}, and fasting blood glucose (FBG) in peripheral blood, as well as the levels of blood and urinary Cd. RBC, Hb, and Ht were measured by the automated hematology analyzer SE-9000 (Sysmex). Serum iron, serum ferritin, HbA_{1C}, and FBG were determined by a colorimetric method (Shino-Test), a chemiluminescent immunoassay (BAYER), a latex agglutination immunoassay (Kyowa Medex), and a glucose oxidase method (Kanto Kagaku), respectively. We also determined the participants' body weight and height, and collected information about their histories of illness and smoking. Subjects with more than 6.0% of HbA_{1C} were diagnosed as diabetic, which is generally acceptable criteria in Japan, and those with less than 5.0 ng/ml of serum ferritin as well as less than 11.0 g/dl of Hb were considered to have iron-deficiency anemia. There was no participant with nephropathy due to DM. As a result, the investigated population included only 29 subjects with DM and 13 with iron deficiency. We selected 42 subjects for our study: 10 individuals with DM and 14 age-matched controls, and 6 individuals with anemia and 12 age-matched controls, all of whom had no history of smoking or other specific illnesses. None of the subjects was receiving specific therapy for DM or anemia. We obtained the subjects' informed consent for participation in this research project after detailed description of research goals.

Confinement of study participants in camps. This study was conducted in the form of a "camp" to completely manage the participants' meals and excretions. We reserved all rooms of a local inn with a hot spa in the heart of the mountains so that the participants could stay together for 7 nights and 8 days without leaving the camp. Smoking was prohibited in the inn during the study to avoid passive as well as active smoking. The participants were asked to eat or drink only what we served to them, including not only three

meals a day, but also refreshments such as confectionary, fruits, and beverages. The meals were prepared in the inn's kitchen using locally harvested foodstuffs to maintain the same Cd exposure level that subjects were exposed to from their ordinary diets. The major Cd source was locally grown rice that contained approximately 0.4 ppm Cd, resulting in a dietary Cd exposure close to the PTWI during the study (the mean and SD were 1.37 and 0.27 μg/kg/day, respectively). Some participants were allowed to take their habitual medicines as usual during the study to maintain their ordinary lifestyles. These drugs included antihypertensives, herbal remedies, mild laxatives, common cold drugs, etc., all of which contained only minimal amounts of Cd. The participants' activities at the inn were unrestricted except for food and excretions. We offered some recreational programs during the study, including lectures on health, aerobic exercises, video movies, etc. The study consisted of two camps; the first was for the participants with DM and their controls, and the second was for those with anemia and their controls. The former participants, who were all postmenopausal, stayed together for 1 week simultaneously. The second group, most of whom were premenopausal, also stayed for a 1-week period but selected one of three scheduled weeks to participate in the study to avoid their menstrual periods.

Collection of samples from the participants. The camps started at 10:00 a.m. on the first day and ended at 10:00 a.m. of the 8th day. The participants were served meals at 7:30, 12:00, and 18:00. Nutritionists checked the amounts that each participant ate, separated into three components: rice, side dishes, and soup. Condiments, including soy sauce, Worcestershire sauce, and mayonnaise, were prepared in small containers for each participant, and participants were allowed to use them according to their own preferences. We determined the amounts of condiments consumed by each individual. Triplicate meals were prepared to weigh the three components separately, and the mean values were used for calculation of Cd concentration. One set of meal components was frozen for later measurement of Cd concentration. The amounts of other foodstuffs and medications consumed were described in information sheets completed by each participant. The condiments, refreshments, and drug samples were preserved for measurement of Cd.

Nurses collected the participants' urine samples every 24 h and recorded the amounts daily. Ten milliliters of urine with one drop of nitric acid was stored in plastic tubes at −80 °C until it was tested for Cd concentration. The nurses collected feces just after defecation, and the total 24-h feces were stored at −80 °C in several plastic petri dishes after determination of their weights. When the total amounts of feces were too large to be stored, the feces were blended with distilled water using an electric mixer, and portions of the sample were stored. The restrooms in the inn were temporarily reconstructed to stop drains to avoid accidental loss of sample materials during the camps.

Measurement of Cd concentration in the samples. All Cd determinations were conducted by Metocean Environment Inc. (Shizuoka, Japan). Peripheral blood samples were decomposed with nitric acid by a microwave device, MDS-2000 (CEM). Raw and boiled rice, side dishes, soups, other solid foodstuffs, and feces were dried under vacuum at −80 °C, and the resulting powdered samples were resuspended in ultrapure water. Cd concentration in these samples was measured using HP 4500 ICP-MS (Yokokawa Analytical Systems). Liquid samples, such as urine, beverages, and sauces, were mixed with nitric acid and held for 24 h, after which Cd concentration was measured by flameless atomic absorption spectrometry, SIMAA 6000 (Perkin-Elmer). Indium (In) and thallium (Tl) were added as internal standards to all samples before treatment. The standard solutions for Cd, In, and Tl were purchased from Wako. The influence of sample matrix on Cd measurement was checked using standard substances, including CRM195 (Institute for Reference Materials and Measurements) and AMI B1701 (National Institute of Occupational Health of Denmark) for peripheral blood, 69071 Level 1 (human urine) (Bio Rad) for urine, and NIES No.10 (the National Institute of Environmental Science in Japan) for rice. Results for all of these standard substances were within reference ranges. All items, such as plastic bottles, tubes, or boxes, that would be in contact with these samples had no detectable Cd contamination.

Calculation of the dietary Cd absorption rate. The individual rate of dietary Cd absorption was calculated from the amounts of total oral Cd intake and total Cd fecal excretion for 1 week. Cd intakes from each meal, condiments, foodstuffs, and drugs were calculated by multiplying the consumed amounts by their Cd concentrations, and then all of the values were summed to determine the individual's weekly Cd intake. The individual amount of daily Cd fecal excretion was calculated by multiplying the weight of the feces by their Cd concentration, and then summed for the individual's weekly Cd excretion. Cd intake in the 8th day's breakfast was excluded from the weekly Cd intake calculation because the Cd was obviously retained in the body. In the case of no fecal excretion during the last 24 h, Cd intake after 10:00 a.m. of the 7th day as well as the 7th day's breakfast were further excluded. Cd excretion during the first 24 h was also excluded from the weekly Cd excretion calculation because it was thought to reflect prestudy Cd intake. In the case of no excretion during the first 24 h, Cd intake during the first 24 h and Cd excretion during the first 48 h were excluded from the calculation.

Statistical analysis. The differences in the mean values of age, BMI, RBC, Hb, Ht, serum iron, serum ferritin, HbA_{1C}, FBG, and blood and urinary Cd levels between subjects with DM or anemia and their matched controls were analyzed by Student's *t* test or Welch's test. The rates of

dietary Cd absorption were presented as the mean values and 95% confidence intervals (CI). The relationships between the rates of Cd absorption and other factors were analyzed by Pearson's correlation coefficients and multiple regression models. Data for weekly urinary Cd excretion were analyzed by repeated measures ANOVA, followed by Fisher's protected least-significance difference analysis for comparison of the data. The values of serum ferritin, HbA_{1C}, and FBG were transformed into base-10 logarithms before statistical analysis because they were considered to follow a log-normal distribution from their normal probability plots. We judged as significant when P value was less than 0.05.

Results

Profiles of the participants and their rates of Cd absorption

One participant suffered from severe diarrhea and three were severely constipated and had only two passages of feces during the first camp. These four were excluded from the

Table 1
Profiles of the study participants for analysis

	Control (N = 13)			Diabetes (N = 7)			P value
	Mean	SD	Range	Mean	SD	Range	
A. The first study							
Age	63.7	5.9	53–73	58.9	3.6	54–65	0.064
Body mass index	24.4	3.3	19.1–31.1	25.8	3.9	20.5–30.5	0.365
Red blood cell (×10/μl)	441.1	32.3	388–495	468.7	38.6	410–531	0.105
Hemoglobin (g/dl)	13.4	1.0	11.9–15.9	14.1	1.1	12.3–15.7	0.188
Hematocrit (%)	41.4	2.7	38.0–48.4	44.2	2.4	39.7–47.0	0.034
Serum iron (μg/dl)	93.3	31.0	53–153	97.4	32.0	61–154	0.782
Serum ferritin (ng/ml)	63.6	44.1	9.9–169	97.7	46.1	39.6–173.0	0.100[a]
Hemoglobin A_{1C} (%)	5.0	0.3	4.3–5.4	7.2	1.6	6.0–10.3	0.004[a]
Blood glucose (mg/dl)	89.7	5.7	82–100	169.3	79.5	117–338	0.008[a]
U-Cd/Cr (μg/g cr.)	5.16	1.82	2.15–8.84	4.90	1.51	2.64–7.43	0.750
B-Cd (μg/L)	4.98	1.38	2.64–7.70	2.18	0.57	1.07–2.74	<0.001
	Control (N = 12)			Anemia (N = 6)			P value
	Mean	SD	Range	Mean	SD	Range	
B. The second study							
Age	38.7	11.8	23–53	44.3	8.4	29–50	0.311
Body mass index	23.1	3.5	18.4–30.5	22.5	3.0	19.4–27.9	0.713
Red blood cell (×10/μl)	444.0	32.6	380–492	417.5	56.8	312–482	0.222
Hemoglobin (g/dl)	13.4	1.1	11.1–15.2	9.1	1.3	7.3–10.6	<0.001
Hematocrit (%)	41.7	3.4	36.2–47.6	32.0	3.7	25.9–35.5	<0.001
Serum iron (μg/dl)	70.0	25.3	30–112	15.0	7.5	7–25	<0.001
Serum ferritin (ng/ml)	23.2	22.5	5–66.4	3.3	1.3	1.4–4.8	<0.001[a]
Hemoglobin A_{1C} (%)	4.9	0.2	4.7–5.3	5.0	0.3	4.6–5.3	0.654[a]
Blood glucose (mg/dl)	89.3	7.4	79–101	94.0	10.7	84–112	0.294[a]
U-Cd/Cr (μg/g cr.)	2.72	1.85	0.88–7.62	2.92	0.95	1.47–4.10	0.817
B-Cd (μg/l)	2.71	1.31	0.51–5.36	3.56	1.25	2.06–5.31	0.209

U-Cd/Cr, urinary cadmium level adjusted by creatinine; B-Cd, blood cadmium level.
[a] Analysed after the values were transformed into base-10 logarithms.

Table 2
The rates of dietary Cd absorption calculated from the total amounts of weekly Cd intake and excretion

A. Individual data

ID	Intake (μg)	Excretion (μg)	Rate (%)
The first study			
Control.1	437.4	495.0	−13.2
Control.2	356.9	487.6	−36.6
Control.3	462.5	378.4	18.2
Control.4	338.8	348.9	−3.0
Control.5	437.4	603.6	−38.0
Control.6	560.5	477.8	14.7
Control.7	533.6	583.1	−9.3
Control.8	494.9	484.0	2.2
Control.9	340.8	416.4	−22.2
Control.10	357.8	425.9	−19.0
Control.11	446.3	573.2	−28.4
Control.12	547.9	616.4	−12.5
Control.13	467.8	456.1	2.5
Diabetes.1	378.7	343.8	9.2
Diabetes.2	339.9	383.4	−12.8
Diabetes.3	461.8	539.7	−16.9
Diabetes.4	550.4	299.0	45.7
Diabetes.5	452.7	563.1	−24.4
Diabetes.6	569.1	639.2	−12.3
Diabetes.7	503.1	545.3	−8.4
The second study			
Control.1	292.5	169.3	42.1
Control.2	529.5	324.5	38.7
Control.3	434.7	371.0	14.6
Control.4	451.1	436.7	3.2
Control.5	509.1	245.9	51.7
Control.6	442.0	222.9	49.6
Control.7	544.8	153.9	71.8
Control.8	573.4	442.5	22.8
Control.9	622.8	433.7	30.4
Control.10	658.7	568.8	13.6
Control.11	473.1	533.6	−12.8
Control.12	607.9	585.9	3.6
Anemia.1	665.9	935.2	−40.4
Anemia.2	585.0	326.3	44.2
Anemia.3	374.2	213.0	43.1
Anemia.4	369.2	509.8	−38.1
Anemia.5	424.2	233.6	44.9
Anemia.6	564.8	407.2	27.9

B. Statistics

	Intake (μg)	Excretion (μg)	Rate (%)
	Mean ± SD	Mean ± SD	Mean ± SD (95% CI)
The first study			
Control ($N = 13$)	444.8 ± 77.8	488.2 ± 85.5	−11.1 ± 17.8 (−21.9, −0.3)
Diabetes ($N = 7$)	465.1 ± 84.5	473.4 ± 129.4	−2.8 ± 23.7 (−24.8, 19.1)
Total ($N = 20$)	451.9 ± 78.6	483.0 ± 99.8	−8.2 ± 19.9 (−17.5, 1.1)
The second study			
Control ($N = 12$)	511.6 ± 101.1	374.1 ± 151.5	27.4 ± 24.4 (12.0, 42.9)
Anemia ($N = 6$)	497.2 ± 124.6	437.5 ± 267.7	13.6 ± 41.4 (−29.9, 57.1)

Table 2 (*continued*)

B. Statistics

	Intake (μg)	Excretion (μg)	Rate (%)
	Mean ± SD	Mean ± SD	Mean ± SD (95% CI)
The second study			
Total ($N = 18$)	506.8 ± 106.0	395.2 ± 192.0	22.8 ± 30.6 (7.6, 38.0)
All controls ($N = 25$)	476.9 ± 94.2	433.4 ± 132.5	7.4 ± 28.6 (−4.4, 19.2)
All subjects ($N = 38$)	477.9 ± 95.4	441.4 ± 155.0	6.5 ± 29.7 (−3.3, 16.2)

analysis. Therefore, 38 subjects were available for analysis: 20 from the first camp (7 diabetics and 13 controls), and 18 from the second camp (6 anemics and 12 controls).

Table 1 shows the profiles of the participants in this study. The mean values of HbA_{1C} and FBG of the DM group were significantly higher than those of the control group in the first study, and the mean values of Hb, Ht, serum iron, and serum ferritin in the anemia group were significantly lower than those of the control group in the second study. Age, BMI, and urinary Cd did not differ significantly between subjects with DM or anemia and their matched controls, but age and urinary Cd were higher in the first study group than in the second. The participants in the first and second studies did not include any anemic or diabetic subjects, respectively. These characteristics of the groups indicate that the participants were selected in an appropriate way to compare the rates of dietary Cd absorption between diabetic or anemic women and healthy ones.

We calculated the Cd absorption rate of each subject from weekly Cd intake and excretion, then compared the mean values and the 95% CI between the groups (Table 2). The rates in the diabetic group and its controls were −2.8% (95% CI: −24.8, 19.1) and −11.1% (95% CI: −21.9, −0.3), respectively, and those in the anemic group and its controls were 13.6% (95% CI: −29.9, 57.1) and 27.4% (95% CI: 12.0, 42.9), respectively. These results indicate that there were no significant differences in dietary Cd absorption between diabetic or anemic women and their respective age-matched control subjects. The rates of Cd absorption in all subjects and all controls were 6.5% (95% CI: −3.3, 16.3) and 7.4% (95% CI: −4.4, 19.2), respectively, which are consistent with previous reports (Friberg et al., 1986; WHO, 1992). There was no significant difference in daily urinary Cd excretion between diabetic or anemic groups and their control groups, respectively (data not shown).

Factors that affect Cd absorption

Although the rates of Cd absorption in the diabetic and anemic groups were not significantly different from those of their controls, the rate in controls for the anemic group was significantly higher than that of the controls for the diabetic group, seemingly reflecting the difference in age

Table 3
The matrix of Pearson's correlation coefficients in the participants (the number is 38)

	Cd absorption rate	Age	BMI	RBC	Hb	Ht	S-Fe	log Ferritin	log Hb A_{1C}	log BG	U-Cd/Cr	B-Cd
Cd absorptional rate	1.000											
Age	−0.669**	1.000										
Body mass index	−0.218	0.275	1.000									
Red blood cell	0.140	−0.036	0.271	1.000								
Hemoglobin	−0.092	0.279	0.280	0.600**	1.000							
Hematocrit	−0.024	0.212	0.265	0.704**	0.973**	1.000						
Serum iron	−0.345*	0.459**	0.371*	0.498**	0.827**	0.772**	1.000					
log Serum ferritin	−0.430**	0.594**	0.297	0.346*	0.721**	0.698**	0.709**	1.000				
log Hemoglobin A_{1C}	−0.208	0.242	0.274	0.380*	0.315	0.331*	0.315	0.456**	1.000			
log Blood glucose	−0.170	0.148	0.344*	0.382*	0.242	0.264	0.300	0.329*	0.947**	1.000		
U-Cd/Cr	−0.466**	0.612**	−0.021	0.004	0.261	0.231	0.288	0.445**	0.177	0.080	1.000	
B-Cd	−0.395*	0.501**	0.020	−0.054	−0.035	−0.075	0.060	0.204	−0.294	−0.376*	0.547**	1.000

U-Cd/Cr, urinary cadmium level adjusted by creatinine; B-Cd, blood cadmium level.
* $P < 0.05$.
** $P < 0.01$.

or urinary Cd levels (Table 2). This result led us to hypothesize that some factors other than clinical diagnosis of anemia or DM, such as age or Cd burden, might affect Cd absorption (Table 1). Thus, we next conducted an individual analysis using all data from the 38 subjects. When we examined the relationships between the rate of Cd absorption and the other factors by simple correlation analysis, age showed the highest Pearson's correlation coefficient, followed in order by urinary Cd, serum ferritin, blood Cd, and serum iron (Table 3). There were no significant relationships between Cd absorption rate and Hb, Ht, HbA_{1C}, or FBG.

Because serum ferritin, serum iron, and Cd levels were also highly related to age (Table 3), it was necessary to adjust these factors for age to determine the actual relationships. Thus, we next analyzed them using multiple regression models, with the rate of Cd absorption as a dependent variable and age, serum ferritin, serum iron, urinary Cd, and blood Cd as independent variables (Table 4). Serum ferritin and serum iron, and urinary Cd and blood Cd were not selected simultaneously out of consideration for their colinearity. In the four kinds of multiple regression models, age always showed significantly high negative standard partial regression coefficients, while serum ferritin, serum iron, and Cd body burden had only small ones. These results indicate that age was an independent factor affecting Cd absorption, and the correlations of Cd absorption with iron storage and Cd burden were confounded by age.

Rates of Cd absorption in groups divided by age

Because age was an independent factor and linearly related to Cd absorption (Fig. 1), it is more appropriate to calculate the rates of Cd absorption and compare them among groups normalized by age than between clinically diagnosed diabetic or anemic groups and healthy groups, as shown in Table 2.

When the subjects were divided into three age groups, 20–39 years (8 subjects), 40–59 years (16 subjects), and 60–79 years (14 subjects), the means and 95% CI of the rates of Cd absorption were 44.0% (31.7, 56.3), 1.0% (−11.9, 13.8), and −5.9% (−18.8, 7.1), respectively (Fig. 2).

Table 4
The effects of age, serum ferritin, serum iron, urinary Cd, and blood Cd on dietary Cd absorptional rate (as a dependent variable) analyzed by the four kinds of multiple regression models (the subject number is 38)

Model	Independent variables										R'
	Age		log Serum ferritin		Serum iron		U-Cd/Cr		B-Cd		
	SPRC	P value	SPRC	P value	SPRC	P value	SPRC	P value	SPRC	P value	
A	−0.593	0.002	−0.040	0.805			−0.085	0.604			0.636
B	−0.587	0.003	−0.063	0.694					−0.088	0.555	0.637
C	−0.592	0.002			−0.048	0.740	−0.089	0.582			0.637
D	−0.589	0.001			−0.069	0.638			−0.096	0.527	0.638

SPRC, standard partial regression coefficient; R', multiple correlation coefficient adjusted for the degrees of freedom; U-Cd/Cr, urinary cadmium level adjusted by creatinine; B-Cd, blood cadmium level.
The dependent variable is the dietary Cd absorption rate in all models. The independent variables are age, log serum ferritin, and U-Cd/Cr in model A; age, log serum ferritin, and B-Cd in model B; age, serum iron, and U-Cd/Cr in model C; and age, serum iron, and B-Cd in model D.

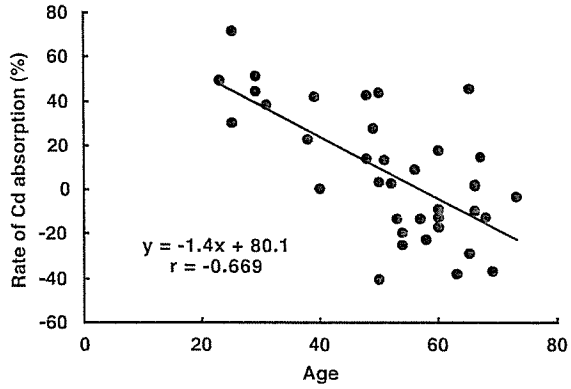

Fig. 1. The relationship between dietary cadmium (Cd) absorption rate and age among 38 female subjects. The solid circles indicate the individual subjects, and the straight line indicates the regression line, presented with the linear regression equation (x: age, y: rate of Cd absorption) and the correlation coefficient (r) ($P < 0.01$).

This indicates that younger women tend to absorb Cd at a much higher rate than do older women, who do not absorb substantially, and even excrete, Cd.

Weekly urinary Cd excretion in the participants

The daily urinary Cd excretion for the week of this study was also analyzed after subdividing the subjects by age group (Fig. 3). While we could not see any significant daily changes in urinary Cd excretion during the study, there was a significant difference among the age groups, consistent with the high correlation between age and creatinine-adjusted urinary Cd level (Table 3). This result indicates that urinary Cd is a stable indicator of Cd body burden, which is higher in older women than in younger ones. However, we did not include the urinary Cd excretion in the calculation of absorption rates because it does not reflect acute oral Cd exposure (Suzuki and Taguchi, 1980).

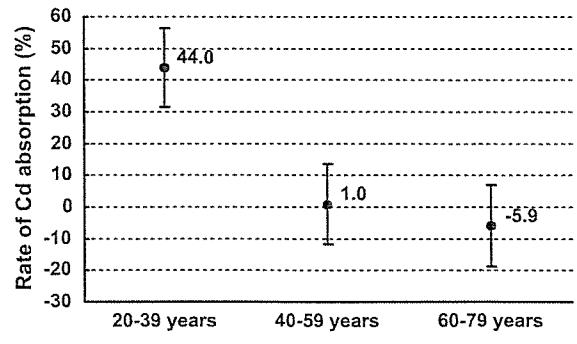

Fig. 2. The rates of dietary cadmium (Cd) absorption in subjects grouped by age. The numbers of subjects aged 20–39, 40–59, and 60–79 years were 8, 16, and 14, respectively. The data are presented as mean values of the rate (solid circles and data labels) with 95% confidence intervals (bars).

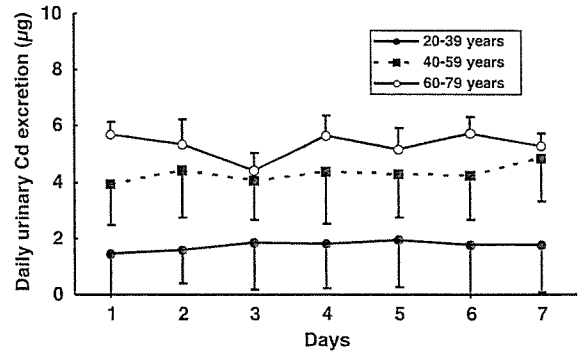

Fig. 3. The daily cadmium (Cd) excretion for a week in subjects grouped by age. The numbers of subjects aged 20–39, 40–59, and 60–79 years were 8, 16, and 14, respectively. The data are presented as mean values of urinary Cd excretion with SD bars.

Discussion

There are two major ways to investigate Cd absorption in humans: (1) the balance study, which estimates Cd absorption as the difference between total Cd intake from foods and excretion of Cd into feces, and (2) the biokinetic study using a radioisotope of Cd. The balance study provides a direct measure of Cd absorption, but requires substantial effort to collect complete food and fecal samples and should be conducted as a confinement study to avoid sample loss. In addition, the rate values calculated have wide ranges, even including negative ones. On the other hand, the biokinetic study is relatively easy, and the biological half-life can be calculated in addition to the absorption rate. However, this type of study actually estimates a retention rate rather than an absorption rate. In addition, it requires a radioisotope marker that may be poorly absorbed, such as chromium, to determine the final point of elimination, but we cannot necessarily know it precisely by this method because of the lack of an ideal marker (Mclellan et al., 1978). Both methods do not provide the net Cd absorption rates through the GIT, but instead provide apparent Cd absorption rates that include hepatoenteric or enteroenteric circulation (Suzuki and Taguchi, 1980). In experiments using human subjects, it is impossible to exclude such internal Cd circulation from the calculation of Cd absorption without taking bile samples invasively by endoscopic or percutaneous transhepatic procedures. In this study, we adopted the balance method to answer JECFA requirements for information on Cd bioavailability actually observed in human beings to establish a reliable dietary intake safety level in the general population (WHO, 2000). Although the rate values showed a wide range as expected, the averages, 6.5% in all subjects and 7.4% in healthy subjects, were consistent with previously reported

values in human subjects (Table 5), indicating that our study was conducted properly.

Although many studies on dietary Cd absorption have been performed previously, only two were balance studies that reported absorption rates (Table 5). One of these was not a confinement study and involved only healthy older adults, whose calculated absorption rate was not differentiated by sex (Bunker et al., 1984). The other was a well-designed confinement study, but the participants were limited to young healthy women (Kikuchi et al., 2003). In contrast, our study participants included 38 women of varied ages who had been environmentally exposed to much higher levels of Cd than those in the previous studies, including women with DM and iron deficiency anemia. Therefore, this is the first balance study to investigate the effects of potentially important factors, such as age, Cd exposure, DM, and anemia, on Cd absorption comprehensively using multiple regression analyses for all individual data. The comparisons between the DM group and its control and the anemic group and its control did not show significant differences in Cd absorption rates, but these analyses could not exclude the effects of confounding factors. We therefore made four suitable multiple regression models to observe the true contributions of these factors to Cd absorption rates. Our analyses demonstrated clearly that younger age was the only independent factor that could accelerate dietary Cd absorption, while DM, iron storage, and Cd burden did not affect it. In addition, we demonstrated a high Cd absorption rate in young women (44% in women aged 20–39 years), which is consistent with a recent investigation of young Japanese women (24–47% in women aged 20–23 years) (Kikuchi et al., 2003). These findings imply that young women are always at high risk from dietary Cd exposure regardless of their iron storage status.

In previous studies, however, iron deficiency status has been reported to be an important factor accelerating dietary Cd absorption in humans (Bunker et al., 1984; Flanagan et al., 1978), although one report showed no significant relation between iron deficiency and Cd absorption (Vanderpool and Reeves, 2001). One possible explanation for this discrepancy is that the numbers of subjects in the former two studies were limited compared with the number in our study, and thus the statistical procedures were much less reliable. The study by Flanagan et al. used 10 subjects with low iron stores and 12 controls, but in fact the numbers of female subjects were only 8 and 4, respectively. In addition, the age distribution in the study group was not balanced: the range was 21–37 years with one woman aged 61 years. The study by Bunker et al. investigated only 12 female subjects, who were all more than 70 years of age. The unbalanced age distribution as well as the small number of subjects could make it difficult to differentiate the effects of age and iron deficiency because iron deficiency is common in young women. In fact, in our study on 38 human subjects, the ferritin level showed a significantly high Pearson's correlation coefficient with the Cd absorption rate (Table 3), which was the same result as that of the study by Bunker et al., but ferritin proved to be much less important than age in multiple regression models (Table 4).

Another reason for the discrepancy between the previous studies and ours may be differences in the criteria for iron deficiency or anemia. Although Flanagan et al. categorized subjects with serum ferritin levels less than 20 ng/ml as iron deficient, the subjects were not necessarily anemic. In the

Table 5
Previous human studies on the dietary Cd absorptional rate

Reference	Subject number	Sex	Age	Study method	Labeled meal	Rate (%)
Rahola et al. (1972)	5	M	19–50	Kinetic (^{115m}Cd)	Calf kidney cortex	5.9 ± 0.5 (SE)
Yamagata et al. (1975)	1	M	53	Kinetic (^{115m}Cd)	Rice	4.4
Koizumi (1975)	1	M	58	Kinetic (cold Cd)	Water	5.33
					Rice	1.53
Flanagan et al. (1978)	10 (low body iron store)	M (2) and F (8)	21–37 and one woman	Kinetic (^{115m}Cd)	Oats and milk	8.9 ± 2.0 (SE)
	12 (normal iron store)	M (8) and F (4)	at 61 years			2.3 ± 0.3 (SE)
Mcllellan et al. (1978)	14	Unknown	21–61	Kinetic (^{115m}Cd)	Oats and milk	4.6 ± 4.0 (SD)
Newton et al. (1984)	7	M	29–61	Kinetic (^{115m}Cd)	Crab sandwiches	2.7 ± 0.9 (SE)
Bunker et al. (1984)	11	M	73–85	Balance	–	−15.0
	12	F	70–86	(5 days, not confined)	–	
Berglund et al. (1994)	23 (high-fiber diet)	F	20–50	Balance	–	no information
	34 (mixed diet)	F	20–50	(4 days, not confined)	–	
Vahter et al. (1996)	17 (shellfish diet)	F	27–48	Balance	–	no information
	34 (mixed diet)	F	20–50	(4 days, not confined)	–	
Vanderpool and Reeves (2001)	14	F	30–70	Kinetic (^{113}Cd)	Sunflower kernels	10.6 ± 4.4 (SD)
Kikuchi et al. (2003)	25	F	20–23	Balance	–	23.7–47.2
				(20 days, confined)		

M: male; F: female.
Kinetic study: rates were calculated from the amounts of Cd remaining in the body or excreted into feces after Cd administration.
Balance study: rates were calculated from the differences between total Cd intake from foods and excretion of Cd into feces.