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Effects of aging on cadmium concentrations and renal dysfunction in inhabitants in cadmium-polluted regions in Japan

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ABSTRACT: The absorption of cadmium (Cd) may lead to Cd-related diseases such as renal tubular dysfunction and bone disease, and it is known to take around 10–30 years to reduce Cd concentrations to half their original levels. Urinary β_2-microglobulin (β_2-MG), N-acetyl-β-D-glucosaminidase (NAG), protein, glucose and albumin were used as indicators of renal dysfunction caused by Cd exposure. Our previous study found that urinary Cd concentrations had increased recently and that age was more strongly associated with urinary β_2-MG concentration than recent Cd body burden. Therefore, the purpose of the present study was to investigate the effect of aging on Cd concentrations and renal dysfunction. The Cd, β_2-MG, NAG, protein, glucose and albumin concentrations in the urine of 40 Japanese subjects (20 females and 20 males) environmentally exposed to Cd were collected. They lived in the Kakehashi River basin and were divided into three age categories: 50–69, 70–79 and 80–99 years. Significant differences in urinary Cd and β_2-MG concentrations were found among age groups, with urinary Cd levels tending to increase with age in both sexes. No significant correlations were found between urinary Cd and any indicators of renal dysfunction. The correlation between age, Cd and indicators of renal dysfunction was observed more clearly in females than in males. Age is more strongly correlated with indicators of renal dysfunction than Cd body burden. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: age; urinary cadmium; indicators of renal dysfunction; Kakehashi river basin; cadmium-polluted regions

Introduction

Cadmium (Cd) is a heavy metal and environmental pollutant, with industrial pollution, contaminated food and water and tobacco smoke considered to be the main sources of Cd exposure in humans (Nordberg *et al.,* 2007; Nawrot *et al.,* 2010). The absorption of Cd may lead to adverse health effects such as liver disease, kidney damage and bone disease, and it is known to take around 10–30 years to reduce Cd concentrations to half their original level (Nordberg *et al.,* 1985; Suwazono *et al.,* 2009). In this regard, Itai-itai ('it hurts-it hurts') disease was recognized by the Japanese Ministry of Health and Welfare in 1969 as being a result of chronic Cd poisoning (WHO, 1992). With regards to chronic exposure to Cd, experiments have shown that approximately 75% of the Cd absorbed is found in the liver and kidneys, with the distribution of Cd concentrations in the kidneys being extremely important as these organs are vital in the event of long-term exposure (Nordberg *et al.,* 1985, 2007; WHO, 1992). β_2-mioglobulin (β_2-MG), which is a low-molecular-weight protein, N-acetyl-β-D-glucosaminidase (NAG), a high-molecular-weight isoenzyme, protein, glucose and albumin in urine have been used as indicators for evaluating the renal dysfunction caused by Cd poisoning (Nogawa *et al.,* 1983, 1986a; Kido *et al.,* 1987, 1988, 1991; Nakagawa *et al.,* 2006; Nordberg *et al.,* 2007, 2008).

In non-Cd-polluted regions, some studies have suggested that the Cd concentration in the renal cortex peaks between the ages of 50 to 70 years, gradually decreasing after that (Tsuchiya *et al.,* 1976; Nogawa *et al.,* 1986a; Lyon *et al.,* 1999). In contrast, urinary Cd and β_2-MG concentrations increase almost linearly with age (Moriguchi *et al.,* 2005). However, our previous studies in a Cd-contaminated region showed that urinary Cd concentrations had increased significantly recently after previously decreasing by half. Similarly, urinary β_2-MG concentrations tended to increase over the 28-year study period in both sexes, with age being more strongly associated with urinary β_2-MG concentration than recent Cd body burden (Hoang *et al.,* 2016).

The Cd pollution in the Kakehashi River basin was improved in 1979 and 1980 by removing the polluted soils and adding unpolluted soils to rice fields. Accordingly, the mean Cd concentration in rice was reduced in 1980 (Kido *et al.,* 2001). However, most inhabitants kept eating rice which was produced from their fields. Moreover, the biological half-life of Cd was too long and Cd induced the renal dysfunction. The process of renal dysfunction is

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irreversible (Kido *et al.,* 1988, 1990; Nordberg *et al.,* 2007;). Therefore, health examinations for an inhabitant after cessation Cd are necessary. In light of our recent results, the aim of the present study was to elucidate the impact of aging on Cd concentration and renal dysfunction by analyzing the urinary Cd, β_2-MG, NAG, protein, glucose and albumin concentrations in urine samples from residents living in Cd-contaminated regions in Japan.

Materials and methods

Selection of study population

This is a long-term study that has been underway since 1986. At the baseline in 1986, all inhabitants over 4 years of age and living in a hamlet located in the Kakehashi River basin in Komatsu City (Ishikawa Prefecture) in Japan, an area previously polluted by Cd, were selected. A total of 214 inhabitants participated (participation rate: 75%). The number of subjects subsequently decreased as a result of death or moving elsewhere. Finally, 41 elderly subjects were selected in the present study. The Cd concentration in the urine from one female could not be determined as a result of an insufficient volume. Thus, the data in this study were based on 40 participants (20 male and 20 female). The mean age of the subjects was 74.60 ± 10.23 (range: 58–98) for males and 74.45 ± 8.50 years (range: 59–85) for females. The mean residence times for males and females were 67.00 ± 15.77 (range: 26–94) and 52.89 ± 13.73 years (range: 26–81), respectively. The health conditions of the subjects, as self-reported using questionnaires, were as follows: hypertension (8 subjects), cardiovascular disease (5 subjects), diabetes mellitus (3 subjects) and cancer (5 subjects; thyroid, bladder, stomach and the liver, plus a double cancer of colon and malignant lymphoma).

Sample collection and urine analysis

Samples were collected three times between August and November 2014. A spot urine sample was collected from each participant and transported in a cool box. Samples were kept frozen (−20°C) until the analysis of Cd, β_2-MG, NAG, protein, glucose, albumin and creatinine concentrations could be performed. As in our previous studies, the Cd concentration was analyzed by flameless atomic absorption spectrophotometry (Honda *et al.,* 1989), urinary NAG was measured using the fluorometric method of Asami (Nogawa *et al.,* 1986b) and urinary albumin was analyzed by radioimmunoassay (Kido *et al.,* 1991). Similarly, the latex agglutination test was used to analyze the β_2-MG concentration, and Jaffe's method was used to determine urinary Cr levels (Bonsnes and Taussky, 1945). A questionnaire was used to collect basic information from subjects, including age, sex, health condition and period of residence.

Calculation and statistical analysis

The urinary Cd, β_2-MG, NAG, protein, glucose and albumin concentrations measured were expressed as corrected for creatinine ($\mu g\ g^{-1}$ creatinine) and transformed to logarithms to improve normality before applying statistical tests. The aim of this study was to clarify the impact of aging on Cd concentrations and renal dysfunction. As such, the subjects were further separated into three age categories (50–69, 70–79 and 80–99 years) to compare urinary Cd, β_2-MG, NAG, protein, glucose and albumin and determine the correlation between urinary Cd and β_2-MG, NAG, protein, glucose and albumin concentrations for males and females separately and combined. The distribution was determined using the Shapiro–Wilk test, Pearson's correlation coefficient and Spearman's rank correlation coefficients were used to calculate the linear correlation between two variables in the case of a normal and non-normal distribution, respectively. Statistical comparisons of the mean differences between groups were calculated using a two-way analysis of variance (ANOVA) and Tukey's Post Hoc Test. Finally, multiple linear regressions were performed to associate each urinary concentration value, such as total protein, glucose, albumin, NAG and β_2-MG, as a dependent variable and Cd concentration and age as an independent variable.

The JMP 12 @ statistical software package (SAS Institute, Cary, NC, USA) and Excel 2010 (Microsoft, Redmond, WA, USA) were used. A significant difference was determined with a *P*-value of ≤ 0.05. Data are shown as geometric means (GM) with geometric standard deviations (GSD).

Ethics in research

This research was approved by the Kanazawa University bioethics committee (approval No. 512). All participants in this study were volunteers, and personal information was kept confidential.

Results

Table 1 shows a comparison of the urinary protein, glucose, albumin, NAG, β_2-MG and Cd concentrations subdivided by sex and age group. There were no significant differences regarding sex and age group except for urinary β_2-MG and Cd for the different age groups. Thus, significant differences in urinary β_2-MG and Cd were found between the 50–69 and 80–99 age groups, although almost all parameters showed an increasing trend from the 50–69 to the 80–99 age groups for both sexes.

Table 2 shows a correlation between age and urinary Cd, protein, glucose, albumin, NAG and β_2-MG concentrations for each sex alone and combined. In females, significant correlations were found between age and urinary protein, NAG and β_2-MG concentrations, with a borderline significance being found between age and urinary albumin and Cd concentrations. Except for the association between age and urinary Cd and β_2-MG concentrations, no significant associations were found in males. Furthermore, the combined sex group clearly showed significant correlations for all concentrations except for urinary glucose. The correlations between age and urinary Cd and β_2-MG concentrations for male and female subjects combined are shown in Figs. 1 and 2, respectively.

Table 3 shows a correlation between urinary Cd and protein, glucose, albumin, NAG, β_2-MG concentrations for each sex alone and combined. No significant correlations were found between urinary Cd and urinary protein, glucose, albumin and β_2-MG concentrations in males and females. Moreover, no significant correlations were found for the combined data. The correlation between urinary Cd and β_2-MG concentrations for male and female subjects combined is shown in Fig. 3.

Table 4 shows the association between urinary protein, glucose, albumin, NAG and β_2-MG concentrations and Cd concentration and age using a multiple regression analysis for each sex alone and combined. Each urinary indicator was used as a dependent variable, and urinary Cd and age were used as independent variables. With regards to age and period of residence, only age was selected as an independent variable to avoid collinearity as a very

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Table 1. Comparison of urinary protein, glucose, albumin, NAG, β_2-MG and Cd concentrations adjusted for creatinine, by sex and age group

		50–69 ($n = 13$)			70–79 ($n = 12$)			80–99 ($n = 15$)			*P*				
	Sex	n	GM	GSD	n	GM	GSD	n	GM	GSD	Sex	Age Group			
U-Protein	Females	6	4.294	1.282	7	10.75	2.878	7	16.49	3.179	0.571	0.187	50–69	70–79	80–99
	Males	7	8.186	4.057	5	4.487	2.805	8	10.64	4.121			A	A	A
U-Glucose	Females	6	17.08	3.169	7	19.09	4.790	7	31.11	3.675	0.392	0.614	50–69	70–79	80–99
	Males	7	34.82	7.660	5	25.77	7.327	8	48.69	6.641			A	A	A
U-Albumin	Females	6	10.38	1.844	7	13.87	4.609	7	32.69	6.228	0.562	0.186	50–69	70–79	80–99
	Males	7	13.44	7.711	5	5.966	6.295	8	23.58	4.401			A	A	A
U-NAG	Females	6	2.972	1.306	7	5.912	2.050	7	6.851	2.333	0.218	0.192	50–69	70–79	80–99
	Males	7	6.653	2.299	5	6.726	2.077	8	7.199	1.954			A	A	A
U-β_2-MG	Females	6	81.685	2.356	7	525.3	15.26	7	1800.7	10.53	0.670	0.007	50–69	70–79	80–99
	Males	7	158.7	4.965	5	125.4	3.276	8	1587.7	16.43			B	A B	A
U-Cd	Females	6	3.498	1.150	7	5.010	1.859	7	5.591	1.432	0.102	0.002	50–69	70–79	80–99
	Males	7	2.681	1.444	5	3.736	1.288	8	5.283	1.354			B	A B	A

GM: geometric mean; GSD: geometric standard deviation.
P: probability of significance.
Subgroups not connected by same letter demonstrate a significant difference.

Table 2. Correlation coefficients between age and urinary Cd, protein, glucose, albumin, NAG and β_2-MG concentrations adjusted for creatinine in males, females and both sexes in 2014

	Age					
	Females ($n = 20$)		Males ($n = 20$)		Total ($n = 40$)	
	r	*P*	*r*	*P*	*r*	*P*
U-Protein	0.751 [b]	0.0003	0.257 [a]	0.289	0.405 [a]	0.013
U-Glucose	0.402 [b]	0.079	0.221 [b]	0.349	0.254 [b]	0.114
U-Albumin	0.437 [b]	0.054	0.298 [a]	0.202	0.338 [a]	0.033
U-NAG	0.493 [a]	0.027	0.242 [a]	0.304	0.354 [a]	0.025
U-β_2-MG	0.559 [a]	0.011	0.557 [a]	0.011	0.553 [a]	0.0002
U-Cd	0.431 [a]	0.058	0.631 [a]	0.003	0.516 [a]	0.0007

[a]Pearson's correlation coefficient.
[b]Spearman's correlation coefficient.
P: probability of significance.

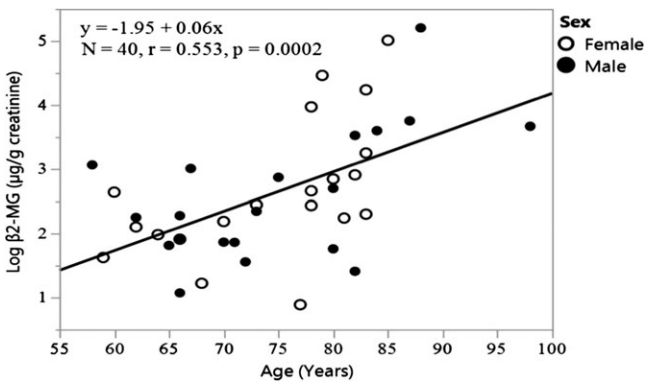

Figure 1. The correlation between age and urinary β_2-MG concentration adjusted for creatinine for male and female subjects combined.

close correlation between age and period of residence was found for the subjects studied ($r = 0.59$, $P < 0.0001$). No significant associations were found between urinary protein, glucose, albumin, NAG and β_2-MG concentrations and Cd. In contrast, significant associations were found between age and urinary protein, albumin and β_2-MG in females and the combined data.

Discussion

The increasing trend in Cd concentrations from the 50–69 to the 80–99 age groups seen in Table 1 can be explained by the impact of age. Various studies in several different countries have shown that Cd excretion increases with age, thereby supporting our findings (Nordberg *et al.,* 1985, 2007; Buchet *et al.,* 1990; WHO, 1992). The results displayed in Table 2 show the significant correlations between age and Cd concentration in males, as well as for the combined data, with a borderline significant correlation being

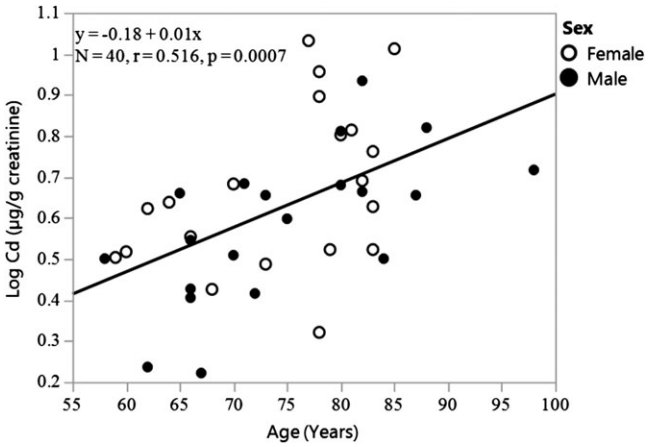

Figure 2. The correlation between age and urinary Cd concentration adjusted for creatinine for male and female subjects combined.

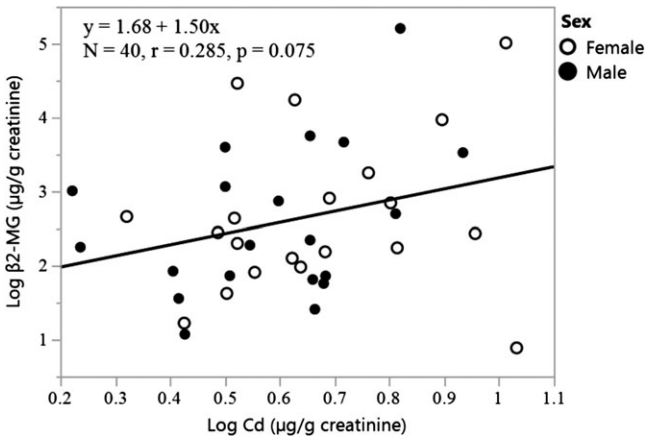

Figure 3. The correlation between urinary Cd and β_2-MG concentrations adjusted for creatinine for male and female subjects combined.

found in females. Once exposed to Cd, several organs tend to accumulate this metal. However, as subjects become older, the internal organs of the body gradually become atrophic and, when damaged, Cd is released into the urine, thus resulting in higher urinary Cd levels. Indeed, a case study on gastric cancer reported that Cd concentrations in urine increased more than 200-fold over only 1 year (Kido *et al.,* 1989a). Five cases of cancer were detected in our study, with one case aged 73 years and the others aged between 80 and 84 years. Furthermore, as the duration of previous Cd exposure was longer in the elderly group, this leads to a greater uptake of Cd than in the younger group and, therefore, higher urinary Cd concentrations. Another study showed that inhabitants exposed to Cd for more than 30 years (males) or more than 20 years (females) excreted higher Cd concentrations in urine (Kido *et al.,* 2001). Moreover, an experimental study in rabbits has shown that age is an aggravating factor in Cd poisoning (Nomiyama *et al.,* 1980). Given these findings, we consider that the combination of aging and Cd exposure affects the urinary Cd concentrations of people living in Cd-polluted regions.

Inhabitants with various diseases were included as subjects, in the present study, as they all lived at home and their health condition was stable, thus meaning that they could undergo the required examination, although two persons died 2 years later in 2016. Moreover, subjects were divided into two groups to compare urinary Cd concentration. The first group included 32 healthy subjects (mean Cd concentration: 4.626; range: 1.667–10.769) and the second group 8 subjects (3 with diabetes mellitus and 5 cancer patients) (mean Cd concentration: 4.831; range: 2.544–8.595). A comparison of the results showed no statistically significant differences between the two groups ($P = 0.858$). In contrast, the epidemiological study reported that the significant negative association between Cd-exposed and blood pressure was observed in inhabitants in Japan (Kurihara *et al.,* 2004). Besides, the studies from Belgium and United States provided that the level of 24-h urinary Cd excreted was found to correlate negatively with low blood pressure (Staessen *et al.,* 1984) and no association was shown between urinary Cd and blood pressure (Whittemore *et al.,* 1991). Furthermore, blood hypertension has not been observed in Itai-Itai disease patients (Kagamimori *et al.,* 1985) and Cd exposure could reduce the risk of prevalence hypertension (Zhang *et al.,* 1996). As such, all subjects were included in the study.

A sex difference was found in the correlation between age, Cd and indicators of renal dysfunction (Tables 2 and 3). In the present study, although this correlation was found more clearly in women than in men, it is nevertheless in accordance with other studies in Cd-contaminated regions (Kido *et al.,* 1989b; Hoang *et al.,* 2016). Females commonly exhibit iron deficiency caused by menstruation-related anemia or childbirth, which could lead to increased Cd absorption (Nogawa and Ishizaki, 1978; Berglund *et al.,*

Table 3. Correlation coefficients between urinary Cd and protein, glucose, albumin, NAG and β_2-MG concentrations adjusted for creatinine in males, females and both sexes in 2014

	U-Cd					
	Females ($n = 20$)		Males ($n = 20$)		Total ($n = 40$)	
	r	*P*	*r*	*P*	*r*	*P*
U-Protein	0.146 [b]	0.564	-0.046 [a]	0.853	0.070 [a]	0.679
U-Glucose	-0.033[b]	0.889	0.016 [b]	0.947	-0.017[b]	0.919
U-Albumin	-0.090 [b]	0.705	-0.065 [a]	0.785	-0.073[a]	0.654
U-NAG	0.423 [a]	0.063	0.094 [a]	0.694	0.219[a]	0.174
U-β_2-MG	0.206 [a]	0.385	0.369 [a]	0.109	0.285[a]	0.075

[a]Pearson's correlation coefficient.
[b]Spearman's correlation coefficient.
P: probability of significance.

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Table 4. The association between urinary protein, glucose, albumin, NAG and β_2-MG concentration adjusted for creatinine and Cd concentration and age in males, females and both sexes in 2014 using multiple regression analysis

	U- Protein			U- Glucose			U- Albumin			U- NAG			U- β_2- MG		
	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2
Females = 20															
Cd	-0.111	0.616	0.416	-0.124	0.633	0.099	-0.363	0.133	0.267	0.258	0.267	0.298	-0.044	0.847	0.416
Age	0.683	0.007		0.348	0.190		0.556	0.027		0.382	0.108		0.577	0.019	
Males = 20															
Cd	-0.298	0.317	0.124	0.069	0.824	0.035	-0.420	0.152	0.195	-0.098	0.751	0.064	0.031	0.907	0.124
Age	0.431	0.155		0.136	0.664		0.563	0.061		0.304	0.329		0.537	0.054	
Total = 40															
Cd	-0.168	0.350	0.185	-0.075	0.689	0.052	-0.337	0.057	0.198	0.049	0.783	0.127	-0.001	0.997	0.185
Age	0.487	0.009		0.258	0.176		0.512	0.005		0.328	0.075		0.553	0.001	

β: standardized coefficients; *P*: *P*-value; R^2: coefficient of determination.

1994). Similarly, men may move away to non-Cd-contaminated regions to find jobs when young, thus reducing their Cd exposure, whereas women are more likely to stay in such regions. Besides, the geometric mean of urinary Cd concentrations in each age group in females (50–69: 3.498 μg g^{-1} creatinine; 70–79: 5.010 μg g^{-1} creatinine; 80–99: 5.591 μg g^{-1} creatinine) for examination in our study were higher than the values of 2.4 μg g^{-1} creatinine for females found in non-polluted regions of Japan (Suwazono *et al.,* 2000; Suwazono *et al.,* 2015). Furthermore, in our follow-up data (which has not been published), the significant simple correlation between urinary Cd concentration in 1986 and urinary β_2-MG concentration in 2014 was shown in females ($r = 0.589$, $P = 0.027$), which could explain why women show a more significant relationship between Cd and renal indicators than men.

Table 2 shows a clear correlation between age and indicators of renal dysfunction, except for urinary glucose in the combined data. This could be explained by the sampling time, which depended on the subjects and could, therefore, result in a different glucose concentration in the urine. Also, the presence of glucose in urine could be as a result of both renal tubular damage caused by Cd exposure and by other health conditions. For example, three cases of diabetes mellitus were detected in this study. Previous studies in residents (aged 20–80 years) from four regions of Belgium with varying levels of Cd contamination showed that diabetes mellitus patients are more susceptible to the harmful effects of Cd in the proximal tubule (Buchet *et al.,* 1990). No significant correlations were found between Cd and the indicators of renal dysfunction (see Table 4), which could be explained by the fact that Cd concentrations fall by half after 30 years (Nordberg *et al.,* 1985; Suwazono *et al.,* 2009). Furthermore, Table 4 shows that age is more strongly associated with renal effect markers than Cd, especially β_2-MG, thus indicating the most useful indicator for assessing renal tubular dysfunction. In contrast, there was no significant correlation between age and NAG. Our previous studies showed that, in the event of Cd exposure, NAG activity increased with increasing β_2-MG concentration even although they have different excretion mechanisms in urine (Nogawa *et al.,* 1986b). In addition, the glomerular function is reflected by total protein and albumin in urine. Thus, severe Cd exposure leads to higher total protein and albumin levels in the urine of Cd-exposed subjects (Kido *et al.,* 1990, 1991).

The increase in Cd concentration with age is clear from Table 1. In non-Cd-polluted regions, some studies have shown that Cd concentrations in the renal cortex peak between the ages of 50 and 70 years, gradually decreasing thereafter (Tsuchiya *et al.,* 1976; Nogawa *et al.,* 1986a; Lyon *et al.,* 1999), thereby suggesting that the trends for study subjects living in Cd-polluted regions and those from non-polluted regions differ. Table 1 also shows that urinary β_2-MG concentrations tend to increase with age. A similar trend was observed in non-polluted regions (Suwazono *et al.,* 2000; Moriguchi *et al.,* 2005). However, there was essentially no difference between them. In non-polluted regions, the β_2-MG concentration increased with age, whereas in Cd-polluted regions the increase in β_2-MG concentrations was explained by a combination of both factors (aging and Cd exposure). Furthermore, in another of our previous studies, Cd-exposed persons with urinary β_2-MG concentrations of more than approximately 1000 μg g^{-1} creatinine tended to present irreversible renal tubular dysfunction (Kido *et al.,* 1988). Studies in non-Cd-polluted regions also showed that NAG should be used to monitor the effects of Cd exposure on renal tubules rather than β_2-MG (Moriguchi *et al.,* 2009). In our opinion, we strongly believe that β_2-MG is the most useful indicator for early detection of renal tubular damage in chronic Cd poisoning. Another study in a non-polluted region supports this point (Suwazono *et al.,* 2000).

With regards to the implications of the present findings for health risk assessment and management, measures to counter Cd pollution, especially the replacement of Cd-polluted soil by non-polluted soil in rice fields, affected both the present environmental situation and the health conditions of inhabitants. However, our findings also showed the significant difference between age subgroups more than 30 years after the cessation of Cd exposure, with higher urinary Cd concentrations and indicators of renal dysfunction found in older people. This means that renal tubular dysfunction is likely to be more severe in people who lived in Cd-contaminated areas and who were, therefore, environmentally exposed to Cd over a long period, as is the case in the present study, where subjects over the age of 80 years present the highest health risk. Moreover, our follow-up study showed that urinary β_2-MG concentrations tended to increase over the 28-year study period in both sexes, with age being more strongly associated with urinary β_2-MG concentration than recent Cd body burden (Hoang *et al.,* 2016). When renal tubular dysfunction is caused by Cd poisoning, irreversible aggravation is inevitable (Kido *et al.,* 1988, 1990; Nordberg *et al.,* 2007;). In addition, a multiple regression analysis showed that the correlation between age, Cd and indicators of renal dysfunction was observed more clearly in females

than in males. Similar Cd-induced adverse health effects have been reported previously, with Itai-Itai, for example, being more commonly observed in women (Nordberg *et al.,* 1985, 2008). As such, health examinations for inhabitants of Cd-polluted regions should be conducted on a regular basis. In addition, as a first step for preventing pollution, measures to control the discharge of pollutants and treatment involving filtration processes are important and necessary to prevent the Cd pollution caused by mining activities.

Conclusion

This study shows that the Cd concentration increases with age, with the latter being more strongly correlated with renal dysfunction than Cd body burden in inhabitants from a Cd-polluted region. The correlation between age, Cd and indicators of renal dysfunction was clearer in females than in males. Renal tubular dysfunction is inevitable after exposure to Cd and worsens, especially in the older group. As such, our research in this Cd-polluted region should continue for several more years.

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Conflict of interest

None of the authors has any conflicts of interest in this study.

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