and C, respectively (1200 in total). In contrast, data on cadmium concentrations in rice samples from the households were used without exclusion, since they are generally used as an indicator not for an individual exposure level, but for a regional one, because of their large yearly fluctuation (Horiguchi et al., 2004).

2.2. Procedures for health examinations

About one week prior to the health examinations, we explained the purpose of the study and its protocol in a group orientation session and obtained written informed consent from all the participants. At the same time, we asked them to fill out two self-administered questionnaires. The first included questions about all their places of residence, origins of the rice they consumed, and medical history including diseases, therapies, and smoking habits. The second was a diet history questionnaire (DHQ) 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 eaten in Japan (Sasaki et al., 1998a). Estimates of intakes for food, energy, and selected nutrients were calculated using ad hoc computer algorithm for the DHQ based on Standard Tables of Food Composition in Japan, which were well validated previously (Sasaki et al., 1998b, 2000). Using the DHQ, we determined each participant's intakes of rice and energy. The rice intake was energy-adjusted using the density method (per 1000 kcal). We also asked the participants to bring small amounts of the polished rice that they consumed. Health examinations were held in the morning, and we measured the weight and height of the participants, and took peripheral blood and urine samples before breakfast. Nurses and nutritionists checked and collected the two questionnaires.

2.3. Analyses of blood and urine samples

We collected peripheral blood in two separate tubes; the first one with heparin for cadmium measurement, and the second one without anticoagulants to obtain serum samples by centrifugation for biochemical measurements. We divided urine samples, which were placed on ice immediately after collection, into three tubes; the first tube contained one drop of 20% sodium carbonate to prevent the destruction of β2MG due to low pH (Donaldson et al., 1989; Horiguchi et al., 2010), the second tube contained one drop of 0.1 mol nitric acid to stabilize cadmium, and the third tube, for measurements of α1MG and creatinine, did not contain any additives. Urinary α1MG and β2MG, and serum and urinary creatinine were assessed by the latex agglutination method (Itoh and Kawai, 1990; Itoh et al., 1988) and an enzymatic method (Guder et al., 1986), respectively. Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan) conducted all biochemical measurements. We adjusted urinary concentrations by creatinine. We calculated estimated glomerular filtration rate (eGFR), as an indicator for renal glomerular function, using the equation for female Japanese: eGFR (mL/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ × 0.739 (Matsuo et al., 2009). For a subject with cadmium nephropathy, we additionally examined red blood cell count, hemoglobin, and hematocrit in peripheral blood collected with EDTA by the automated hematology analyzer SE-9000 (Sysmex, Hyogo, Japan), inorganic phosphorus, iron, ferritin, and erythropoietin in serum by the enzymatic method (Berti et al., 1988), colorimetric method (Makino et al., 1988), chemiluminescent immunometric assay (Dupuy et al., 2009), and radioimmunoassay (Sherwood and Goldwasser, 1979), respectively, inorganic phosphorus in urine by the enzymatic method, and bone mineral density in the non-dominant forearm using dual energy X-ray absorption (DTX-200, Osteometer MediTech, Inc., Hawthorne, CA, USA). Tubular reabsorption of phosphorus (TRP) was calculated using the following formula: (1 − urinary phosphorus × serum creatinine/urinary creatinine × serum phosphorus) × 100 (%).

2.4. Determination of cadmium concentrations in whole blood, urine, and rice

We measured blood and urinary cadmium concentrations as indicators for recent exposure and renal accumulation levels, respectively (Nordberg et al., 2007). Cadmium concentrations in whole blood and rice were measured using HP 4500 ICP-MS (Yokogawa Analytical Systems, Tokyo, Japan) after decomposition by microwaving with nitric acid, and urinary cadmium concentrations were determined using flameless atomic absorption spectrometry (SIMAA 6000; Perkin-Elmer, Norwalk, CT, USA) after mixing urine samples with nitric acid and holding them for 24 h. Ultrapur nitric acid (Kanto Kagaku, Tokyo, Japan) and nitric acid for Ultratrace Analysis (Wako Pure Chemical Industries, Osaka, Japan) were used. Indium and thallium were added to all samples as internal standards. The standard solutions for cadmium, indium, and thallium were purchased from Wako Pure Chemical Industries. The influence of the sample matrix on cadmium measurements was checked using standards, including CRM195 (Institute for Reference Materials and Measurements) for peripheral blood, 69,071 Level 1 (human urine) (Bio Rad) for urine, and NIES No. 10 (the National Institute of Environmental Science in Japan) for rice. For the certified values of blood, urinary, and rice concentration of standards (5.06 ± 0.15 μg/L, 6.0 (4.8–7.2) μg/L, and 0.32 ± 0.02 μg/L, respectively), the actual measured values were 4.86, 4.92, 4.95, and 4.98 μg/L (coefficient of variation (CV): 1.04%), 5.8, 6.0, 6.3, 6.3, and 6.0 μg/L (CV: 3.57%), and 0.28, 0.30, and 0.30 μg/L (CV: 3.94%), respectively. The limit of quantitation (LOQ) was calculated at ten-times the standard deviation of the reagent blank measurements (n = 5). No item in contact with the samples, including plastic bottles, tubes, and syringes, had detectable cadmium contamination. IDEA Consultants, Inc. (Metocean Environment, Inc.) (Shizuoka, Japan), which has successfully participated in the German External Quality Assessment Scheme (G-EQUAS) intercomparison program for blood and urinary cadmium measurement in the field of occupational and environmental medicine, conducted these measurements of cadmium concentrations.

2.5. Statistical analysis

Age and eGFR, which followed a normal distribution, were presented as arithmetic means with arithmetic standard deviations (SDs). The other data (i.e., cadmium levels in rice, blood and urine, and urinary α1MG and β2MG levels) were presented as medians with 25th and 75th percentiles, since they often included extraordinarily high values. For multiple comparison, the Bonferroni–Holm correction was performed to compare the means, and Steel's test or Steel–Dwass's test was performed to compare the medians. After excluding subjects with extremely low or high energy intake (≤1000 or ≥3500 kcal; n = 18), the data of energy-adjusted rice intakes were presented as medians, which were applied by the Kruskal–Wallis test. The Kruskal–Wallis test and Jonckheere–Terpstra test, or the one-way analysis of variance (ANOVA) and trend test using the cumulative χ^2 method were used to examine trends in the age- and urinary cadmium-dependent changes of the above data. The χ^2 test, with Yates correction if applicable, was used to compare the proportions of rice with a cadmium concentration above the safety standard. The Cochran–Armitage test was used to examine trends of the prevalence rates of subjects with high urinary cadmium or β2MG levels. In multiple regression analyses for urinary β2MG, we made two models using blood or urinary cadmium in addition to age as independent variables, because of collinearity between them. We used the Smirnov–Grubbs' test to judge whether values of the variables or residuals were outliers or not. In those models, the data of urinary β2MG, blood and urinary cadmium, and urinary creatinine were converted into base-10 logarithms, and urinary variables were not adjusted by creatinine level, which was added as an independent variable instead (Barr et al., 2005). We judged

Table 1
Cadmium concentrations in rice (μg/g) from the three areas.

	Area A	Area B			Area C	
Cropped year	2006	2000[a]	2001[b]	2002	2003	2004
Number	241	359	643	114	240	198
Median	0.070	0.176[c]	0.149[c]	0.177[c]	0.156[c]	0.085[c]
(25–75th percentile)	(0.043–0.105)	(0.108–0.273)	(0.092–0.238)	(0.113–0.275)	(0.081–0.254)	(0.047–0.154)
Range[d]	<0.005–0.330	0.012–1.358	<0.010–0.971	0.031–0.730	<0.020–0.687	0.008–0.669
% of >0.4 μg/g	0.0	11.4	8.4	12.3	9.2	1.5

[a] One-year stored rice in 2001.
[b] Newly harvested rice in 2001 (570) and one-year stored rice in 2002 (73).
[c] Significant difference with the value in area A ($p < 0.05$, Steel's test).
[d] The limit of quantitation was 0.010, 0.020, and 0.005 μg/g in 2001, 2002 to 2003, and 2004 to 2006, respectively.

significance of factors not only by their p values, but also their partial correlation coefficients (PCCs) as well as the standard regression coefficients (βs), since the statistical is inclined to produce false positives at higher degrees of freedom (Armitage and Berry, 1994; Horiguchi et al., 2004). Values less than LOQ or the minimal measurable limit were replaced with half the value for the statistical calculation. Statistical analyses were performed using SPSS release 17.0 (SPSS Japan, Tokyo, Japan) based on the basic management of data by Excel Tokei ver. 6.0 (Esumi, Tokyo, Japan).

3. Results

3.1. Cadmium concentrations in rice

Cadmium concentrations in rice from three areas are shown in Table 1. Rice from areas B and C were further divided by the cropped years to observe the yearly fluctuation. Medians were significantly higher in areas B and C than in area A, although the level in area C in 2004 was around 54% of that in 2003 ($p < 0.05$). While there was no rice with a cadmium concentration above 0.4 μg/g in area A, around 10% of such rice was detected in area B without significant fluctuations ($p = 0.192$). On the other hand, the rate in area C decreased markedly from 9.2% in 2003 to 1.5% in 2004 ($p = 0.001$). The median values of rice consumption in the areas A, B, and C were almost the same levels, although they were statistically different (184, 209, and 190 g/1000 kcal, respectively, $p = 0.002$).

Table 2
Blood and urinary cadmium levels in 1200 female farmers from three areas.

	Area A	Area B	Area C
Number (age, years old)			
All ages	222 (61.9 ± 7.5)	623 (59.1 ± 8.6[a])	355 (57.5 ± 8.1[a])
	Range 42–79	Range 40–78	Range 41–77
40–49 years	15 (46.2 ± 2.0)	99 (45.4 ± 2.9)	67 (46.1 ± 2.6)
50–59 years	63 (55.3 ± 2.8)	201 (54.5 ± 2.9)	138 (54.6 ± 2.7)
60–69 years	114 (64.8 ± 2.7)	265 (64.6 ± 2.7)	123 (63.8 ± 2.8[a])
70–79 years	30 (72.5 ± 2.4)	58 (72.7 ± 2.2)	27 (72.7 ± 2.6)
Blood cadmium (μg/L)			
All ages	2.15 (1.60–2.90)	3.83 (2.80–5.22)[b]	3.47 (2.39–5.19)[b]
	Range 0.76–6.90	Range 0.55–13.1	Range 0.74–31.2
40–49 years	2.40 (1.70–3.40)	3.62 (2.60–5.07)[b]	2.80 (1.88–4.55)
50–59 years	2.00 (1.50–2.90)	3.23 (2.43–4.77)[b]	2.89 (1.98–3.98)[b,c]
60–69 years	2.10 (1.70–2.80)	4.14 (3.05–5.28)[b]	3.98 (2.85–6.02)[b]
70–79 years	2.15 (1.88–2.70)	4.49 (3.64–5.99)[b]	6.25 (5.02–8.82)[b,c]
K–W test[d]/J–T test[e]	$p = 0.64$/$p = 0.76$	$p < 0.001$/$p < 0.001$	$p < 0.001$/$p < 0.001$
Urinary cadmium (μg/g cr.)			
All ages	3.03 (2.37–4.31)	4.38 (3.16–6.07)[b]	6.24 (4.42–8.97)[b,c]
	Range 1.04–16.7	Range 0.51–27.3	Range 0.35–29.7
40–49 years	2.32 (1.86–2.75)	3.73 (2.54–5.28)[b]	4.32 (2.58–5.70)[b]
50–59 years	2.98 (2.09–4.42)	4.24 (2.88–5.84)[b]	6.00 (4.29–7.80)[b,c]
60–69 years	3.33 (2.49–4.54)	4.59 (3.56–6.62)[b]	7.62 (5.84–10.1)[b,c]
70–79 years	2.99 (2.34–4.07)	4.90 (3.48–6.66)[b]	9.34 (7.22–13.4)[b,c]
K–W test/J–T test	$p = 0.035$/$p = 0.093$	$p = 0.001$/$p < 0.001$	$p < 0.001$/$p < 0.001$

Data of age are presented as the arithmetic mean ± SD, and data of cadmium are presented as the median (25–75th percentile).
[a] Significant difference with the value in area A ($p < 0.05$, Bonferroni–Holm correction).
[b] Significant difference with the value in area A ($p < 0.05$, Steel–Dwass's test).
[c] Significant difference with the value in area B ($p < 0.05$, Steel–Dwass's test).
[d] Kruskal–Wallis test.
[e] Jonckheere–Terpstra trend test.

3.2. Blood and urinary cadmium levels in three areas

We stratified the study subjects by age to exclude its confounding effects on cadmium accumulation or renal function (Tables 2 and 3). The female farmers in areas B and C were slightly younger than those in area A, but mean ages for each age-specific subgroup were similar among the three areas.

While blood and urinary cadmium levels in area A did not increase with age, those in areas B and C showed significant age-dependent increases (Table 2). Blood cadmium levels in almost all age-specific subgroups were significantly higher in areas B and C than in area A, and significantly higher in area C than in area B at 70–79 years old. Urinary cadmium levels were also significantly higher in areas B and C than in area A at all ages, and significantly higher in area C than in area B at 50–79 years old. Notably, the median at 70–79 years in area C was 9.34 μg/g cr., more than three times that in area A and very close to the threshold for cadmium nephropathy (10 μg/g cr.). The proportion of subjects with a urinary cadmium level of more than the threshold was 1.4%, 5.5%, and 15.8% in areas A, B, and C, respectively, and showed an increasing trend in the order ($p < 0.001$).

3.3. Urinary α1MG and β2MG levels in three areas

Urinary α1MG and β2MG levels showed age-dependent increasing trends in all areas (Table 3). Especially, there were significant sudden rises of the protein levels from 60–69 to 70–79 year-old groups in area C ($p < 0.05$ by Steel–Dwass's test). While there were no significant differences in urinary α1MG or β2MG levels at 40–59 years among the three areas, both urinary proteins at 60–69 years in areas B and urinary β2MG at 60 years or more in area C were significantly higher than in area A. Notably, the urinary β2MG level at 70–79 years of age in area C was 493 μg/g cr., about 2.6 times that in area A, and exceeded the minimal cutoff value for an adverse renal effect of cadmium, 300 μg/g cr. (Bernard, 2004; Elinder et al., 1985). On the other hand, there were no significant differences in eGFR levels among the areas at 60–79 years (Table 3).

Furthermore, we stratified the subjects in each age group into three groups based on urinary β2MG levels, 300 and 1000 μg/g cr., which are generally used as cutoff values for "incipient cadmium tubulopathy"

Table 3
Urinary α_1-microglobulin and β_2-microglobulin, and estimated glomerular filtration rate (eGFR) levels in 1200 female farmers from three areas.

	Area A	Area B	Area C
Urinary α_1-microglobulin (mg/g cr.)			
All ages	4.43 (2.73–6.96)	4.78 (3.09–8.22)	4.58 (2.75–7.42)
	Range ND[a]–24.1	Range ND–56.0	Range ND–48.6
40–49 years	2.74 (1.44–3.51)	2.86 (1.95–4.69)	3.48 (2.18–5.62)
50–59 years	4.32 (2.57–6.81)	4.64 (3.11–7.80)	4.24 (2.60–6.20)
60–69 years	4.55 (2.74–6.98)	5.70 (3.85–9.01)[b]	5.61 (3.55–7.85)
70–79 years	6.51 (3.60–11.8)	6.90 (3.34–12.7)	11.3 (3.79–17.5)
K–W test[c]/J–T test[d]	$p < 0.001/p < 0.001$	$p < 0.001/p < 0.001$	$p < 0.001/p < 0.001$
Urinary β_2-microglobulin (μg/g cr.)			
All ages	135 (84–210)	149 (98–251)[b]	150 (100–287)[b]
	Range ND–1220	Range ND–5690	Range ND–15,300
40–49 years	102 (92–116)	111 (78–158)	116 (70–167)
50–59 years	137 (88–190)	146 (97–252)	141 (92–222)
60–69 years	138 (80–210)	160 (109–262)[b]	172 (115–307)[b]
70–79 years	188 (105–389)	224 (116–474)	493 (174–1523)[b,e]
K–W test/J–T test	$p = 0.042/p = 0.016$	$p < 0.001/p < 0.001$	$p < 0.001/p < 0.001$
eGFR (mL/min/1.73 m^2)			
All ages	79.8 ± 14.8	81.7 ± 14.7	78.6 ± 14.3[f]
	Range 30.6–130	Range 43.5–144	Range 28.2–133
40–49 years	92.8 ± 15.0	88.1 ± 15.8	84.5 ± 15.9
50–59 years	82.0 ± 12.5	83.7 ± 13.1	80.0 ± 12.3[f]
60–69 years	78.7 ± 15.3	80.0 ± 14.1	76.4 ± 12.5
70–79 years	73.4 ± 13.0	71.8 ± 14.1	67.1 ± 18.3
ANOVA/trend test	<0.001/<0.001	<0.001/<0.001	<0.001/<0.001

Data of urinary protein levels are presented as the median (25–75th percentile), and data of eGFR are presented as the arithmetic mean ± SD.
[a] Not detected (α_1-microglobulin, less than 0.9 mg/L; β_2-microglobulin, less than 70 μg/L).
[b] Significant difference with the value in area A ($p < 0.05$, Steel–Dwass's test).
[c] Kruskal–Wallis test.
[d] Jonckheere–Terpstra trend test.
[e] Significant difference with the value in area B ($p < 0.05$, Steel–Dwass's test).
[f] Significant difference with the value in area B ($p < 0.05$, Bonferroni–Holm correction).

and "irreversible proteinuria", respectively (Bernard, 2004; Elinder et al., 1985; Iwata et al., 1993), and observed the prevalence (Fig. 2). The proportions of subjects with 300 μg/g cr. or more in the 60–69 and 70–79 year-old groups showed significant increasing trends from areas A to C ($p = 0.013$ and $p = 0.006$, respectively). In area C, there was a 75-year-old woman diagnosed with "overt cadmium nephropathy" (over 10,000 μg/g cr. of urinary β2MG) (Aoshima, 1987; Bernard, 2004; Shiroishi et al., 1977). As demonstrated in Table 4, she showed very high cadmium accumulation (blood cadmium, 31.2 μg/L; urinary cadmium, 18.8 μg/g cr.), renal tubular dysfunction (urinary β2MG, 15,300 μg/g cr.; TRP, 77.9%) along with decreased glomerular function (eGFR, 37.8 mL/min/1.73 m^2), and osteoporosis (T% of young adult mean, 47.4%), but not iron deficiency nor renal anemia (serum iron, 67 μg/dL; serum ferritin, 27 ng/mL; serum erythropoietin, 33.6 mIU/mL).

3.4. Urinary protein levels of all subjects stratified by age and urinary cadmium levels

Since values of blood and urinary cadmium distribute very widely in each area, especially that areas B and C include quite a few subjects whose values of them are similar with those of area A (Table 2), comparison between the three areas does not show a real relationship between cadmium exposure and its effects. Therefore, we combined the subjects from the three areas and stratified them according to age and urinary cadmium levels, to make clearer the age-relevant cadmium effects on renal function (Tables 5 and 6). First we divided the subjects in each age-specific group by the 25th, 50th, and 75th percentiles of urinary cadmium, which were around 3.0, 4.5, and 6.5 μg/g cr., respectively. Then we further divided the groups with the highest urinary cadmium by 10 μg/g cr., resulting in five subgroups, because subjects with extremely high urinary β2MG values were scattered mainly over 10 μg/g cr. at 70–79 years (Fig. 3).

The stratification revealed different urinary cadmium-dependent changes of urinary α1MG and β2MG levels among four age-specific subgroups (Table 6). The subgroup of 40–49 years did not show significant dose–response association between urinary cadmium and either urinary α1MG ($p = 0.191$) or β2MG ($p = 0.454$). On the other hand, there were significant, although mild, linear dose–response trends of urinary α1MG levels in the subgroup of 50–59 years ($p = 0.002$), and urinary β2MG levels in the subgroups of 50–59 years ($p = 0.005$) and 60–69 years ($p < 0.001$). In addition, urinary β2MG in the subgroup of 70–79 years showed quite a different dose–response increasing trend from other age-specific subgroups ($p = 0.033$): the value in the subgroup of 10 μg/g cr. or more was by far the highest compared with other subgroups, to the level of incipient cadmium tubulopathy, which is exemplified in Fig. 4. On the other hand, eGFR levels did not show any urinary cadmium-dependent changes (Table 6).

To further clarify the differences in the renal effects of cadmium among age groups, we performed multiple regression analyses for the urinary β2MG in each age group, using age and blood or urinary cadmium as independent variables (Table 7). Although there were a few subjects who had extremely high values of urinary β2MG, the multiple regression models yielded significant multiple correlation coefficients, and there were no outliers in residuals except for one subject in the subgroup of 40–49 years. Blood and urinary cadmium were not significant variables in the subgroups of 40–49 and 50–59 years. On the other hand, they were significant in the subgroups of 60–69 and 70–79 years, especially that βs and PCCs of blood and urinary cadmium were much higher than those of age in the subgroup of 70–79 years.

4. Discussion

Rice from areas B and C was highly contaminated by cadmium, partly exceeding the safety standard, 0.4 μg/g (Codex, 2008). While rice from area B showed high cadmium contamination constantly from 2000 to 2002, the level in area C was largely down from 2003 to 2004 (Table 1). This change could be explained by the fact that the JAs in these areas and the prefectural administrative agency had just started guidance for farmers from 2002 to take measures to lower cadmium absorption from the soil; the flooding of paddy fields before and after heading during August (Arao et al., 2010). Still, it is estimated that cadmium levels in rice in area C had been much higher for decades since the mean concentration was reported as 0.66 μg/g in 1970s in area C (Saito et al., 1977). These results suggest that the farmers ingested cadmium high enough to induce renal tubular dysfunction through home-harvested rice constantly from birth until around 2003.

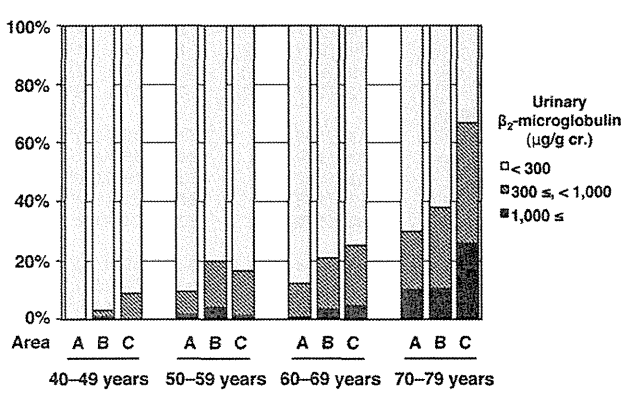

Fig. 2. Prevalence of subjects with a high urinary β_2-microglobulin level in three areas.

Table 4
Clinical characteristics of a subject with overt cadmium nephropathy.

Personal background		
Living history	In area C throughout her life	
Work history	Farmer from 15 years old	
Smoking history	None	
Current symptom	Back pain	
Current treatment	None	
Medical history	Hysterectomy at 65 years (no history of renal disease)	
Medical test items	Test values	Reference ranges
Age (years old)	75	
Height (cm)	132.5	
Weight (kg)	41.6	
Body mass index	23.7	
Blood cadmium (g/L)	31.2	
Urinary cadmium (μg/g cr.)	18.8	
Urinary α_1-microglobulin (mg/g cr.)	48.6	
Urinary β_2-microglobulin (μg/g cr.)	15,300	
TRP[a] (%)	77.9	>80[b]
Serum creatinine (mg/dL)	1.09	0.47–0.79
eGFR[c] (mL/min/1.73 m^2)	37.8	30–59: moderate decrease[d]
Bone mineral density (g/cm^2)	0.288	
T% of bone mineral density (%)[e]	47.4	≥70.0[f]
Z% of bone mineral density (%)[g]	82.1	
Red blood cell (10^4/mm^3)	411	380–500
Hemoglobin (g/dL)	11.2	11.5–15.0
Hematocrit (%)	37.5	34.8–45.0
Serum iron (μg/dL)	67	40–180
Serum ferritin (ng/mL)	27	4.0–64.2
Serum erythropoietin (mIU/mL)	33.6	9.1–32.8

[a] Tubular reabsorption of phosphorus.
[b] According to the criteria for cadmium-induced renal tubular dysfunction from Ministry of the Environment Government of Japan.
[c] Estimated glomerular filtration rate.
[d] According to the criteria for chronic renal disease from Kidney Disease: Improving Global Outcomes (KDIGO).
[e] The ratio of young adult mean (20–44 years old).
[f] According to the criteria for osteoporosis from Japanese Society for Bone and Mineral Research.
[g] The ratio of the same age mean.

In accordance with the change of oral cadmium exposure, blood cadmium levels, reflecting recent exposure, were relatively lower in area C than in area B except for the 70–79 year-old group (Table 2). In contrast, area C showed much higher urinary cadmium levels, meaning high accumulation in the kidneys, than in area B. The high blood cadmium level observed at 70–79 years in area C might come from a high body burden in addition to recent oral exposure, since an increasing proportion of blood cadmium after long-term exposure is related to the body burden (Nordberg et al., 2007). These results suggest that previous cadmium exposure levels would have been much higher in the upstream area where mines existed than in the downstream. The area would be one of the most severely polluted ones in Japan today, since the urinary cadmium level in the 70–79 year-olds, 9.34 μg/g cr., was very close to the level in female farmers at 64–69 years in the Jinzu River Basin in 2003, 9.85 μg/g cr. (Horiguchi et al., 2010).

Renal function at 60 years or more was decreased in the polluted areas, especially showing an incipient cadmium tubulopathy level of urinary β2MG along with one subject with overt cadmium nephropathy in the 70–79 years group in area C. These are reasonable results considering the urinary cadmium level close to the threshold for cadmium nephropathy. Therefore, Japanese farmers residing in such cadmium-polluted areas are at risk of renal dysfunction through consumption of home-harvested rice. It is necessary to take appropriate countermeasures for it, such as the flooding of paddy fields or checking cadmium concentration in home-harvested rice.

We demonstrated the age-relevant different relationships between urinary cadmium and its renal effects. While no significant association was observed at 40–49 years, there were mild linear dose–response relationships at 50–69 years. Similar observation has been reported in populations exposed to low environmental levels of cadmium (Buchet et al., 1990; Ohno et al., 2007; Suwazono et al., 2000), but it is a notable finding that the renal function was within a normal range over a "threshold" of urinary cadmium level. Cadmium, binding to metallothionein, a low-molecular weight, cysteine-rich protein whose expression is induced by cadmium (Kägi, 1991), is filtrated through glomeruli and reabsorbed at proximal tubular cells in a megalin-mediated way, which is inhibitory to β2MG uptake (Klassen et al., 2004; Wolff et al., 2006). Therefore, the increase in urinary cadmium–metallothionein without overt nephropathy might lead to parallel increase in urinary β2MG excretion. In contrast, the relationship at 70–79 years showed a more distinct dose–response decrease in renal function, with an abrupt deterioration to the level of incipient cadmium tubulopathy over a threshold urinary cadmium level. These results suggest that cadmium, even after accumulating over this "threshold", does not affect the kidneys at younger ages, but causes renal deterioration abruptly at older ages. Incidentally, we reported in a study of the Jinzu River Basin that there were a slight,

Table 5
Blood and urinary cadmium levels in 1200 female farmers stratified by age and urinary cadmium levels.

	Urinary cadmium (μg/g cr.)					Total
	<3.0	3.0≤, <4.5	4.5≤, <6.5	6.5≤, <10	10≤	
Number (age, years old)						
All ages	288 (57.2 ± 9.1)	305 (59.0 ± 8.5[a])	294 (59.4 ± 8.1[a])	220 (60.1 ± 7.6[a])	93 (62.5 ± 7.4[a])	1200 (59.2 ± 8.4)
40–49 years	66 (45.4 ± 2.7)	49 (45.4 ± 3.0)	37 (45.9 ± 2.6)	20 (46.7 ± 2.2)	9 (47.9 ± 1.8)	181 (45.8 ± 2.7)
50–59 years	105 (54.5 ± 3.2)	92 (54.6 ± 2.8)	110 (54.8 ± 2.7)	76 (54.6 ± 2.7)	19 (56.2 ± 2.3)	402 (54.7 ± 2.8)
60–69 years	90 (64.5 ± 2.9)	137 (64.2 ± 2.7)	123 (65.0 ± 2.8)	102 (64.2 ± 2.7)	50 (64.5 ± 2.5)	502 (64.5 ± 2.8)
70–79 years	27 (72.7 ± 2.4)	27 (72.7 ± 2.6)	24 (72.6 ± 2.1)	22 (72.6 ± 2.4)	15 (72.9 ± 2.4)	115 (72.7 ± 2.3)
Blood cadmium (μg/L)						
All ages	2.33 (1.69–3.30)	3.01 (2.16–4.10)[a]	3.53 (2.58–4.86)[a]	4.68 (3.25–6.23)[a]	6.36 (4.16–8.54)[a]	3.34 (2.31–4.83)
40–49 years	2.34 (1.70–3.43)	2.94 (2.15–4.03)	4.06 (3.10–5.26)[a]	6.12 (4.25–7.93)[a]	5.93 (2.84–8.05)[a]	3.25 (2.28–4.61)
50–59 years	2.30 (1.62–3.15)	2.81 (2.00–3.85)[a]	2.98 (2.34–4.04)[a,b]	3.82 (2.87–5.70)[a,b]	6.47 (3.40–8.45)[a]	2.96 (2.11–4.20)
60–69 years	2.36 (1.68–3.37)	3.06 (2.21–4.06)[a]	3.61 (2.65–4.90)[a]	4.70 (3.60–6.26)[a]	6.02 (4.59–8.17)[a]	3.58 (2.50–4.96)
70–79 years	2.24 (1.70–3.64)	3.54 (2.24–4.82)	5.05 (3.28–6.09)[a]	5.43 (4.23–6.73)[a]	8.52 (6.36–13.1)[a]	4.13 (2.60–6.18)[b]
Urinary cadmium (μg/g cr.)						
All ages	2.34 (1.83–2.66)	3.79 (3.39–4.15)[a]	5.39 (4.93–5.93)[a]	7.79 (7.10–8.87)[a]	11.9 (10.5–13.7)[a]	4.54 (3.06–6.61)
40–49 years	2.21 (1.78–2.55)	3.78 (3.28–4.20)[a]	5.22 (4.81–5.66)[a]	7.77 (7.20–9.00)[a]	11.8 (10.6–13.6)[a]	3.78 (2.40–5.26)
50–59 years	2.42 (1.82–2.73)	3.88 (3.39–4.10)[a]	5.39 (5.01–5.90)[a]	7.66 (6.99–8.78)[a]	12.3 (10.4–14.3)[a]	4.54 (2.95–6.17)[b]
60–69 years	2.38 (1.91–2.61)	3.81 (3.44–4.15)[a]	5.39 (4.93–5.94)[a]	7.80 (7.09–8.89)[a]	11.9 (10.6–13.3)[a]	4.86 (3.46–7.10)[b]
70–79 years	2.35 (1.83–2.72)	3.70 (3.43–4.00)[a]	5.80 (5.28–6.07)[a,b]	8.02 (7.16–9.36)[a]	13.4 (10.7–14.6)[a]	4.76 (3.07–7.65)[b]

Data of age are presented as the arithmetic mean ± SD, and data of cadmium are presented as the median (25–75th percentile).
[a] Significant difference with the value in the group with urinary cadmium < 3 μg/g cr. ($p < 0.05$, Steel's test).
[b] Significant difference with the value at 40–49 years ($p < 0.05$, Steel's test).

Table 6
Urinary α_1-microglobulin and β_2-microglobulin, and estimated glomerular filtration rate (eGFR) levels in 1200 female farmers stratified by age and urinary cadmium levels.

	Urinary cadmium (μg/g cr.)					Total
	<3.0	3.0≤, <4.5	4.5≤, <6.5	6.5≤, <10	10≤	
Urinary α_1-microglobulin (mg/g cr.)						
All ages	4.09 (2.49–6.59)	4.62 (2.72–7.62)[a]	4.74 (3.11–7.63)[a]	5.13 (3.34–8.20)[a]	6.56 (3.57–11.1)[a]	4.65 (2.90–7.71)
40–49 years	2.78 (1.94–4.11)	2.68 (2.14–4.64)	3.21 (2.30–5.89)	2.67 (1.63–4.12)	3.48 (2.19–6.98)	2.90 (2.05–4.73)
50–59 years	3.90 (2.46–6.54)[b]	4.24 (2.45–6.49)[b]	4.57 (3.15–7.07)	4.98 (3.19–8.56)[b]	5.73 (3.58–7.83)	4.44 (2.78–6.89)[b]
60–69 years	4.77 (3.57–7.53)[b]	5.48 (3.52–9.29)[b]	5.01 (3.44–8.06)[b]	5.80 (3.72–8.03)[b]	6.41 (3.64–11.1)	5.25 (3.59–8.44)[b]
70–79 years	7.87 (3.56–11.8)[b]	6.58 (3.65–12.2)[b]	5.71 (2.47–12.4)	6.57 (3.34–11.7)[b]	12.7 (7.21–21.2)[b]	7.87 (3.56–12.7)[b]
Urinary β_2-microglobulin (μg/g cr.)						
All ages	127 (88–198)	141 (83–208)[a]	150 (95–259)[a]	161 (110–307)[a]	231 (128–415)[a]	147 (95–248)
40–49 years	111 (84–144)	104 (68–155)	126 (69–210)	110 (66–143)	160 (90–296)[a]	111 (77–160)
50–59 years	134 (89–195)	136 (81–188)	134 (93–280)	174 (101–314)[b]	194 (109–283)	142 (92–225)[b]
60–69 years	141 (83–205)	152 (90–221)[b]	160 (111–233)	171 (112–307)[a,b]	214 (124–415)[a]	158 (100–262)[b]
70–79 years	257 (114–406)[b]	165 (107–512)[b]	178 (98–443)	264 (133–782)[b]	498 (247–2452)[a,b]	248 (126–601)[b]
eGFR (mL/min/1.73 m^2)						
All ages	80.0 ± 13.9	79.4 ± 14.2	81.5 ± 15.3	80.4 ± 14.1	82.0 ± 17.0	80.5 ± 14.6
40–49 years	88.3 ± 13.9	82.7 ± 13.0	89.6 ± 17.6	85.3 ± 17.1	97.6 ± 26.7	87.2 ± 15.9
50–59 years	80.6 ± 11.5	81.9 ± 14.0	83.0 ± 12.9	83.7 ± 12.9	81.1 ± 13.4	82.2 ± 12.8
60–69 years	75.2 ± 12.4	78.5 ± 14.5	79.3 ± 14.7	79.6 ± 13.6	83.1 ± 14.2[c]	78.8 ± 14.1
70–79 years	73.4 ± 17.2	69.2 ± 10.6	73.7 ± 19.3	68.7 ± 11.3	70.1 ± 15.1	71.1 ± 15.0

Data of urinary protein levels are presented as the median (25–75th percentile), and data of eGFR are presented as the arithmetic mean ± SD.
[a] Significant difference with the value in the group with urinary cadmium <3 μg/g cr. ($p < 0.05$, Steel's test).
[b] Significant difference with the value at 40–49 years ($p < 0.05$, Steel's test).
[c] Significant difference with the value in the group with urinary cadmium <3 μg/g cr. ($p < 0.05$, Bonferroni–Holm correction).

dose-dependent decrease in renal function and severe dysfunction that occurs sporadically (Horiguchi et al., 2010). However, significant relevance of aging to the different renal effects of cadmium was clearly demonstrated in this study for the first time. Still, there were some subjects at 70–79 years who had no renal tubular dysfunction despite urinary cadmium levels over the threshold, suggesting an individual difference in duration of cadmium exposure, induction of metallothionein, etc.

Therefore, it may be necessary to evaluate these two types of cadmium renal effects separately. Since the mild linear dose-dependent renal effect of cadmium was within the normal range, its toxicological significance might be not high. In addition, there were decreasing trends of urinary creatinine and body weight levels along with the increases in urinary β2MG levels at less than 70 years old (not shown), which might overestimate the effect. In fact, multiple regression analyses including urinary creatinine as an independent variable showed no significant effects of cadmium exposure on renal functions at younger ages (Table 7). Nevertheless, a benchmark dose approach could be applied for it (Crump, 1984), and actually much lower values of the benchmark dose lower confidence limits (BMDLs) than the traditional threshold for cadmium nephropathy have been reported in populations without overt cadmium nephropathy. For example, the BMDL of urinary cadmium for urinary α1MG was 0.5–0.8 μg/g cr. in Swedish women whose mean urinary cadmium level was 0.76 μg/g cr. (Suwazono et al., 2006), and the BMDL of urinary cadmium for urinary β2MG was 1.4 μg/g cr. in Japanese women whose mean urinary cadmium level was 1.3 μg/g cr. (Suwazono et al., 2011). On the other

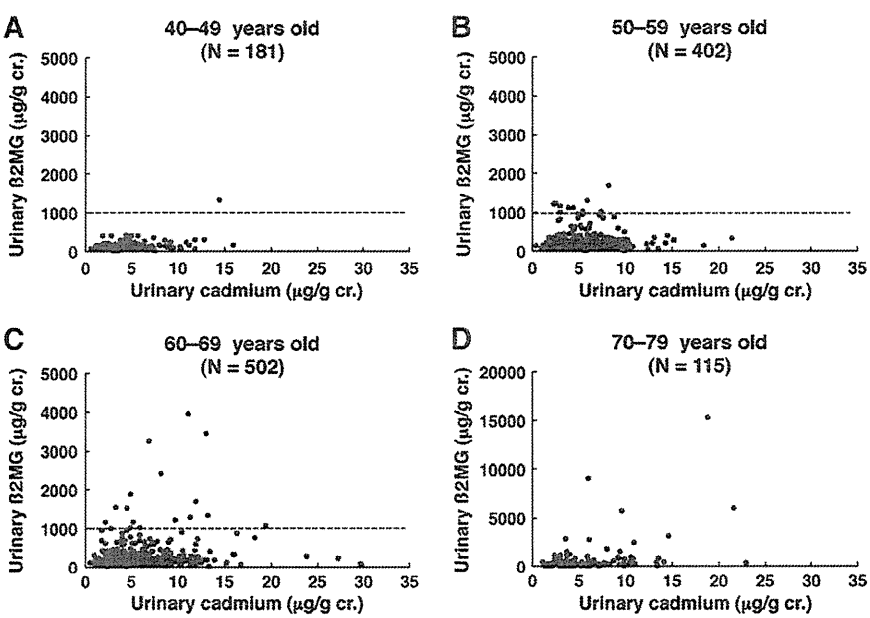

Fig. 3. Relationships between urinary cadmium and β_2-microglobulin (β2MG) levels in the groups of 40–49 (A), 50–59 (B), 60–69 (C), and 70–79 years old (D). The dotted lines indicate 1000 μg/g cr. of urinary β_2-microglobulin (irreversible proteinuria).

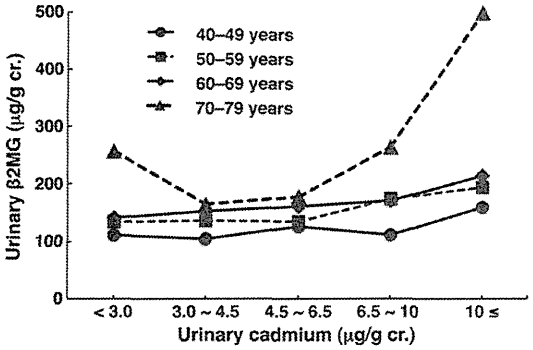

Fig. 4. *Dose relationships between urinary cadmium and β_2-microglobulin (β2MG) levels in four age-specific subgroups. Markers indicate medians.*

hand, the traditional threshold, 10 μg/g cr., was originally estimated from the critical level of cadmium in the renal cortex for the appearance of tubular proteinuria (Bernard et al., 1979). Besides, it has clinical and toxicological significance that leads to *itai itai* disease due to the continuous urinary loss of phosphorus and calcium (Nordberg et al., 2007), and has been supported for decades (Ikeda et al., 2003; Roels et al., 1993). The findings in this study suggest that, although this traditional threshold for cadmium nephropathy remains reasonable, age should be taken into account for it.

The relationship between urinary cadmium levels and renal function at 70–79 years showed a *J*-like curve: urinary β2MG levels in the subgroups with 3.0–4.5/4.5–6.5 μg/g cr. were slightly lower, although not significant, than that in the subgroup of less than 3.0 μg/g cr., and that in the subgroup of 10 μg/g cr. or more was by far the highest (Fig. 4). This may suggest that the deterioration of renal function with aging would be suppressed by a moderate level of exposure to cadmium, while renal dysfunction develops quickly at levels exceeding the threshold. One plausible explanation for the suppressive effect of cadmium on renal dysfunction is the involvement of metallothionein. Since metallothionein protects against the toxicity of not only cadmium but also other hazardous agents (Klaassen et al., 2009), the renal cells might retain their function through cadmium-induced production of metallothionein. More detailed investigations including measurements of metallothionein are necessary to test this possibility.

There may have been several limitations to this study. Since the JAs had information about the head of each farming household registered, but not about how many female members there were in each age group, we could not know the precise numbers of female members at 40 years or more, the eligible age for this study. But "rates of application per household" were roughly estimated as about 5% in area A and about 10% in areas B and C. Since the participants were collected by self-application, there might be selection bias. However, they were recruited equally from every farming hamlet in each area to make the bias minimal. Actually, the resultant cadmium levels differed significantly between the areas, indicating selection bias to be within the range of JA members. Although there were small numbers of female farmers in the divided groups at 70–79 years, which might affect the power in multiple comparison analyses, we got similar and reliable results by the multiple regression analyses. We adjusted concentrations of urinary substances by urinary creatinine, which might be amplified among elderly populations due to loss of muscle mass (Moriguchi et al., 2005). The age-dependent increasing trends were observed in a similar way without adjustment by urinary creatinine while there were age-dependent decreasing trends of urinary creatinine and body weight levels, suggesting the amplification of concentrations of urinary substances by urinary creatinine adjustment would be minimal. The comparisons between the areas or between urinary cadmium-stratified groups in 70–79 year-old groups would also be minimally affected by creatinine adjustment, since the values of urinary substances in area C and in the subgroup of 10 μg/g cr. or more of urinary cadmium were still significantly higher than other groups without the adjustment, and urinary creatinine and body weight levels did not show significant differences. Therefore, it is suggested that our data were not heavily influenced by sampling, selection, or confounding bias.

Table 7
Multiple regression analyses on log urinary β_2-microglobulin in female farmers at each age group.

Age group	Independent variables	β[a]	*p* value	PCC[b]	R[c]
40–49 years	Age	0.106	0.078	0.132	0.626
(N = 181)	log Blood cadmium	0.094	0.114	0.118	($p < 0.001$)
	log Urinary creatinine	0.633	<0.001	0.627	
	Age	0.112	0.067	0.137	0.621
	log Urinary cadmium	0.075	0.369	0.068	($p < 0.001$)
	log Urinary creatinine	0.582	<0.001	0.459	
50–59 years	Age	0.132	0.004	0.144	0.441
(N = 402)	log Blood cadmium	0.069	0.125	0.077	($p < 0.001$)
	log Urinary creatinine	0.443	<0.001	0.440	
	Age	0.124	0.007	0.135	0.443
	log Urinary cadmium	0.113	0.067	0.092	($p < 0.001$)
	log Urinary creatinine	0.363	<0.001	0.280	
60–69 years	Age	0.125	0.002	0.136	0.409
(N = 502)	log Blood cadmium	0.189	<0.001	0.202	($p < 0.001$)
	log Urinary creatinine	0.340	<0.001	0.350	
	Age	0.142	0.001	0.153	0.391
	log Urinary cadmium	0.184	<0.001	0.157	($p < 0.001$)
	log Urinary creatinine	0.228	<0.001	0.193	
70–79 years	Age	0.125	0.130	0.143	0.499
(N = 115)	log Blood cadmium	0.454	<0.001	0.468	($p < 0.001$)
	log Urinary creatinine	0.208	0.012	0.235	
	Age	0.152	0.088	0.161	0.359
	log Urinary cadmium	0.382	0.001	0.307	($p < 0.001$)
	log Urinary creatinine	−0.024	0.835	−0.020	

[a] Standard regression coefficient.
[b] Partial correlation coefficient.
[c] Multiple correlation coefficient adjusted for the degrees of freedom.

5. Conclusions

In cadmium-polluted areas in Japan, female farmers had been exposed to cadmium through constant consumption of home-harvested rice. Consequently, they showed high levels of cadmium accumulation and deterioration of renal function at older ages, including one case of cadmium nephropathy. Therefore, Japanese farmers in the polluted areas are at risk of cadmium-induced renal dysfunction. In addition, aging is significantly relevant to the different renal effects of cadmium: a mild linear dose-dependent effect in the population aged less than 70 years and an abrupt deteriorating effect in the aged population at a dose over the threshold of 10 μg/g cr. It may be necessary to evaluate these two types of cadmium renal effects separately.

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References

Aoshima K. Epidemiology of renal tubular dysfunction in the inhabitants of a cadmium-polluted area in the Jinzu River basin in Toyama Prefecture. Tohoku J Exp Med 1987;152:151–72.

Arao T, Ishikawa S, Murakami M, Abe K, Maejima Y, Makino T. Heavy metal contamination of agricultural soil and countermeasures in Japan. Paddy Water Environ 2010;8:247–57.

Armitage P, Berry G. Statistical inference. Statistical methods in medical research. 3rd ed. Oxford: Blackwell Science; 1994. p. 93-153.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005;113:192–200.

Bernard A. Renal dysfunction induced by cadmium: biomarkers of critical effects. Biometals 2004;17:519–23.

Bernard A, Buchet JP, Roels H, Masson P, Lauwerys R. Renal excretion of proteins and enzymes in workers exposed to cadmium. Eur J Clin Invest 1979;9:11–22.

Berti G, Fossati P, Tarenghi G, Musitelli C, Melzi d'Eril GV. Enzymatic colorimetric method for the determination of inorganic phosphorus in serum and urine. J Clin Chem Clin Biochem 1988;26:399–404.

Buchet JP, Lauwerys R, Roels H, Bernard A, Bruaux P, Claeys F, et al. Renal effects of cadmium body burden of the general population. Lancet 1990;336:699–702.

Codex. Codex general standard for contaminants and toxins in foods. http://www.mhlw.go.jp/shingi/2008/07/dl/s0708-3c.pdf2008. [CODEX STAN 193–1995, Rev.3-2007].

Crump KS. A new method for determining allowable daily intakes. Fundam Appl Toxicol 1984;4:854–71.

Donaldson MD, Chambers RE, Woolridge MW, Whicher JT. Stability of α_1-microglobulin, β_2-microglobulin and retinol binding protein in urine. Clin Chim Acta 1989;179:73–7.

Dupuy AM, Debarge L, Poulain M, Badiou S, Rossi M, Cristol JP. Determination of serum ferritin using immunoturbidimetry or chemiluminescent detection in comparison with radioimmunoassay a compendium of a methodological juxtaposition. Clin Lab 2009;55:207–15.

Elinder CG, Edling C, Lindberg E, Kågedal B, Vesterberg O. β_2-Microglobulinuria among workers previously exposed to cadmium: follow-up and dose–response analyses. Am J Ind Med 1985;8:553–64.

Guder WG, Hoffmann GE, Hubbuch A, Poppe WA, Siedel J, Price CP. Multicentre evaluation of an enzymatic method for creatinine determination using a sensitive colour reagent. J Clin Chem Clin Biochem 1986;24:889–902.

Health and Welfare Statistics Association. Life expectancy. Journal of health and welfare statistics, 54 (9). Tokyo: Health and Welfare Statistics Association; 2007. p. 68–9.

Horiguchi H, Oguma E, Sasaki S, Miyamoto K, Ikeda Y, Machida M, et al. Dietary exposure to cadmium at close to the current provisional tolerable weekly intake does not affect renal function among female Japanese farmers. Environ Res 2004;95:20–31.

Horiguchi H, Aoshima K, Oguma E, Sasaki S, Miyamoto K, Hosoi Y, et al. Latest status of cadmium accumulation and its effects on kidneys, bone, and erythropoiesis in inhabitants of the formerly cadmium-polluted Jinzu River Basin in Toyama, Japan, after restoration of rice paddies. Int Arch Occup Environ Health 2010;83:953–70.

Ikeda M, Ezaki T, Tsukahara T, Moriguchi J, Furuki K, Fukui Y, et al. Threshold levels of urinary cadmium in relation to increases in urinary β_2-microglobulin among general Japanese populations. Toxicol Lett 2003;137:135–41.

Itoh Y, Kawai T. Human α_1-microglobulin: its measurement and clinical significance. J Clin Lab Anal 1990;4:376–84.

Itoh Y, Enomoto H, Kawai T. A latex agglutination test for urinary β_2-microglobulin. Contrib Nephrol 1988;68:172–8.

Iwata K, Saito H, Moriyama M, Nakano A. Renal tubular function after reduction of environmental cadmium exposure: a ten-year follow-up. Arch Environ Health 1993;48:157–63.

Kägi JH. Overview of metallothionein. Methods Enzymol 1991;205:613–26.

Klaassen CD, Liu J, Diwan BA. Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 2009;238:215–20.

Klassen RB, Crenshaw K, Kozyraki R, Verroust PJ, Tio L, Atrian S, et al. Megalin mediates renal uptake of heavy metal metallothionein complexes. Am J Physiol Renal Physiol 2004;287:F393–403.

Kojima S, Haga Y, Kurihara T, Yamawaki T, Kjellström T. A comparison between fecal cadmium and urinary β_2-microglobulin, total protein, and cadmium among Japanese farmers. An epidemiological study of cooperation between Japan and Sweden. Environ Res 1977;14:436–51.

Makino T, Kiyonaga M, Kina K. A sensitive, direct colorimetric assay of serum iron using the chromogen, nitro-PAPS. Clin Chim Acta 1988;171:19–27.

Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–92.

Moriguchi J, Ezaki T, Tsukahara T, Fukui Y, Ukai H, Okamoto S, et al. Effects of aging on cadmium and tubular dysfunction markers in urine from adult women in non-polluted areas. Int Arch Occup Environ Health 2005;78:446–51.

Nordberg GF, Nogawa K, Nordberg M, Friberg LT. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook on the toxicology of metals. 3rd ed. Burlington, Vermont, USA: Academic Press; 2007. p. 445–86.

Ohno T, Sakamoto M, Kurosawa T, Dakeishi M, Iwata T, Murata K. Total mercury levels in hair, toenail, and urine among women free from occupational exposure and their relations to renal tubular function. Environ Res 2007;103:191–7.

Roels H, Bernard AM, Cárdenas A, Buchet JP, Lauwerys RR, Hotter G, et al. Markers of early renal changes induced by industrial pollutants. III. Application to workers exposed to cadmium. Br J Ind Med 1993;50:37–48.

Saito H, Shioji R, Hurukawa Y, Nagai K, Arikawa T. Cadmium-induced proximal tubular dysfunction in a cadmium-polluted area. Contrib Nephrol 1977;6:1–12.

Sasaki S, Yanagibori R, Amano K. 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 1998a;8:203–15.

Sasaki S, Yanagibori R, Amano K. Validity of a self-administered diet history questionnaire for assessment of sodium and potassium: comparison with single 24-hour urinary excretion. Jpn Circ J 1998b;62:431–5.

Sasaki S, Ushio F, Amano K, Morihara M, Todoriki O, Uehara Y, et al. Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. J Nutr Sci Vitaminol 2000;46:285–96.

Sherwood JB, Goldwasser E. A radioimmunoassay for erythropoietin. Blood 1979;54:885–93.

Shiroishi K, Kjellström T, Kubota K, Evrin PE, Anayama M, Vesterberg O, et al. Urine analysis for detection of cadmium-induced renal changes, with special reference to β_2-microglobulin. A cooperative study between Japan and Sweden. Environ Res 1977;13:407–24.

Suwazono Y, Kobayashi E, Okubo Y, Nogawa K, Kido T, Nakagawa H. Renal effects of cadmium exposure in cadmium nonpolluted areas in Japan. Environ Res 2000;84:44–55.

Suwazono Y, Sand S, Vahter M, Filipsson AF, Skerfving S, Lidfeldt J, et al. Benchmark dose for cadmium-induced renal effects in humans. Environ Health Perspect 2006;114:1072–6.

Suwazono Y, Nogawa K, Uetani M, Kido T, Nakagawa H. Reassessment of the threshold of urinary cadmium by using hybrid approach in a cadmium non-polluted area in Japan. Int J Hyg Environ Health 2011;214:175–8.

Tsuchiya K. Causation of Ouch-Ouch Disease (Itai-Itai Byō) — an introductory review. I. Nature of the disease. Keio J Med 1969;18:181–94.

WHO. Cadmium. WHO Food Additive series, 46. Geneva: World Health Organization; 2000.

Wolff NA, Abouhamed M, Verroust PJ, Thévenod F. Megalin-dependent internalization of cadmium–metallothionein and cytotoxicity in cultured renal proximal tubule cells. J Pharmacol Exp Ther 2006;318:782–91.