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# Seventeen-Year Observation on Urinary Cadmium and $\beta_2$ -Microglobulin in Inhabitants after Cessation of Cadmium-Exposure in Japan

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**Abstract** The purpose of this study is to clarify the change and relationship of urinary cadmium (Cd) and  $\beta_2$ -microglobulin ( $\beta_2$ -MG) concentrations of inhabitants in Cd-polluted areas after soil restoration. The urinary Cd and  $\beta_2$ -MG concentrations of 25 males and 28 females did not show a significant change, 22 years after the Cd-polluted soil was restored. Once exposed to Cd, it was found to remain in the body, 22 years after the Cd-polluted soil was restored. However, this did not influence renal tubular dysfunction in most of the younger generation compared with elders heavily exposed to Cd.

**Keywords** Urinary-Cadmium, Urinary- $\beta_2$ -Microglobulin, Biological half life, Soil restoration

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Cadmium (Cd), atomic No 48, atomic weight 112 is a mineral found in mines. Cd is a by-product that is released to the environment when zinc is refined from the mines (Wada 1986). Residents in mining areas have been exposed to chronic Cd poisoning for long periods of time, for example the Kakehashi river basin (Ishikawa Prefecture) population were exposed for about 100 years. Kakehashi river basin inhabitants use the river water that Cd is released into for farming vegetables, rice etc. Hence, people were directly exposed by consuming farming foods as well as fish from the river. This oral exposure resulted in kidney dysfunction, osteomalacia, osteoporosis, liver injury and hypertension (Kasuya. 1985; Saito et al. 1993). The most common characteristic of Cd exposure is renal tubular dysfunction. An indicator of renal tubular dysfunction is urinary  $\beta_2$ -microglobulin ( $\beta_2$ -MG), a low molecular weight protein which is used as an indicator and judged by the limit 1000  $\mu\text{g/gCr}$  (Nogawa et al. 1983; Aoshima et al. 1988; Kubota et al. 1985). Due to Cd being released to the Jinzu river (Toyama Prefecture, Japan), inhabitants in the surrounding areas have been exposed for a long time. Itai-itai disease is the severest disease cause by chronic Cd poisoning. The direct translation of the Japanese phrase “Itai-itai” is based on the patient’s response “Ouch-ouch” to the many fractures in their bodies by osteomalacia (Aoshima, Kasuya 1993). The ministry of Health in Japan and Welfare recognized Itai-itai disease as the first mining-related illness in April 1968 (Matsunami 2006). There are six Cd-polluted areas in Japan. The largest Cd polluted area is Jinzu

43 river basin, followed by Kakehashi river basin (Nakagawa, Nishijo 1999).

44 In 1981 the Ishikawa Prefecture Government decided to restore the soil in the most Cd-polluted  
45 Kanehira areas around Kakehashi river basin (Ishikawa Prefecture 1975) and performed a physical  
46 examination on Kakehashi river basin inhabitants of over 50 years of age (Ishikawa Prefecture  
47 South Kaga Public Health 1997). It was evident from the results obtained that the biological  
48 half-life of Cd is 10-30 years (Friberg 1985) and Cd concentration increased with age (Aoshima,  
49 Kasuya 1993). The results showed that 14.3% men and 18.7% women were over the 1000  $\mu\text{g/g.Cr}$   
50 limit of urinary  $\beta_2\text{-MG}$ . However, only 6% men and 5% women were over the limit in the control  
51 area. This shows a significant difference (Kido 1995). In 1986, five years after soil restoration  
52 (Kido et al. 2002) were apprehensive about the effects of Cd on inhabitants 50 years and under, who  
53 were not examined in 1981 by Ishikawa Prefecture Government. Further tests were performed 4  
54 times over a 17 year period from 1986 to 2003, in inhabitants of most Cd -polluted area of  
55 Kakehashi River basin. The purpose of this study is to clarify the change and relationship of urinary  
56 Cd and  $\beta_2\text{-MG}$  concentrations of inhabitants in Cd- polluted areas after soil restoration.

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## 58 **Materials and Method**

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60 In 1981 the Ishikawa Prefecture Government restored the soil in the most Cd-polluted area in the  
61 Kakehashi river basin. Five years after soil restoration in 1986, investigations were carried out to  
62 determine the influence of Cd on the inhabitant health, and 3 further tests were carried out in 1991,  
63 1999 and 2003, over 17 years. The subjects were 53 inhabitants who received physical  
64 examinations 4 times during 17 years. The 53 inhabitants (50 years and under in 1986) included 25  
65 men and 28 women. The mean age of the subjects was 40.2 years old (men: 38.5, women: 42.1) in  
66 1986.

67 The research process was spread into two parts; 1) Urinary test 2) Questionnaire. The urinary test  
68 involved measuring concentration of urinary creatinine (Cr), Cd and  $\beta_2\text{-MG}$  in early morning urine.  
69 The concentration of urinary indicators was then corrected by Cr. Urinary  $\beta_2\text{-MG}$ , Cd and Cr were  
70 measured by Radioimmunoassay (RIA), Flameless Atomic Absorption Spectrophotometry (Honda  
71 et al 1989) and Jaffé methods respectively. The questionnaire involved obtaining basic data from  
72 subjects by gender, age, period of residence and Brinkman index.

73 Urinary Cd and  $\beta_2\text{-MG}$  concentrations were converted into a logarithm scale. The results were  
74 statistically analyzed using the one-way layout ANOVA, t-test, Pearson correlation coefficient, and  
75 multiple regression analysis. The significant difference was  $p \leq 0.05$  The software used for analysis  
76 was SPSS12.OJ for Windows.

77 Subject participation in this research was voluntary. An agreement was signed regarding this  
78 matter. The examination performed in 1999 and 2003 were accepted by the Kanazawa Medical  
79 University Bioethical committee. To protect privacy of the individual, only numbers (no names)  
80 were used during examination.

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## 84 **Results and Discussion**

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86 Table 1 shows that there is no significant difference in urinary Cd concentration ( $\mu\text{g/g.Cr}$ ) for men  
87 and women during a 17 year period, even though both of them showed decreasing tendency. It can  
88 be seen from table 2 that there is significant difference ( $p=0.000$ ) in men during 1991-1999 and  
89 1999-2003. However, in women there is no significant difference during a 17 year period. It can be  
90 seen from table 3 that there is a significant difference in 1986 ( $p=0.000$ ), 1991 ( $p=0.002$ ), 1999  
91 ( $p=0.003$ ) and 2003 ( $p=0.000$ ), when comparing the urinary Cd concentration between men and  
92 women. On the other hand, urinary  $\beta_2$ -MG concentration shows a significant difference between  
93 men and women in 1986 ( $p=0.000$ ), 1999 ( $p=0.022$ ) and 2003 ( $p=0.003$ ), but there is no significant  
94 difference in 1991 ( $p=0.174$ ). In both cases it can be clearly seen that the geometric mean is higher  
95 in females than males, except for mean urinary  $\beta_2$ -MG concentration in 1991. Table 4 shows that in  
96 25 males there is a significant correlation with urinary Cd and  $\beta_2$ -MG concentrations in 1999  
97 ( $p=0.044$ ). On the other hand, in 28 females there is a significant correlation between urinary Cd  
98 and  $\beta_2$ -MG concentration in 1986 ( $p=0.010$ ) and 2003 ( $p=0.033$ ). Urinary Cd and  $\beta_2$ -MG  
99 concentrations showed significant relationship in 2003. Fig 1 shows that only two subjects were  
100 found to exceed the critical urinary  $\beta_2$ -MG limit ( $1000 \mu\text{g/g.Cr}$ ) in 2003. Table 5 shows that there is  
101 no significant regression between urinary  $\beta_2$ -MG and urinary Cd, age, period of residence,  
102 Brinkman index in 25 males and 28 females, except for significant association in females between  
103 urinary  $\beta_2$ -MG and age ( $p=0.036$ ) as well as Brinkman index ( $p=0.040$ ) in 2003.

104 As shown in table 1 there is no significant difference in urinary Cd concentration in 25 males and  
105 28 females, 22 years after cessation of Cd exposure (soil restoration in 1981, first investigation 5  
106 years after in 1986 until 2003). This shows that once exposed to Cd it remains in the body for a long  
107 time. This agrees with the biological half life of Cd which is 10-30 years (Nordberg 1985). This  
108 shows that once exposed to Cd, irreversible injury (renal tubular dysfunction) was noted to occur  
109 when urinary  $\beta_2$ -MG excretion exceeded  $1000 \mu\text{g/g.Cr}$  (Cai 2001; Iwata et al. 1993; Kido et al.  
110 1988).

111 As shown in table 2 in males there is no significant difference in urinary  $\beta_2$ -MG concentration  
112 over 17 years. However, between 1991-1999 and 1999-2003 ( $p=0.000$ ) there is a significant  
113 decrease and increase respectively. At the moment there is no plausible reason for this "V" shaped  
114 curve. But the geometric means of urinary  $\beta_2$ -MG concentration during 17 years were within the  
115 normal range. This can be explained by the physiological changes of the human body. Urinary  
116  $\beta_2$ -MG is a sensitive indicator of renal tubular dysfunction (Nogawa et al. 1983; Aoshima et al.  
117 1988; Kubota et al. 1985). Previous studies have shown that there is a significant relationship  
118 between urinary Cd and  $\beta_2$ -MG (Kido et al. 1988; Honda et al. 1982). It can be seen from table 3  
119 that females have a higher geometric mean urinary Cd and  $\beta_2$ -MG concentration relative to males  
120 (Kido et al. 2001). This study shows that urinary Cd still remain in the body 22 years after cessation  
121 of exposure. Moreover, urinary  $\beta_2$ -MG remains in the body for the same period of time as Cd  
122 (Tables 1 and 2). Therefore, there is a significant correlation between urinary Cd and  $\beta_2$ -MG  
123 concentration in males in 1999 ( $p=0.004$ ) and in females in 1986 ( $p=0.010$ ) and 2003 ( $p=0.033$ )  
124 (Table 4). In multiple regression analysis with urinary  $\beta_2$ -MG, urinary Cd, age, period of residence  
125 and Brinkman index are contributing factors for Cd exposed subjects. The results in table 5 show

126 that in 2003 there is a significant relationship between urinary  $\beta_2$ -MG and age ( $p=0.036$ ) and also  
127 Brinkman index ( $p=0.040$ ) in females. Even though Cd remained in the body for 22 years after  
128 cessation of Cd exposure it did not influence renal tubular dysfunction. This is because when the  
129 first investigation was conducted in 1986, 5 years after cessation of Cd exposure, the ages of all 53  
130 subjects were under 50 years. At this time their exposure to Cd was less than that of inhabitants 50  
131 years and over. Only four subjects were found to exceed the critical urinary  $\beta_2$ -MG limit (1000  
132  $\mu\text{g/g.Cr}$ ) during 17 years, as shown in figure 1.

133 Recently, not many long term epidemiological studies have been performed internationally. So it  
134 is imperative that long term epidemiological studies should be carried out such as our investigation.  
135 This study has thoroughly examined urinary Cd and  $\beta_2$ -MG in inhabitants after cessation of Cd  
136 exposure. In Cd-polluted areas of Japan, this is the first report that shows inhabitants having no  
137 adverse health effects. Countermeasure of replacement of Cd-polluted soil might contribute to  
138 younger inhabitants rather than the elder people in the former Cd-polluted areas. Even though the  
139 seventeen year observation data shows no significance in relationships between urinary Cd and  
140  $\beta_2$ -MG due to the aforementioned reasons, further investigations will be carried out to confirm these  
141 present results.

142 This manuscript can be concluded by the following points:

- 143 i) The change of urinary Cd and  $\beta_2$ -MG concentrations in 25 males and 28 females were not  
144 significant during 17 years after the Cd-polluted soil was restored even though they showed  
145 decreasing tendency.
- 146 ii) The comparison with urinary Cd and  $\beta_2$ -MG concentrations between men and women  
147 showed a significant difference during a 17 year period (1986, 1991, 1999 and 2003), except  
148 for urinary  $\beta_2$ -MG concentrations in 1991. The geometric mean was higher in females than  
149 males.
- 150 iii) Once exposed to Cd, it was found to remain in the body, 22 years after the Cd-polluted soil  
151 was restored. However, this did not influence renal tubular dysfunction generally..

152  
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154 had to endure physical examinations over a period of 17 years. We sincerely wish the inhabitants of  
155 the Kanehira area to be well.

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169 **Table 1** Change in urinary Cd concentration ( $\mu\text{g/g.Cr}$ ) in males and females  
 170 (one- way layout ANOVA)

Year	Males (N=25)		Female (N=28)	
	GM	GSD	GM	GSD
1986	3.02	2.14	5.50	2.19
1991	2.75	2.00	5.50	2.30
1999	2.19	1.66	3.80	2.09
2003	1.95	1.74	4.27	2.00

GM: Geometric Mean

GSD: Geometric Standard Deviation

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174 **Table 2** Change in urinary  $\beta_2$ -MG concentration ( $\mu\text{g/g.Cr}$ ) in males and females  
 175 (one-way layout ANOVA)

Year	Males (N=25)		Females (N=28)	
	GM	GSD	GM	GSD
1986	30.20	8.32	83.18	4.07
1991	89.13	2.51	128.83	2.95
1999	12.88	6.46	51.29	10.23
2003	57.54	2.24	131.83	3.02

GM: Geometric Mean

GSD: Geometric Standard Deviation p: p-value

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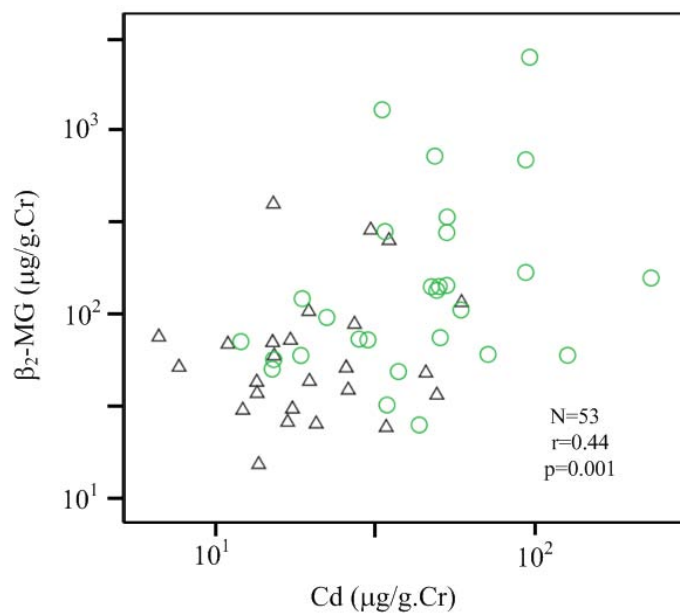
179

180 **Table 3** Comparison with urinary Cd and  $\beta_2$ -MG concentration ( $\mu\text{g/g.Cr}$ ) in 25 males and  
 181 28 females (t-test)

Year	Gender	urinary Cd ( $\mu\text{g/g.Cr}$ )			urinary $\beta_2$ -MG ( $\mu\text{g/g.Cr}$ )		
		GM	GSD	p-value	GM	GSD	p-value
1986	M	3.05	2.16	p=0.000	29.90	8.36	p=0.000
	F	5.52	2.19		83.12	4.09	
1991	M	2.79	2.01	p=0.002	88.19	2.47	p=0.174
	F	5.47	2.27		128.94	2.94	
1999	M	2.17	1.66	p=0.003	12.84	6.49	p=0.022
	F	3.79	2.10		50.90	10.15	
2003	M	2.00	1.73	p=0.000	57.50	2.20	p=0.003
	F	4.30	2.00		133.05	3.01	

182 GM: Geometric Mean

GSD: Geometric Standard Deviation



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Figure. 1 Scatter plot of correlation between urinary Cd and  $\beta_2$ -MG concentration for 28 females ( $\circ$ ) and 25 males ( $\triangle$ ) in 2003 (Pearson correlation method; r: Pearson correlation coefficient).

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191 **Table 4** The correlation coefficient between urinary Cd and  $\beta_2$ -MG concentration  
192 in females and males (Pearson correlation method)

	1986	1991	1999	2003
Males	r=0.202	r=0.183	r=0.349	r=0.155
(N=25)	p=0.167	p=0.191	p=0.044	p=0.229
Females	r=0.435	r=0.241	r=0.093	r=0.351
(N=28)	p=0.010	p=0.108	p=0.320	p=0.033

193 r : Pearson correlation coefficient                      p : p-value

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**Table 5** Multiple regression analysis of urinary  $\beta_2$ -MG and Age, urinary Cd, period of residence, Brinkman index in males and females

Males (N=25)									
	1986		1991		1999		2003		
	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value	
Age	-0.142	0.682	-0.037	0.914	-0.174	0.501	-0.073	0.804	
Cd	0.174	0.593	-0.114	0.775	0.268	0.284	0.038	0.907	
Period of residence	0.145	0.651	0.241	0.426	0.137	0.632	0.254	0.425	
Brinkman index	0.125	0.674	0.444	0.132	0.401	0.119	0.118	0.689	
$R^2$	0.055		0.140		0.232		0.056		
Females (N=28)									
	1986		1991		1999		2003		
	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value	
Age	0.111	0.684	0.247	0.410	0.301	0.177	0.426	0.036	
Cd	0.294	0.327	-0.056	0.858	-0.181	0.386	0.015	0.941	
Period of residence	0.112	0.625	9.225	0.340	0.397	0.053	0.244	0.208	
Brinkman index	-0.037	0.847	-0.083	0.676	-0.211	0.237	-0.346	0.040	
$R^2$	0.435		0.134		0.322		0.435		

$\beta$ : Standardized partial regression coefficient

$R^2$ : Coefficient of determination



229 **References**

230

231 Aoshima K, Iwata K, Kasuya M (1988) Environmental Exposure to Cadmium and Effects on Human Health Part 1.

232 Renal tubular function in inhabitants of the cadmium-polluted Jinzu River basin in Toyama Prefecture. *Jap J Hyg* 43:853-863 (Abstract in  
233 English)

234 Aoshima K, Kasuya M (1993) Itai-Itai disease – a natural history of osteomalacia caused by urinary tubular dysfunction during exposure to general  
235 environmental cadmium. *J Med Treat* 75: 1031-1035 (in Japanese)

236 Cai Y, Aoshima K, Katoh T, Teranishi H, Kasuya M (2001) Renal tubular dysfunction in male inhabitants of cadmium-polluted area in Toyama,  
237 Japan—an eleven-year follow-up study. *J Epidemiol* 11(4): 180-189

238 Friberg, L.( 1985) Cadmium and Health. CRC Press, Boca Raton, Florida:1-5

239 Honda R, Yamada Y, Tsurutani I, Kobayashi E, Ishizaki M, Nogawa K (1982) Significance of Low Molecular Proteinuria in Cadmium Poisoning.  
240 Kanazawa Medical University Journal 7(3): 142-151 (Abstract in English)

241 Honda R, Tsurutani I, Ishizaki M, Kido T, Yamada Y, Nogawa K (1989) Fláméeles Atomic Absorption Spectrophotometry of Cadmium and Lead in  
242 Blood and Urine. Kanazawa Medical University Journal 14(3): 337-347 (Abstract in English)

243 Ishikawa Prefecture (1975) In measurement of soil in farmlands of polluted areas, Ishikawa Prefecture Laws and Regulations.  
244 ([www.pref.ishikawa.jp/reiki/reiki\\_honbun/enkaku/i1010488001.html](http://www.pref.ishikawa.jp/reiki/reiki_honbun/enkaku/i1010488001.html)) (in Japanese)

245 Ishikawa Prefecture South Kaga Public Health Center (1997) Research and Induction about Local Health and Environment during 9 Heisei years.  
246 Report in Ishikawa Prefecture South Kaga Public Health : 82-88 (in Japanese)

247 Iwata K, Saito H, Moriyama M, Nakano A (1993) Renal Tubular Function After Reduction of Environmental Cadmium Exposure: A Ten-Year  
248 Follow-up. *Arch Environ Health* 48(3):157-163

249 Kasuya, M (1985) Liver injury by cadmium. *KanTan Sui* 10:923-928 (in Japanese)

250 Kido T, Honda R, Tsuritani I, Yamaya H, Ishizaki M, Yamada Y, Nogawa K (1988). Progress of renal dysfunction in  
251 inhabitants environmentally exposed to cadmium. *Arch Environ Health* 43(3):213-217

252 Kido T (1995) Studies on health effects of cadmium exposure in the general environment. *Jap J Hyg* 49(6):960-972

253 Kido T, Nishijo M, Nakagawa H, Kobayashi E, Nogawa K, Tsuritani I, et al. (2001) Urinary Finding of Inhabitants in a Cadmium-Polluted Area Ten  
254 Years After Cessation of Cadmium Exposure. *Hokuriku J Public Health* 27(2): 1-4.

255 Kido T, Naganuma R, Takasaki I, Nishijo M, Nakagawa H, Kobayashi E, Nogawa K (2002) Eighteen years observation of renal function in  
256 inhabitants exposure to cadmium in Kakehashi River basin, Ishikawa Prefecture. *Environ Health Report* 66:336-339 (Abstract in English)

257 Kubota Y, Teranishi H, Aoshima K, Kato T, Nishijo M, Kasuya M (1985) A Study on Normal Range of Urinary Protein Fraction Concentrations,  
258 Albumin,  $\beta_2$ -Microglobulin and  $\alpha_1$ -Microglobulin Among Community Inhabitants. *Hokuriku J Pub Health* 12: 27-32 (Abstract in English)

259 Nogawa K, Yamada Y, Honda R, Ishizaki A, Tsuritani I, Kawano S, Kato T(1983) The relationship between Itai-Itai disease among inhabitants of the  
260 Jinzu River basin and cadmium in rice. *Toxicol Lett* 17(3):263-266

261 Nakagawa H, Nishijo M (1999) Dose-response relationship and mortality in inhabitants of cadmium polluted areas, Advance in Prevention of  
262 Environmental Cadmium Pollution and Countermeasures. *Eiko Laboratory* : 31-34

263 Nordberg GF, Kjellstrom T, Nordberg M (1985) Cadmium and Health. A toxicological and Epidemiological Appraisal. CRC Press, Boca Raton,  
264 Florida Ch.6

265 Matsunami J (2006) Review of Itai-Itai disease. *Katura Syobou*: 2-38 (in Japanese)

266 Saito H, Takebayashi S, Harada K, Hara K, Iwata K, Segawa S (1993) Chronic Cadmium Poisoning. Nagasaki University (second internal medicine),  
267 54-117. (in Japanese)

268 Wada T (1986) Metals and Humans. Asakura publishers: 246

269