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 luteinizing 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.

Acknowledgments

We express special gratitude for the cooperation of Toyo Medic Co., Ltd. and Ohgida Hospital.

References

Akiba, T., Endou, H., Koseki, C., Sakai, F., 1980. Localization of 25-hydroxyvitamin D_3-1α-hydoxylase activity in the mammalian kidney. Biochem. Biophys. Res. Commun. 94, 313–318.

Alfvén, T., Elinder, C.G., Carlsson, M.D., Grubb, A., Hellstrom, L., Persson, B., Pettersson, C., Spang, G., Schutz, A., Järup, L., 2000. Low-level cadmium exposure and osteoporosis. J. Bone Miner. Res. 15, 1579–1586.

Aoshima, K., Kato, T., Teranishi, H., Horiguchi, H., Kasuya, M., 1993. Abnormalities of calcium, phosphorus and vitamin D metabolism with proximal renal tubular dysfunction in subjects environmentally exposed to cadmium. Jpn. J. Hyg. 47, 1009–1020 (Japanese with English abstract).

Armitage, P., Berry, G., 1994. Statistical inference. In: Statistical Methods in Medical Research, 3rd Edition. Blackwell Science, Oxford, pp. 93–153.

Bhattacharyya, M.H., Whelton, B.D., Stern, P.H., Peterson, D.P., 1988. Cadmium accelerates bone loss in ovariectomized mice and fetal rat limb bones in culture. Proc. Natl. Acad. Sci. USA 85, 8761–8765.

Buchet, J.P., Lauwerys, R., Roels, H., Bernard, A., Bruaux, P., Claeys, F., Ducoffre, G., Plaen, P.D., Staessen, J., Amery, A., Lijnen, P., Thijs, L., Rondia, D., Sartor, F., Remy, A.S., Nick, L., 1990. Renal effects of cadmium body burden of the general population. Lancet 336, 699–702.

Donaldson, M.D., Chambers, R.E., Woolridge, M.W., Whicher, J.T., 1989. Stability of α_1-microglobulin, β_2-microglobulin and retinol binding protein in urine. Clin. Chim. Acta 179, 73–77.

Friberg, L., Kjellstrom, T., Nordberg, G.F., 1986. Cadmium. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds.), Handbook on the Toxicology of Metals, 2nd Edition. Elsevier, Amsterdam, pp. 130–184.

Honda, R., Tsuritani, I., Noborisaka, Y., Suzuki, H., Ishizaki, M., Yamada, Y., 2003. Urinary cadmium excretion is correlated with calcaneal bone mass in Japanese women living in an urban area. Environ. Res. 91, 63–70.

Horiguchi, H., Teranishi, H., Niiya, K., Aoshima, K., Katoh, T., Sakuragawa, N., Kasuya, M., 1994. Hypoproduction of erythropoietin contributes to anemia in chronic cadmium intoxication: clinical study on Itai-itai disease in Japan. Arch. Toxicol. 68, 632–636.

Horiguchi, H., Oguma, E., Sasaki, S., Miyamoto, K., Ikeda, Y., Machida, M., Kayama, F., 2004. Dietary exposure to cadmium at close to the current Provisional Tolerable Weekly Intake does not affect renal function among female Japanese farmers. Environ. Res.

Ikeda, M., Ezaki, T., Tsukahara, Y., Moriguchi, J., Furuki, K., Furui, Y., Ukai, H., Okamoto, S., Sakurai, H., 2003. Threshold levels of urinary cadmium in relation to increases in urinary β_2-microglobulin among general Japanese populations. Toxicol. Lett. 137, 135–141.

Ishizaki, A., Fukushima, M., 1968. Studies on "Itai-itai" disease (Review). Jpn. J. Hyg. 23, 271–285 (Japanese with English abstract).

Järup, L., Alfvén, T., Persson, B., Toss, G., Elinder, C.G., 1998. Cadmium may be a risk factor for osteoporosis. Occup. Environ. Med. 55, 435–439.

Kasuya, M., Aoshima, K., Katoh, T., Teranishi, H., Horiguchi, H., Kitagawa, M., Hagino, S., 1992a. Natural history of Itai-itai disease: a long-term observation on the clinical and laboratory findings in patients with Itai-itai disease. In: Cook, M.E., Hiscock, S.A., Morrow, H., Volpe, R.A. (Eds.), Proceedings of the Seventh International Cadmium Conference New Orleans. Cadmium Association, London, pp. 180–192.

Kasuya, M., Teranishi, H., Aoshima, K., Katoh, T., Horiguchi, H., Morikawa, Y., Nishijo, M., Iwata, K., 1992b. Water pollution and by cadmium and the onset of Itai-itai disease. Water Sci. Technol. 25, 149–156.

Miyahara, T., Tanaka, M., Takeuchi, M., Mori-uchi, S., Miyata, M., Magai, M., Sugure, A., Matsushita, M., Kozuka, H., Kuze, S., 1992. Stimulative effects of cadmium on bone resorption in neonatal parietal bone resorption. Toxicology 73, 93–99.

Nogawa, K., Tsuritani, I., Kido, T., Honda, R., Yamada, Y., Ishizaki, M., 1987. Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1α, 25-dihydroxyvitamin D level. Int. Arch. Occup. Environ. Health 59, 21–30.

Ogoshi, K., Nanzai, Y., Moriyama, T., 1992. Decrease in bone strength of cadmium-treated young and old rats. Arch. Toxicol. 66, 315–320.

Roels, H., Bernard, A., Buchet, J.P., Goret, A., Lauwerys, R., Chettle, D.R., Harvey, T.C., Haddad, I.A.L., 1979. Critical concentration of cadmium in renal cortex and urine. Lancet 1 (8109), 221.

Ruth, K.S., Foreman, J.W., Segal, S., 1981. The Fanconi's syndrome and mechanisms of tubular transport dysfunction. Kidney Int. 20, 705–716.

Sasaki, S., Yanagibori, R., Amano, K., 1998. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. J. Epidemiol. 8, 203–215.

Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R., 1999. For the public health and environmental exposure to cadmium (PheeCad) study group, environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Lancet 353, 1140–1144.

Teranishi, H., Horiguchi, H., Morikawa, Y., Nishijo, M., Iwata, K., Katoh, T., Aoshima, K., Kasuya, M., Kanai, M., 1992. A fifteen-year follow-up study on renal dysfunction among people living in cadmium-polluted area. Water Sci. Technol. 25, 157–164.

The Committee on Standard Criteria of Primary Osteoporosis, 1996. The standard criteria of primary osteoporosis revised in 1996. Osteoporosis Jpn. 4, 643–653.

World Health Organization (WHO), 1992. Environmental Health Criteria 134: Cadmium. World Health Organization, Geneva.

World Health Organization (WHO), 2000. WHO Food Additive Series 46: Cadmium. World Health Organization, Geneva.

Yamagata, N., Shigematsu, I., 1970. Cadmium pollution in perspective. Bull. Inst. Publ. Health 19, 1–27.