study by Bunker et al., the lowest serum ferritin level was as high as 19.0 ng/ml, implying that no subjects with iron deficiency anemia were included, because there were no anemic subjects whose serum ferritin levels were more than 10 ng/ml among the 600 participants in our health checkups. In contrast, we adopted much more stringent criteria, less than 5.0 ng/ml of serum ferritin as well as less than 11.0 g/dl of Hb, to assess the risk of Cd exposure among women with iron deficiency anemia. In fact, the mean values of serum ferritin and Hb in the anemic group in our study were 3.3 ng/ml and 9.1 g/dl, respectively (Table 1). In other words, there have been no previous studies that actually investigated dietary Cd absorption rates in humans with true iron deficiency anemia.

Although the reason why Cd absorption is so highly accelerated in young women is not yet known, we speculate that estrogen might play an important role. Estrogen could induce the gene for transferrin, a well-known iron transporter (Vyhlidal et al., 2002) that might also work as a Cd transporter, because Cd and ferrous iron often show a similar distribution. Some types of metal transporters, such as natural-resistance-associated macrophage protein-2 (Nramp2) or divalent metal transporter 1 (DMT1), have been reported to associate with Cd absorption (Elisma and Jumarie, 2001; Park et al., 2002), although it is unknown whether they could be induced by estrogen. Further studies would be necessary to elucidate this relationship.

On the other hand, older subjects in our study showed zero to negative Cd balance, consistent with a previous report in which the rate was calculated as −15% in healthy elderly people (Bunker et al., 1984). This result indicates that older people would absorb only a small amount of Cd from foods via the GIT or even excrete Cd into feces through the intestinal mucosa or biliary and pancreatic juices, although the amount is generally estimated to be small (Cherian and Vostal, 1977; Ishihara et al., 1987). The mechanism for the age difference is not yet known, but the capacity to preserve Cd in hepatic tissue might decrease in older people. In fact, it has been reported that the amount of Cd accumulated in the body decreases after age 50 (Schroeder et al., 1967).

Considering the zero to negative absorption rate at older ages, the high rate of Cd absorption at younger ages would not contribute so much to Cd accumulation throughout a subject's life. Actually, the average absorption rate in subjects of all ages in our study was only 6.5% (Table 2). However, the difference in Cd absorption rate between age groups could be applied to risk management for environmental Cd exposure, for example, by establishing a special tolerable intake level for young women in addition to the current PTWI for the general population. The theoretical dietary Cd absorption rate at each age can be calculated from the linear regression equation, $y = -1.4x + 80.1$ (Fig. 1, Table 6).

Not only iron storage status, but also urinary and blood Cd levels, which are often used as indicators of Cd burden in the body, made only a very low contribution to the Cd absorption rate in multiple regression models in spite of high Pearson's correlation coefficients. The apparent high correlation between Cd burden and Cd absorption must be due to the confounding effect of aging because Cd burden gradually increases with aging. Even though metallothionien, a cysteine-rich, low-molecular-weight protein that has a high affinity for Cd (Templeton and Cherian, 1991), might have been induced in the intestinal mucosa of the older participants with relatively high Cd burden, it would not have affected the uptake of Cd from the intestinal lumen (Ohta and Cherian, 1991).

Table 6
Theoretical Cd absorption rates at the indicated age

Age	Cd absorption rate (%)[a]
20	52.1
30	38.1
40	24.1
50	10.1
60	−3.9
70	−17.9

[a] The rates were calculated by substituting age into the linear regression equation (80.1 − 1.4 × age).

It is obvious that DM does not affect dietary Cd absorption because neither FBG nor HbA_{1c} showed any significant correlation with the Cd absorption rate even by simple correlation analysis. Exposure to Cd could increase susceptibility to diabetic nephropathy, but glucose intolerance or a high blood glucose level does not increase dietary Cd absorption. On the contrary, our study showed that the mean blood Cd level in diabetic subjects was significantly lower than that in controls (Table 1); the biological basis for this is not yet understood.

In conclusion, age was an independent factor affecting dietary Cd absorption in Cd-exposed Japanese female farmers and the absorption rates were highly accelerated among younger women. High correlations of dietary Cd absorption with iron storage status and Cd burden were due to the confounding effect of age, and DM had no effect on Cd absorption. These results will contribute to risk management for environmental Cd exposure, especially oral exposure from Cd-contaminated foods, for example, by allowing the establishment of a specific tolerable intake level for young women based on differences in the absorption rate between age groups.

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Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers ☆

Hyogo Horiguchi,[a,b] Etsuko Oguma,[b] Satoshi Sasaki,[c] Kayoko Miyamoto,[a] Yoko Ikeda,[a] Munehito Machida,[a] and Fujio Kayama[a,b,*]

[a] *Division of Environmental Immunology and Toxicology, Department of Health Science, Jichi Medical School, Yakushiji 3311-1, Minami-Kawachi, Kawachi-Gun, Tochigi 329-0498, Japan*

[b] *Core Research for Evolutional Science and Technology, Japan Science Technology Cooperation (JST-CREST), Honmachi 4-1-8, Kawaguchi 332-0012, Japan*

[c] *National Institute of Health and Nutrition, Toyama 1-23-1, Shinjuku-Ku, Tokyo 162-8636, Japan*

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Abstract

Some recent research suggests that environmental exposure to cadmium, even at low levels, may increase the risk of osteoporosis, and that the bone demineralization is not just a secondary effect of renal dysfunction induced by high doses of cadmium as previously reported. To investigate the effect of exposure to cadmium at a level insufficient to induce kidney damage on bone mineral density (BMD) and bone metabolism, we conducted health examinations on 1380 female farmers from five districts in Japan who consumed rice contaminated by low-to-moderate levels of cadmium. We collected peripheral blood and urine samples and medical and nutritional information, and measured forearm BMD. Analysis of the data for subjects grouped by urinary cadmium level and age-related menstrual status suggested that cadmium accelerates both the increase of urinary calcium excretion around the time of menopause and the subsequent decrease in bone density after menopause. However, multivariate analyses showed no significant contribution of cadmium to bone density or urinary calcium excretion, indicating that the results mentioned above were confounded by other factors. These results indicate that environmental exposure to cadmium at levels insufficient to induce renal dysfunction does not increase the risk of osteoporosis, strongly supporting the established explanation for bone injury induced by cadmium as a secondary effect.

Keywords: Cadmium; Bone; Kidney; Female; Japanese

1. Introduction

Cadmium (Cd) is a toxic heavy metal that induces the deterioration of renal tubular function, which can be detected by the increase in urinary excretion of low molecular weight proteins such as α_1-microglobulin (α1-MG) or β_2-microglobulin (β2-MG), after sustained environmental exposure (Friberg et al., 1986; WHO, 1992). The most severe form of chronic intoxication, called "Itai-itai disease," was endemic among female farmers in the heavily Cd-polluted area of the Jinzu River basin in Japan and was characterized by renal tubular dysfunction, renal anemia, and multiple bone fractures due to osteomalacia, which lead to generalized severe pain (the Japanese word "itai" means "ouch") (Yamagata and Shigematsu, 1970; Kasuya et al., 1992a, b; Horiguchi et al., 1994). The mechanism of the bone injury has been acknowledged as an acquired Fanconi's syndrome: the decrease of the bone calcium (Ca) pool is accelerated by the continuous loss of Ca and

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*Corresponding author. Division of Environmental Immunology and Toxicology, Department of Health Science, Jichi Medical School, 3311-1 Yakushiji, Minami-Kawachi, Kawachi-Gun, Tochigi 329-0498 Japan. Fax: +81-285-44-8465.

E-mail address: kayamaf@jichi.ac.jp (F. Kayama).

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phosphorus into urine and/or by the disturbance of vitamin D metabolism in the kidneys due to renal tubular dysfunction (Ishizaki and Fukushima, 1968; Akiba et al., 1980; Ruth et al., 1981; Nogawa et al., 1987; Aoshima et al., 1993). In other words, Cd primarily damages the kidneys, followed by osteomalacia as a secondary effect. In fact, Itai-itai disease developed only among the large number of the inhabitants with Cd-induced nephropathy, and vitamin D treatment alleviated the osteomalacia (Kasuya et al., 1992a).

However, some recent epidemiological studies in Europe have challenged this accepted mechanism, suggesting that Cd can induce osteoporosis at much lower exposure levels than previously reported (Järup et al., 1998; Staessen et al., 1999; Alfvén et al., 2000). In addition to these human studies, there are some experimental studies to suggest the direct Cd effect on bone (Bhattacharyya et al., 1988; Ogoshi et al., 1992; Miyahara et al., 1992). This implies that bone demineralization could be induced as a primary Cd effect before the occurrence of renal damage. If this were true, it would constitute a major public health problem among the Japanese, who have a higher Cd body burden than most other people do because they consume Cd-contaminated rice as a staple food. The conflicting views have prompted the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to call for additional studies on the impact of lifetime exposure to Cd on the development of osteoporosis (WHO, 2000). Consequently, we undertook a cross-sectional epidemiological study of 1380 female Japanese farmers with dietary exposure to Cd at levels insufficient to induce renal dysfunction in order to investigate the effects of Cd exposure at these levels on bone mineral density (BMD) and bone metabolism.

2. Materials and methods

2.1. Study populations

We conducted health examinations on female Japanese farmers from five districts of Japan in 2001–2002 as previously described, named "Japanese Multicentered Environmental Toxicants Study" (JMETS) (Horiguchi et al., 2004). Briefly, we investigated 202, 202, 203, 204, and 569 participants—1380 in total—from one non-contaminated district (A) as a control, where highly Cd-contaminated rice has never been detected, and four Cd-contaminated districts (B, C, D, and E), where such rice is often detected, respectively. The participants were thought to have been exposed to the sustained levels of Cd through the consumption of the rice grown in their own fields ever since birth. The geometric means (GMs) of urinary Cd levels (Cd-U) were 2.6, 3.5, 3.2, 3.2, and 4.1 μg/g cr. in the five districts, respectively, with only 1% over the threshold at which Cd induces renal dysfunction (10 μg/g cr.) (Roels et al., 1979; Ikeda et al., 2003). In addition, the GM of urinary β2-MG in the total population was less than 150 μg/g cr., with less than 3% showing β_2-microglobulinuria (over 1000 μg/g cr. of urinary β2-MG, a cutoff value considered as the threshold of irreversible tubular dysfunction; Teranishi et al., 1992). Thus, the Cd exposure levels of the investigated subjects can be described as "moderate" and insufficient to induce renal dysfunction.

2.2. Health examinations

At the examinations, we collected peripheral blood and second-morning urine samples before breakfast and measured the participants' weight, height, grasping power, and BMD. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared. The grasping power of a participant's non-dominant hand was measured three times with a hand dynamometer, and the highest value was used as an indicator of physical activity. We measured BMD by dual energy X-ray absorption (DXA) in the participant's nondominant forearm with a DTX-200 (Osteometer), which scanned DXA at distal sites of the radius and ulna between the 8 and 24 mm points. Subjects with BMD less than 80% of the young adult mean (YAM) (20–44 years old) were classified as having "decreased BMD" according to the criteria of the Japanese Society for Bone and Mineral Research (The Committee on Standard Criteria of Primary Osteoporosis, 1996).

We obtained medical information about each participant's health status, including present and past medical history, using a self-administered questionnaire. We investigated the intakes of Ca and vitamin D by another questionnaire, named diet history questionnaire (DHQ), which was designed to determine food and nutrient intake levels in the previous month with regard to the quantity and semi-quantitative frequency of consumption of 110 food items commonly consumed in Japan (Sasaki et al., 1998).

2.3. Analysis of blood and urine samples

Two urinary proteins, α1-MG and β2-MG, Ca, and creatinine (Cr) in urine were determined by a latex agglutination method, the *o*-cresolphthalein complexone method, and the Jaffé reaction method, respectively, with one drop of sodium hydrogen carbonate solution added to the β2-MG sample just after collection to prevent destruction by low pH (Donaldson et al., 1989). Luteinizing hormone (LH), bone-specific alkaline phosphatase (BAP), and bone Gla protein (BGP) in serum

were measured by immunoradiometric assay (IRMA), ELISA, and IRMA, respectively. *N*-telopeptide cross-linked collagen type 1 (NTx) and deoxypyridinoline (D-Pyr) in urine were determined by ELISA. The heparinized whole blood samples were decomposed with nitric acid by a microwave device, after which Cd concentrations (Cd-B) were measured using HP 4500 series ICP-MS (Yokokawa Analytical Systems). We measured Cd-U by flameless atomic absorption spectrometry, SIMAA 6000 (Perkin–Elmer), after holding urine samples with nitric acid for 24 h. All items, such as plastic bottles, tubes, or syringes, which would be in contact with these samples, were shown before use to be free of any detectable Cd contamination. Metocean Environment Inc. (Shizuoka, Japan) conducted all Cd determinations.

2.4. Statistical analysis

We used data of 1243 subjects for the analysis, excluding 137 from the original participants for the following reasons: past or current smoking, 52; chronic renal failure treated with hemodialysis, 1; collagen diseases, 13; spinal caries, 1; oophorectomy, 5; insufficient ovary growth, 1; early menopause, 7; hyperthyroidism, 7; use of oral contraceptives, 4; hormone replacement therapy, 40; steroid hormone therapy, 2; extremely high serum β2-MG, 1; insufficient urine sample volume, 3. When values were less than the limits of detection (blood Cd, 0.4 /μg/L; urinary Cd, 0.3 μg/L; urinary α1-MG, 0.9 mg/L; urinary β2-MG, 70 μg/L), we used half values for statistical calculation. Data that appeared to follow a normal distribution are presented as arithmetic means (AM) and arithmetic standard deviations (ASD). GM and geometric standard deviations (GSD) were used for data with a log-normal distribution, which were also converted into base-10 logarithms before the following statistical analysis. Single regression analysis as well as Bonferroni's multiple comparison procedure following one-way ANOVA were used to test the trend in Cd-U-divided subgroups. The prevalence of subjects with decreased BMD among the subgroups was examined by the χ^2-test, followed by Bonferroni's multiple comparison procedure. In multiple regression models, we selected age, BMI, grip power, Cd-B, Cd-U, α1-MG, β2-MG, Ca-U, and Ca and vitamin D intakes as independent variables. We further added dummy variables for the five districts with district A as a reference into the models. We judged a factor as significant when the standard partial regression coefficient (SPRC) showed a relatively high value with a partial correlation coefficient (PCC) greater than 0.2, since the statistical P value is inclined to produce false positives at higher degrees of freedom (Armitage and Berry, 1994).

3. Results

3.1. Grouping subjects based on Cd-U and age-related menstrual status

We first divided the population using three cutoff values of Cd-U (2.5, 3.5, and 5.0 μg/g cr.) into four subgroups with similar numbers of participants, although the mean ages increased Cd-U-dependently, reflecting the close relation between age and Cd accumulation (Table 1). Since age and menstrual status could affect bone metabolism, we further divided each Cd-U-based subgroup into four classes by age-related menstrual status: premenopause (41–48 years old), perimenopause (49–55), younger postmenopause (56–65), and older postmenopause (66–75). Subjects less than 41 years of age or more than 75 years of age were excluded because of small numbers. We verified menstrual status using serum LH, the secretion of which increases in response to the decrease of estrogen levels due to menopause. All subjects in the premenopausal class had menses and low LH, but the perimenopausal class included both subjects with and without menstruation, showing higher LH levels with wider ASD. All subjects in both postmenopausal classes had sustained high LH levels and no menstrual periods. As a result, each age class lost difference in mean age between Cd-U-divided subgroups. On the other hand, the increasing trends of Cd exposure, indicated by Cd-B and Cd-U, did not disappear even after age-classification (Table 2). Thus, this grouping method allowed us to observe the effects of Cd exposure not confounded by age and menstrual status.

3.2. Cd effect on BMD

We first observed the effects of Cd and age-related menstrual status on BMD (Table 3). The BMD levels declined age-dependently in every Cd-U subgroup, with a notable sudden drop from peri- to postmenopause. Although BMD levels showed a clear, negative correlation with Cd-U when subjects of all ages were considered together, this correlation disappeared when the pre- and perimenopausal groups were considered individually, but remained significant in both postmenopausal groups. These results indicate that Cd exposure might accelerate bone demineralization after menopause, although the effect of aging on the decline of BMD was much stronger than the effect of Cd exposure. We observed a similar, but weaker and statistically nonsignificant, trend in the prevalence of subjects with decreased BMD (Table 4). BMI, however, which is well known to affect BMD, showed significant decreasing trends along with Cd-U after menopause in a very similar pattern to BMD (Table 3), suggesting that the observed Cd effect might be confounded by it.

Table 1
Grouping of the study population by urinary Cd, age, and menstrual status

Age classes	Urinary Cd (μg/g cr.)				
	<2.5	⩾2.5, <3.5	⩾3.5, <5.0	⩾5.0	Total
All subjects					
Number	323	272	321	327	1243
Age	51.9±10.1	55.1±8.8*	58.6±8.4*	60.1±8.1*	56.5±9.4
Maximum age	76	76	78	75	78
Minimum age	30	32	36	36	30
Serum LH	18.2±14.8	20.5±13.1	23.0±13.1	22.8±11.6	21.2±13.3
Premenopause (41–48 y.o.)					
Number	97	56	35	30	218
Age	45.0±2.2	45.0±2.3	45.5±2.4	45.1±2.4	45.1±2.3
Serum LH	9.7±11.7	8.7±11.8	9.5±15.8	8.7±10.1	9.3±12.2
Perimenopause (49–55 y.o.)					
Number	80	88	82	69	319
Age	51.8±1.9	52.0±1.8	51.9±1.7	52.7±2.0	52.1±1.9
Serum LH	27.4±17.1	24.2±13.7	26.6±15.7	26.2±12.1	26.0±14.8
Younger postmenopause (56–65 y.o.)					
Number	68	77	123	129	397
Age	60.8±2.7	60.9±2.9	61.2±2.8	61.1±2.9	61.1±2.8
Serum LH	23.5±8.9	25.6±10.0	26.1±9.9	25.5±11.0	25.4±10.1
Older postmenopause (66–75 y.o.)					
Number	38	40	73	97	248
Age	68.9±2.2	68.5±2.6	68.7±2.2	68.9±2.5	68.8±2.4
Serum LH	24.0±10.3	20.9±7.6	21.7±7.1	21.7±8.4	21.9±8.3

Note. The values of age and serum lutcinizing hormone (LH) (mIU/mL) are presented as arithmetic mean±arithmetic standard deviation.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).

Table 2
Cd concentrations in peripheral blood and urine in subgroups divided by urinary Cd levels and age classes

Age classes	Urinary Cd (μg/g cr.)					Regression coefficients
	<2.5	⩾2.5, <3.5	⩾3.5, <5.0	⩾5.0	Total	
Peripheral blood Cd (μg/L)						
All subjects	1.72 (1.94) (range 0.62–7.47)	2.33 (1.69)* (ND-7.89)	2.71 (1.78)* (ND-8.69)	3.75 (1.62)* (0.92–13.07)	2.54 (1.89) (ND-13.07)	0.394**
Premenopause (41–48 y.o.)	1.57 (1.94)	2.21 (1.67)*	3.58 (1.58)*	4.45 (1.61)*	2.26 (2.00)	0.590**
Perimenopause (49–55 y.o.)	1.72 (1.97)	2.22 (1.80)*	2.54 (1.70)*	3.42 (1.54)*	2.37 (1.85)	0.348**
Younger postmenopause (56–65 y.o.)	1.85 (2.00)	2.42 (1.59)*	2.67 (1.71)*	3.62 (1.66)*	2.72 (1.81)	0.337**
Older postmenopause (66–75 y.o.)	2.12 (1.72)	2.50 (1.69)	2.56 (2.04)	4.02 (1.58)*	2.96 (1.85)	0.369**
Urinary Cd (μg/g cr.)						
All subjects	1.66 (1.46) (range ND-2.50)	2.98 (1.10)* (2.50–3.50)	4.17 (1.10)* (3.50–4.98)	6.75 (1.29)* (5.01–27.26)	3.46 (1.78) (ND-27.26)	—
Premenopause (41–48 y.o.)	1.57 (1.45)	3.02 (1.09)*	4.21 (1.11)*	6.67 (1.32)*	2.66 (1.81)	—
Perimenopause (49–55 y.o.)	1.67 (1.37)	2.97 (1.11)*	4.11 (1.11)*	6.49 (1.24)*	3.31 (1.68)	—
Younger postmenopause (56–65 y.o.)	1.66 (1.60)	2.94 (1.10)*	4.18 (1.10)*	6.78 (1.31)*	3.90 (1.74)	—
Older postmenopause (66–75 y.o.)	1.86 (1.34)	3.03 (1.10)*	4.17 (1.11)*	6.97 (1.30)*	4.28 (1.67)	—

Note: The values are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).
ND, not detected (blood Cd, less than 0.4 μg/L; urinary Cd, less than 0.3 μg/L).

Table 3
BMD and BMI in subgroups divided by urinary Cd levels and age classes

Age classes	Urinary Cd (μg/g cr.)					Regression coefficients
	<2.5	⩾2.5, <3.5	⩾3.5, <5.0	⩾5.0	Total	
BMD (g/cm^2)						
All subjects	0.449 (0.078) (range 0.185–0.623)	0.430 (0.077)* (0.213–0.624)	0.412 (0.083)* (0.210–0.644)	0.392 (0.086)* (0.219–0.680)	0.421 (0.084) (0.185–0.680)	−0.011**
Premenopause (41–48 y.o.)	0.489 (0.051)	0.484 (0.051)	0.495 (0.050)	0.490 (0.052)	0.489 (0.051)	0.001
Perimenopause (49–55 y.o.)	0.466 (0.064)	0.455 (0.063)	0.470 (0.065)	0.460 (0.075)	0.463 (0.066)	0.000
Younger postmenopause (56–65 y.o.)	0.407 (0.078)	0.399 (0.072)	0.388 (0.063)	0.378 (0.070)*	0.390 (0.070)	−0.006**
Older postmenopause (66–75 y.o.)	0.362 (0.073)	0.359 (0.069)	0.349 (0.068)	0.332 (0.062)*	0.346 (0.067)	−0.006**
BMI						
All subjects	23.9 (3.5) (range 16.8–34.9)	23.7 (3.0) (17.3–32.8)	23.7 (3.3) (15.7–41.5)	23.6 (3.2) (15.7–36.7)	23.7 (3.3) (15.7–41.5)	−0.042
Premenopause (41–48 y.o.)	23.7 (3.3)	23.1 (3.1)	22.6 (3.0)	23.5 (3.1)	23.3 (3.2)	−0.033
Perimenopause (49–55 y.o.)	23.6 (3.3)	23.1 (3.0)	23.8 (4.1)	24.0 (3.1)	23.6 (3.4)	0.114
Younger postmenopause (56–65 y.o.)	24.5 (3.5)	24.3 (2.8)	23.8 (3.1)	23.5 (3.2)	23.9 (3.2)	−0.205**
Older postmenopause (66–75 y.o.)	25.1 (3.1)	24.7 (3.1)	24.1 (2.9)	23.6 (3.3)	24.2 (3.2)	−0.289**

Note: The values are presented as arithmetic mean (arithmetic standard deviation).
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).

Table 4
Prevalence of subjects with decreased bone mineral density (<80% of Japanese young adult mean)

Age classes	Urinary Cd (μg/g cr.)										P value (χ^2 test)
	< 2.5		⩾2.5, <3.5		⩾3.5, <5.0		⩾5.0		Total		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
All subjects											
Total	323	100.0	272	100.0	321	100.0	327	100.0	1243	100.0	0.000
⩾80%	260	80.5	198	72.8	211	65.7	169	51.7	838	67.4	
<80%	63	19.5	74	27.2	110	34.3*	158	48.3*	405	32.6	
Premenopause (41–48 y.o.)											
Total	97	100.0	56	100.0	35	100.0	30	100.0	218	100.0	0.782
⩾80%	96	99.0	55	98.2	35	100.0	30	100.0	216	99.1	
<80%	1	1.0	1	1.8	0	0.0	0	0.0	2	0.9	
Perimenopause (49–55 y.o.)											
Total	80	100.0	88	100.0	82	100.0	69	100.0	319	100.0	0.083
⩾80%	74	92.5	76	86.4	76	92.7	56	81.2	282	88.4	
<80%	6	7.5	12	13.6	6	7.3	13	18.8	37	11.6	
Younger postmenopause (56–65 y.o.)											
Total	68	100.0	77	100.0	123	100.0	129	100.0	397	100.0	0.147
⩾80%	38	55.9	42	54.5	71	57.7	57	44.2	208	52.4	
<80%	30	44.1	35	45.5	52	42.3	72	55.8	189	47.6	
Older postmenopause (66–75 y.o.)											
Total	38	100.0	40	100.0	73	100.0	97	100.0	248	100.0	0.272
⩾80%	15	39.5	15	37.5	24	32.9	24	24.7	78	31.5	
<80%	23	60.5	25	62.5	49	67.1	73	75.3	170	68.5	

*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).

3.3. Cd effect on bone metabolism

We next looked into the effect of Cd on bone metabolism (Table 5). Urinary Ca level (Ca-U), which reflects Ca loss from bones, increases significantly along with Cd-U in every age class, with especially notable rises at higher Cd-U levels in perimenopause. The pattern of BAP, an osteogenic marker, was similar to

Table 5
Urinary Ca excretion and markers of bone metabolism in subgroups divided by urinary Cd levels and age classes

Age classes	Urinary Cd (μg/g cr.)					Regression coefficients
	<2.5	⩾2.5, <3.5	⩾3.5, <5.0	⩾5.0	Total	
Urinary Ca/Cr (mg/g cr.)						
All subjects	102.2 (1.80) (range 9.7–619.4)	118.1 (1.76)* (12.3–405.9)	128.9 (1.83)* (12.2–451.7)	149.7 (1.77)* (10.7–1022.2)	123.8 (1.82) (9.7–1022.2)	9.167**
Premenopause (41–48 y.o.)	87.2 (1.67)	94.7 (1.90)	98.4 (1.94)	109.4 (1.65)	93.7 (1.77)	4.279**
Perimenopause (49–55 y.o.)	117.2 (1.82)	122.8 (1.64)	140.5 (1.66)*	161.9 (1.66)*	133.4 (1.71)	9.725**
Younger postmenopause (56–65 y.o.)	118.5 (1.73)	126.1 (1.74)	130.4 (1.89)	143.9 (1.64)	131.6 (1.76)	4.866**
Older postmenopause (66–75 y.o.)	125.3 (1.76)	144.2 (1.68)	131.9 (1.82)	169.8 (1.90)*	146.5 (1.84)	7.953**
BAP						
All subjects	25.0 (1.5) (range 9.0–145.0)	26.6 (1.4)* (10.1–73.4)	29.3 (1.4)* (9.1–74.3)	30.8 (1.4)* (9.8–81.1)	27.9 (1.45) (9.0–145)	1.165**
Premenopause (41–48 y.o.)	20.0 (1.4)	19.7 (1.4)	19.5 (1.4)	19.2 (1.3)	19.7 (1.4)	−0.157**
Perimenopause (49–55 y.o.)	27.2 (1.4)	26.8 (1.4)	28.8 (1.4)	28.7 (1.4)	27.8 (1.4)	0.380
Younger postmenopause (56–65 y.o.)	31.9 (1.4)	30.6 (1.4)	32.0 (1.4)	32.9 (1.3)	32.0 (1.4)	0.291
Older postmenopause (66–75 y.o.)	31.5 (1.3)	32.4 (1.4)	32.6 (1.3)	34.6 (1.4)	33.2 (1.4)	0.593**
Urinary NTx (nmol/mmol cr.)						
All subjects	42.3 (1.7) (range 12.2–552.0)	48.7 (1.6)* (13.6–175.0)	56.0 (1.6)* (8.8–220.0)	61.7 (1.6)* (14.4–200.0)	51.8 (1.7) (8.8–552.0)	3.803**
Premenopause (41–48 y.o.)	31.0 (1.5)	33.2 (1.4)	31.0 (1.6)	31.8 (1.6)	31.7 (1.5)	0.016
Perimenopause (49–55 y.o.)	48.6 (1.5)	50.0 (1.6)	56.6 (1.6)*	65.8 (1.6)*	54.4 (1.6)	3.782**
Younger postmenopause (56–65 y.o.)	57.5 (1.6)	57.2 (1.5)	62.0 (1.5)	66.1 (1.5)*	61.5 (1.5)	1.859**
Older postmenopause (66–75 y.o.)	55.1 (1.5)	63.3 (1.4)	63.8 (1.5)	67.3 (1.5)	63.6 (1.5)	2.048

Note: Cr, creatinine; BAP, bone-specific alkaline phosphatase; NTx, *N*-telopeptide cross-linked collagen type 1. Data are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).

that of BMD, whereas NTx, an osteoclastic marker, corresponded to that of Ca-U. Other markers, BGP and D-Pyr, did not show any significant increasing trends with Cd-U (not shown). Thus, Cd might accelerate urinary Ca excretion and the subsequent decrease of BMD due to high bone turnover after menopause. However, urinary α1-MG and β2-MG showed the increasing trends along with Cd-U in the parallel patterns with Ca-U, although the levels were much less than the threshold of irreversible renal tubular dysfunction (Table 6). These results suggest that the increase of Ca-U by Cd exposure observed in the grouping analyses might in fact be due to the decreased renal tubular reabsorptional ability.

3.4. Multivariate analyses

Since these results suggest that the grouping analyses could not exclude confounding factors sufficiently, we performed multivariate analyses for BMD and Ca-U using possible confounding factors as well as Cd exposure as independent variables. Because of collinearity, we considered it inappropriate to include both Cd-B and Cd-U as markers for Cd exposure or both urinary α1-MG and β2-MG as indicators of renal tubular function in the same model. We therefore made four multiple regression models, each including one of the markers for Cd exposure and one of the indicators of renal tubular function, as shown in Table 7. In every model on BMD, age and BMI were the first and second significant factors, respectively, but neither Cd-B nor Cd-U was significant. Multiple logistic regression models also did not indicate any significant contribution of Cd exposure to decreased BMD (Table 8). These results indicate that Cd exposure would have no actual contribution to BMD, suggesting that the Cd effect on BMD observed in the grouping analyses is confounded by other factors. On the other hand, the multiple regression models on Ca-U revealed significance only for α1-MG and β2-MG (Table 7), indicating that the observed Cd-U-dependent Ca-U increase was confounded strongly by renal tubular function.

4. Discussion

The grouping analyses allowed us to see the effect of Cd on bones independent of age and menstrual status, but other possible confounding factors could not be excluded sufficiently. This indicates the necessity of multivariate analyses to explore the real causes of osteoporosis in our population. We deliberately made

Table 6
Urinary proteins in subgroups divided by urinary Cd levels and age classes

Age classes	Urinary Cd (μg/g cr.)					Regression coefficients
	<2.5	⩾2.5, <3.5	⩾3.5, <5.0	⩾5.0	Total	
Urinary α1-MG/Cr (mg/g cr.)						
All subjects	3.68 (1.95) (range ND-30.72)	4.39 (1.91)* (ND-19.35)	5.17 (1.93)* (ND-37.33)	5.74 (1.94)* (0.86–56.04)	4.70 (1.97) (ND-56.04)	0.403**
Premenopause (41–48 y.o.)	3.07 (1.78)	3.52 (1.88)	3.04 (1.84)	3.44 (1.59)	3.23 (1.79)	0.047
Perimenopause (49–55 y.o.)	4.05 (1.95)	4.15 (1.85)	4.32 (1.73)	4.82 (1.96)	4.30 (1.87)	0.166**
Younger postmenopause (56–65 y.o.)	4.47 (1.85)	4.89 (1.85)	5.63 (1.86)*	5.87 (1.86)*	5.34 (1.87)	0.279
Older postmenopause (66–75 y.o.)	5.30 (1.85)	6.28 (1.91)	7.01 (1.89)*	7.48 (1.89)*	6.76 (1.90)	0.403
Urinary β2-MG/Cr (μg/g cr.)						
All subjects	118.5 (1.93) (range ND-1235.77)	133.5 (2.11) (ND-1555.56)	153.3 (2.39)* (ND-9352.03)	187.9 (2.43)* (ND-5911.11)	146.7 (2.26) (ND-9352.03)	13.840**
Premenopause (41–48 y.o.)	101.9 (1.84)	112.2 (2.06)	99.7 (1.85)	114.0 (1.84)	105.7 (1.90)	1.749
Perimenopause (49–55 y.o.)	126.6 (1.81)	131.6 (2.07)	123.0 (1.93)	159.8 (2.09)	133.6 (1.98)	6.654
Younger postmenopause (56–65 y.o.)	132.9 (2.02)	128.7 (2.09)	164.6 (2.20)	174.6 (2.14)*	154.2 (2.15)	9.348
Older postmenopause (66–75 y.o.)	173.5 (2.16)	195.2 (2.25)	202.3 (3.11)	279.1 (2.85)*	222.8 (2.76)	20.622**

Note: α1-MG, α_1-microglobulin; β2-MG, β_2-microglobulin; Cr, creatinine. Data are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).
ND, not detected (α1-MG, less than 0.9 mg/L; β2-MG, less than 70 μg/L).

Table 7
Multiple regression analyses on BMD and urinary Ca excretion ($n = 1243$)

Dependent variable	Independent variables	Model 1			Model 2			Model 3			Model 4		
		SPRC	PCC	*P* value	SPRC	PCC	*P* value	SPRC	PCC	*P* value	SPRC	PCC	*P* value
BMD	Age	−0.573	−0.541	0.000	−0.582	−0.556	0.000	−0.577	−0.540	0.000	−0.586	−0.553	0.000
	BMI	0.286	0.367	0.000	0.287	0.368	0.000	0.286	0.367	0.000	0.288	0.368	0.000
	Grip	0.106	0.129	0.000	0.108	0.131	0.000	0.106	0.129	0.000	0.108	0.131	0.000
	Cd-B*	−0.004	−0.005	0.869	−0.004	−0.005	0.867						
	Cd-U/Cr*							0.015	0.019	0.509	0.012	0.015	0.601
	α1-MG/Cr*	−0.045	−0.057	0.044				−0.047	−0.059	0.038			
	β2-MG/Cr*				−0.030	−0.038	0.180				−0.031	−0.039	0.167
	Ca-U/Cr*	−0.087	−0.115	0.000	−0.088	−0.114	0.000	−0.089	−0.117	0.000	−0.090	−0.116	0.000
	Ca intake/E	−0.001	−0.001	0.976	−0.001	−0.001	0.964	−0.001	−0.001	0.960	−0.001	−0.002	0.951
	VD intake/E	0.020	0.027	0.337	0.019	0.026	0.359	0.021	0.028	0.325	0.020	0.027	0.349
	District B	−0.069	−0.073	0.011	−0.070	−0.073	0.010	−0.071	−0.074	0.009	−0.071	−0.074	0.009
	District C	−0.056	−0.059	0.039	−0.055	−0.058	0.041	−0.058	−0.061	0.031	−0.057	−0.060	0.035
	District D	−0.063	−0.065	0.022	−0.064	−0.065	0.022	−0.065	−0.067	0.019	−0.065	−0.067	0.019
	District E	−0.133	−0.117	0.000	−0.130	−0.114	0.000	−0.140	−0.125	0.000	−0.136	−0.121	0.000
		$R' = 0.717$			$R' = 0.717$			$R' = 0.717$			$R' = 0.717$		
Ca-U/Cr*	Age	0.116	0.098	0.001	0.114	0.101	0.000	0.108	0.090	0.002	0.104	0.090	0.002
	BMI	0.051	0.053	0.063	0.047	0.050	0.081	0.051	0.053	0.063	0.047	0.050	0.079
	Grip	−0.085	−0.078	0.006	−0.089	−0.083	0.003	−0.077	−0.071	0.013	−0.080	−0.075	0.008
	Cd-B*	0.100	0.092	0.001	0.094	0.088	0.002						
	Cd-U/Cr*							0.095	0.093	0.001	0.094	0.093	0.001
	α1-MG/Cr*	0.216	0.207	0.000				0.206	0.196	0.000			
	β2-MG/Cr*				0.276	0.274	0.000				0.270	0.267	0.000
	Ca intake/E	0.031	0.031	0.271	0.042	0.043	0.130	0.029	0.030	0.300	0.040	0.041	0.148
	VD intake/E	0.009	0.009	0.742	0.021	0.022	0.440	0.008	0.008	0.787	0.020	0.020	0.476
	District B	0.139	0.110	0.000	0.145	0.117	0.000	0.118	0.093	0.001	0.125	0.100	0.000
	District C	0.074	0.058	0.040	0.080	0.065	0.023	0.073	0.058	0.041	0.080	0.064	0.024
	District D	0.126	0.098	0.001	0.130	0.103	0.000	0.098	0.076	0.008	0.103	0.081	0.005
	District E	0.169	0.112	0.000	0.153	0.103	0.000	0.178	0.120	0.000	0.160	0.110	0.000
		$R' = 0.376$			$R' = 0.413$			$R' = 0.376$			$R' = 0.414$		

Note: SPRC, standard partial regression coefficient; PCC, partial correlation coefficient; R', multiple correlation coefficients adjusted for the degrees of freedom; BMD, bone mineral density; BMI, body mass index; Cd-B, blood Cd level; Cd-U, urinary Cd level; α1-MG, urinary α1-microglobulin; β2-MG, urinary β2-microglobulin; Ca-U, urinary Ca level; Cr, creatinine; Ca intake/E, energy adjusted calcium intake; VD intake/E, energy adjusted vitamin D intake. The SPRCs of the four districts (B, C, D, and E) represent contrasts between each district and a reference area, district A.
*Converted into base-10 logarithms for analysis.

Table 8
Multiple logistic regression analysis on the subjects with reduced BMD (the subject number is 1243)

Dependent variable	Independent variables	Model 1			Model 2			Model 3			Model 4		
		SPRC	*P* value	OR (95% CI)	SPRC	*P* value	OR (95% CI)	SPRC	*P* value	OR (95% CI)	SPRC	*P* value	OR (95% CI)
The subjects with reduced BMD	Age	1.856	0.000	1.218 (1.185–1.251)	1.879	0.000	1.220 (1.188–1.254)	1.867	0.000	1.219 (1.186–1.252)	1.888	0.000	1.222 (1.189–1.255)
	BMI	−0.672	0.000	0.814 (0.769–0.862)	−0.675	0.000	0.813 (0.768–0.861)	−0.669	0.000	0.815 (0.770–0.862)	−0.672	0.000	0.814 (0.769–0.862)
	Grip	−0.259	0.008	0.949 (0.913–0.986)	−0.264	0.007	0.948 (0.912–0.985)	−0.256	0.008	0.949 (0.913–0.987)	−0.261	0.007	0.949 (0.913–0.986)
	Cd-B	0.097	0.305	1.058 (0.950–1.178)	0.099	0.297	1.059 (0.951–1.179)						
	Cd-U/Cr							0.021	0.795	1.009 (0.942–1.080)	0.028	0.725	1.012 (0.946–1.083)
	α 1-MG/Cr	0.022	0.782	1.005 (0.971–1.040)				0.020	0.808	1.004 (0.970–1.040)			
	β 2-MG/Cr				−0.080	0.313	1.000 (0.999–1.000)				−0.080	0.311	1.000 (0.999–1.000)
	Ca-U/Cr	0.256	0.003	1.003 (1.001–1.005)	0.282	0.001	1.003 (1.001–1.005)	0.259	0.002	1.003 (1.001–1.005)	0.284	0.001	1.003 (1.001–1.005)
	Ca intake/E	−0.001	0.990	0.990 (0.212–4.614)	−0.008	0.926	0.930 (0.200–4.329)	−0.005	0.951	0.953 (0.205–4.425)	−0.012	0.887	0.895 (0.193–4.153)
	VD intake/E	−0.025	0.758	0.740 (0.109–5.036)	−0.030	0.709	0.693 (0.101–4.755)	−0.027	0.737	0.720 (0.105–4.909)	−0.032	0.694	0.679 (0.099–4.663)
	District B	0.185	0.091	1.668 (0.922–3.018)	0.175	0.108	1.626 (0.899–2.941)	0.178	0.104	1.639 (0.903–2.975)	0.168	0.125	1.595 (0.879–2.895)
	District C	0.182	0.105	1.661 (0.899–3.066)	0.169	0.132	1.603 (0.868–2.963)	0.192	0.087	1.709 (0.926–3.155)	0.179	0.110	1.648 (0.892–3.044)
	District D	0.187	0.114	1.688 (0.882–3.229)	0.176	0.138	1.636 (0.854–3.131)	0.183	0.124	1.670 (0.869–3.211)	0.171	0.151	1.615 (0.839–3.106)
	District E	0.417	0.002	2.336 (1.363–4.002)	0.405	0.003	2.280 (1.331–3.908)	0.460	0.000	2.553 (1.525–4.274)	0.447	0.001	2.485 (1.486–4.157)
Correlation ratio (R^2)			0.413			0.413			0.411			0.412	

Note: SPRC, standard partial regression coefficient; OR, Odd's ratio; YAM, Japanese youg adult mean; BMD, bone mineral density; BMI, body mass index; Cd-B, blood Cd level; Cd-U, urinary Cd level; α-MG, urinary α1-microglobulin; β2-MG, urinary β2-microglobulin; Ca-U, urinary Ca level; Cr, creatinine; Ca intake/E, energy adjusted calcium intake; VD intake/E, energy adjusted vitamin D intake. The dependent variable is divided into two groups, reduced BMD (<80%) and normal BMD (≥80%). The SPRCs and ORs of the four districts (B, C, D and E) represent contrasts between each district and a reference area, district A.

suitable multiple regression models with several independent variables, demonstrating that Cd exposure at a level insufficient to induce renal dysfunction had no effect on BMD or urinary Ca excretion. In short, Cd would not induce bone mineral loss without renal dysfunction.

This result conflicts with those of the OSCAR study in Sweden (Alfvén et al., 2000) and the CadmiBel study in Belgium (Staessen et al., 1999), which proposed that low-dose Cd exposure would increase the risk of osteoporosis. This discrepancy may be partly due to differences among the investigated populations, including subject numbers, ethnic characteristics, or exposure routes: The European studies analyzed around 300–500 Caucasian women exposed to Cd mainly via inhalation, while our current study consisted of more than 1200 dietary-Cd-exposed Japanese women. But much more important must be the difference in Cd exposure levels: mean Cd-U of the investigated subjects in the European studies and our study were about 0.5 and 3.5 μg/g cr., respectively (incidentally, Cd-U levels of patients with Itai-itai disease were around 20 μg/g; cr. Kasuya et al., 1992b). It is unthinkable that the people with much lower Cd exposure are more at risk of osteoporosis, even if the difference in the race or exposure routes is taken into consideration.

This inconsistency must be derived from the misinterpretation of statistics, since careful inspection could not detect any essential differences between the results. In the OSCAR study, the negative correlation between Cd-U and BMD indicated by multiple regression analysis was actually not statistically significant as judged by the 95% CI, but age and weight were significantly correlated with BMD, corresponding to our result. On the other hand, the multiple regression analysis in the CadmiBel study showed a "statistically significant" relationship between BMD and urinary Cd excretion, but it probably had no "practical significance" because of the large number. For example, in our multiple regression models on Ca-U (Table 7), Cd-B and Cd-U showed statistical significance ($P<0.05$) despite very low PCC (<0.1), indicating that significance test with the large number of degrees of freedom would produce a false positive (Armitage and Berry, 1994; Horiguchi et al., 2004). In such cases, comparison of the SPRC between variables should take precedence over P values to judge the "practical significance". Therefore, the reasonable interpretation of their analysis result would be "the contributions of age and body size were much larger than Cd exposure," which reconciles to ours. There is another study reporting that Cd-U was correlated with BMD in the general Japanese female population (Honda et al., 2003), but the authors also judged the significance only by statistical P values despite the large number of degrees of freedom. Therefore, the results of the multiple regression analysis in this study were actually almost the same as ours, too; age and menstrual status were the most important factors, followed by weight, and Cd-U was the least important. In short, age and body weight are always the most important contributors to BMD in the general population without high Cd pollution.

Our study also demonstrated that urinary Ca excretion was primarily affected by renal tubular function, not by Cd exposure, among women without Cd-induced renal tubular dysfunction. This means that Ca excretion is independent of Cd but dependent on renal function. Therefore, Cd exposure at even higher levels sufficient to induce renal dysfunction would increase Ca excretion primarily due to the deterioration of renal tubular reabsorption (Aoshima et al., 1993). This result further supports our contention that bone mineral loss occurs after Cd-induced renal injury severely progresses. This conflicts with the CadmiBel study again, which suggested that calciuria would be a sensitive renal tubular biomarker for a low degree of Cd exposure in the general population (Buchet et al., 1990). However, their multiple regression models did not include renal tubular function as an independent variable.

The classical explanation that Cd-induced bone injury is a secondary effect of renal dysfunction still seems to be the most reasonable. Therefore, urinary low molecular weight proteins should be still the most sensitive and significant indicators to detect the adverse effects of Cd in the general population. The prospective study of this population will provide additional useful information for risk management of environmental exposure to Cd.

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