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Estimation of Benchmark Rice Cadmium Doses as Threshold Values for Abnormal Urinary Findings with Adjustment for Consumption of Jinzu River Water

Etsuko Kobayashi · Yasushi Suwazono · Mirei Dochi · Ryumon Honda · Teruhiko Kido

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Abstract We performed this study to determine whether both eating cadmium (Cd)-polluted rice and drinking and/or cooking with Jinzu River water are associated with renal tubular dysfunction. A multiple logistic regression analysis of retrospective data indicated that both factors may contribute to this condition. Estimated threshold values of rice Cd concentration in men were 0.13–0.27 ppm and 0.09–0.18 ppm in women, without adjustment for use of Jinzu River water. The additional influence of drinking and/or cooking with Jinzu River water was estimated to be about 0.008 ppm.

Keywords Cadmium · Renal tubular dysfunction · Rice cadmium concentration · Drinking river water · Threshold level of cadmium concentration in rice · Benchmark dose approach

Long-term exposure to environmental cadmium (Cd) shows a strong association with renal dysfunction (Nogawa 1981; Nomiyama 1986). The inhabitants of Cd-polluted areas in Japan obtain 1/2–2/3 of their total Cd intake from their staple food, rice (Tsuchiya and Iwao 1978). Several studies have shown a dose-response relationship between rice Cd concentration (RCd), as a Cd-exposure index, and renal tubular dysfunction as a health-effect index in the Jinzu River basin, the region most severely and extensively polluted (Nogawa and Ishizaki 1979; Osawa et al. 2001; Watanabe et al. 2002). Although the ingestion of Cd-polluted rice almost certainly contributed to the renal tubular dysfunction observed in this region, these studies did not consider the potential contribution of Cd consumption in Jinzu River water. After 1990, adverse health effects from environmental Cd exposure, especially from Cd-polluted foods, have been reported from countries other than Japan, including China and Thailand (Cai et al. 1990, 1992, 1995; Simmons et al. 2005). To protect people from the health effects of eating Cd-polluted rice, it is important to determine threshold RCd levels for individual rice consumption, and to adjust these levels for the use of Cd-polluted water. We investigated the influence of both eating Cd-polluted rice and drinking and/or cooking with Jinzu River water on the risk of renal tubular dysfunction in exposed individuals.

E. Kobayashi (✉) · Y. Suwazono · M. Dochi
Department of Occupational and Environmental Medicine, Graduate School of Medicine (A2), Chiba University, 1-8-1 Inohana, Chuohku, Chiba 260-8670, Japan
e-mail: nqi27069@nifty.com; ekoba@faculty.chiba-u.jp

R. Honda
Department of Nursing, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

T. Kido
Department of Community Health Nursing, Kanazawa University School of Health Sciences, 5-11-80 Kodatsuno, Kanazawa 920-0942, Japan

Materials and Methods

In 1967 and 1968, health examinations were conducted among the entire population of the Jinzu River basin, a non-Jinzu River basin, and a region receiving a mixed water supply. All participants were aged $\geq$30 years. In both years 13,183 individuals (6,155 men and 7,028 women) received urinary tests, for a participation rate of 90.3%. From this large group, we selected 3,078 subjects (1,527 men and 1,551 women) who had resided for a total of 30 years or longer in their present hamlet and were 50 or more years old, and in whose hamlet the RCd was known.

The urine samples were collected early in the morning and analyzed later in the same day by semi-quantitative determination of protein (Kingsbury-Clark method, Fukushima and Sakamoto 1974a), and glucose (Benedict's reaction method, Fukushima and Sakamoto 1974b). We used the abnormal urinary findings (proteinuria, glucosuria, or proteinuria with glucosuria) as indices of renal tubular dysfunction. The cut-off values for proteinuria and glucosuria were 10 mg/dL and 1/32%, respectively. In the health examinations, we also conducted a questionnaire survey for self-reported data, which we later confirmed in personal interviews of all participants. The questionnaire included two questions concerning the use of Jinzu River water, which were "Have you used Jinzu River water for drinking?" and "Have you used Jinzu River water for cooking?" Subjects who answered "yes" to either question were counted as users of Jinzu River water for drinking and/or cooking. Users of Jinzu River water for drinking and/or cooking included 43.6% and 40.5% of men and women, respectively. Among all users, 97.2% answered "yes" to both questions. Subjects who answered "no" to both questions were counted as non-users.

From 1971 to 1976, the Toyama Prefecture Department of Health (1976) determined RCd (unpolished non-glutinous rice) in the entire endemic district of the Jinzu River basin. This was the largest and most systematic survey to measure RCd in the endemic area. The area was divided into about 2,500 sub-areas of 2.5 ha. A sample of rice was taken in the center of each sub-area, and its Cd-concentration determined by atomic absorption spectrometry after wet ashing with HNO_3/H_2SO_4 and extraction with DDTC (Diethyldithiocarbamic Acid)/MIBK (Methyl Isobutyl Ketone). We obtained the raw data for 2,446 rice samples and calculated the mean RCd in the individual hamlets. Hamlets were divided into 78 units (5 or more rice sample) in which RCd ranged from 0.02 to 1.06 ppm. The RCd in an individual hamlet was used as the index of external Cd exposure for the entire population of the hamlet.

Baseline data for age, RCd, and the prevalence of users of Jinzu River water for drinking and/or cooking were calculated according to gender and negative or positive urinary findings (Table 1).

Multiple logistic regression analysis was performed according to gender, using abnormal urinary findings as the criterion variables. As explanatory variables, age and RCd were used in model A, and age, RCd and user/non-user status for Jinzu River water, in model B (Table 2). Age and RCd were continuous variables. Odds ratios for age and RCd were estimated per 1 year and per 0.1 ppm increment, respectively.

The threshold level of RCd was calculated using a benchmark dose (BMD) approach as defined by Crump (1984, 1995). The BMD can be defined as the exposure that corresponds to a certain increase in the probability of an adverse response compared to the background at zero exposure. The BMD low (BMDL) is defined as the value corresponding to the lower 95% confidence interval of the BMD and can be used to evaluate dose-response relationships as a replacement for the no observed adverse effect level (NOAEL) or the lowest observable adverse effect level (LOAEL; Gaylor et al. 1998). The BMDL was calculated using the profile likelihood method (Crump 1984; Filipsson et al. 2003). In the present study, the profile likelihood method was used together with the multiple logistic models (Budtz-Jørgensen et al. 2001). The BMD/BMDL values for RCd were calculated, with BMR at 5% and 10%, among the male or female population, assuming the mean age (62.5 years in men and 62.1 years in women; Table 3). The analyses were performed with SPSS, version 12.0 J (multiple logistic regressions, SPSS Japan Inc., Tokyo, Japan) and Microsoft Excel 2003 (BMDL calculation, Microsoft Corporation, Redmond, WA, USA). p values ≤ 0.05 were considered to be statistically significant.

Results and Discussion

Baseline data for age, RCd, and prevalence of users of Jinzu River water for drinking and/or cooking are shown in Table 1, according to gender and normal/abnormal urinary findings. Subjects with abnormal urinary findings were more likely to be users of Jinzu River water for drinking and/or cooking than subjects with normal findings.

Results of multiple logistic regression analysis according to gender are shown in Table 2. In model A, odds ratios for age and RCd were statistically significant for all abnormal urinary findings in both men and women. The odds ratios for RCd for 0.1 ppm increments were 1.104–1.337 in men and 1.247–1.392 in women. In model B, odds ratios for age and RCd were also statistically significant for all abnormal urinary findings in both sexes (odds ratios were 1.105–1.323 in men and 1.238–1.374 in women); however, the odds ratios of user/non-user status of Jinzu River water for drinking and/or cooking were not significant for glucosuria, or for proteinuria with glucosuria in men. The odds ratios with statistical significance were 1.426 in men and 1.451–1.683 in women. From this we conclude that eating the Cd-polluted rice and drinking and/or cooking with Jinzu River water both influence the occurrence of renal tubular dysfunction, especially in women. Eating the Cd-polluted rice also appeared to exert a greater impact on the occurrence of renal tubular dysfunction as compared to drinking and/or cooking with Jinzu River water, because in men the odds ratio for user/non-user status was significant only for proteinuria.

Table 1 Baseline data according to gender and prevalence of abnormal urinary findings

		Urinary finding (−)				Urinary finding (+)			
		N	*M*	Min.	Max.	N	*M*	Min.	Max.
Men	Proteinuria	1,065				462			
	Age (year)		60.6	50	91		66.9	50	95
	RCd (ppm)		0.31	0.02	1.06		0.43	0.02	1.06
	RW (+) (%)		40.0				51.9		
	Glucosuria	1,113				414			
	Age (year)		62.1	50	94		63.5	50	95
	RCd (ppm)		0.33	0.02	1.06		0.38	0.02	1.06
	RW (+) (%)		43.4				44.2		
	Proteinuria with glucosuria	1,327				200			
	Age (year)		61.7	50	94		67.7	50	95
	RCd (ppm)		0.33	0.02	1.06		0.47	0.08	0.95
	RW (+) (%)		41.9				55.0		
Women	Proteinuria	987				564			
	Age (year)		60.1	50	92		65.6	50	88
	RCd (ppm)		0.28	0.02	1.06		0.41	0.02	1.06
	RW (+) (%)		35.4				49.5		
	Glucosuria	1,130				421			
	Age (year)		61.0	50	88		65.1	50	92
	RCd (ppm)		0.30	0.02	1.06		0.41	0.02	1.06
	RW (+) (%)		37.3				48.9		
	Proteinuria with glucosuria	1,268				283			
	Age (year)		61.1	50	92		66.6	50	86
	RCd (ppm)		0.30	0.02	1.06		0.46	0.02	1.06
	RW (+) (%)		37.6				53.4		

RCd, Cadmium concentration in rice; RW (+), Positive reply to either "Have you used Jinzu River water for drinking?" or "Have you used the Jinzu River water for cooking?" Proteinuria $\geq$ 10 mg/dL. Glucosuria $\geq$ 1/32%; N, Number of subjects examined; *M*, Arithmetic mean; Min., minimum value; Max., Maximum value

Table 2 Results of multiple logistic regression analysis according to model and gender

Model	Criterion variable	Explanatory variable	Odds ratio (95% confidence interval)		
			Men	Women	
A	Proteinuria	Age (year)	1.094 (1.078–1.256)	1.092 (1.076–1.107)	$(x + 1)/x$
		RCd (ppm)	1.329 (1.256–1.407)	1.342 (1.271–1.417)	$(x + 0.1)/x$
		Constant	0.001	0.001	
	Glucosuria	Age (year)	1.017 (1.004–1.030)	1.060 (1.046–1.075)	$(x + 1)/x$
		RCd (ppm)	1.104 (1.049–1.163)	1.247 (1.183–1.315)	$(x + 0.1)/x$
		Constant	0.091	0.004	
	Proteinuria with glucosuria	Age (year)	1.078 (1.060–1.097)	1.082 (1.064–1.099)	$(x + 1)/x$
		RCd (ppm)	1.337 (1.247–1.434)	1.392 (1.308–1.481)	$(x + 0.1)/x$
		Constant	0.000	0.000	
B	Proteinuria	Age (year)	1.093 (1.078–1.109)	1.092 (1.076–1.108)	$(x + 1)/x$
		RCd (ppm)	1.318 (1.245–1.396)	1.335 (1.263–1.410)	$(x + 0.1)/x$
		RW (%)	1.426 (1.118–1.817)	1.683 (1.334–2.123)	(+)/(−)
		Constant	0.000	0.001	
	Glucosuria	Age (year)	1.017 (1.004–1.030)	1.060 (1.045–1.074)	$(x + 1)/x$
		RCd (ppm)	1.105 (1.049–1.164)	1.238 (1.174-1.305)	$(x + 0.1)/x$
		RW (%)	0.970 (0.770–1.222)	1.451 (1.144–1.841)	(+)/(−)
		Constant	0.091	0.004	
	Proteinuria with glucosuria	Age (year)	1.077 (1.059–1.096)	1.081 (1.064–1.099)	$(x + 1)/x$
		RCd (ppm)	1.323 (1.232–1.420)	1.374 (1.291–1.463)	$(x + 0.1)/x$
		RW (%)	1.373 (0.997–1.889)	1.620 (1.221–2.150)	(+)/(−)
		Constant	0.000	0.000	

Model A, Factor of drinking and/or cooking river water (RW) is not contained in explanatory variable; Model B, Factor of drinking and/or cooking river water (RW) is contained in explanatory variable; RCd, Cadmium concentration in rice; RW (−), Negative reply to both "Have you used Jinzu River water for drinking?" and "Have you used Jinzu River water for cooking?" RW (+), Positive answer to either question; Proteinuria ≥ 10 mg/dL. Glucosuria ≥ 1/32%

To determine threshold values for RCd, the BMD/BMDL values were calculated with BMR at 5% and 10%, and without (model A) or with (model B) consideration of the use of Jinzu River water for drinking and/or cooking (user/non-user status; Table 3). With the BMR at 5% or 10% in model A, the BMD/BMDL values of RCd for proteinuria at the mean age were 0.14/0.13 or 0.25/0.23 ppm, respectively, in men, and 0.11/0.09 or 0.20/0.17 ppm, respectively, in women. The values for glucosuria were 0.28/0.22 or 0.53/0.42 ppm in men, and 0.16/0.13 or 0.30/0.23 ppm in women. The values for proteinuria with glucosuria were 0.30/0.27 or 0.47/0.43 ppm in men, and 0.22/0.18 or 0.35/0.29 ppm in women. With the BMR at 5% or 10%, and user of Jinzu River water status positive in model B, the BMD/BMDL values of RCd for proteinuria at the mean age were 0.13/0.12 or 0.36/0.21 ppm in men, and 0.10/0.08 or 0.18/0.15 ppm in women. For non-users of Jinzu River water, the values were 0.16/0.14 or 0.28/0.25 ppm in men, and 0.13/0.10 or 0.23/0.19 ppm in women. With the BMR at 5% or 10%, and status of Jinzu River water either user or non-user in model B, the BMD/BMDL values of RCd for glucosuria at the mean age were 0.15/0.11 or 0.28/0.21 ppm, and 0.19/0.14 or 0.33/0.26 ppm in women. The values for proteinuria with glucosuria were 0.18/0.15 or 0.31/0.25 ppm, and 0.25/0.20 or 0.40/0.32 ppm in women. Since the odds ratios for user/non-user status of Jinzu River water as an explanatory variable were not statistically significant, the values could not be calculated for glucosuria, and for proteinuria with glucosuria in men, in model B.

We previously calculated the threshold values of RCd using the same data as in the present study (Osawa et al. 2001; Watanabe et al. 2002). Osawa et al. (2001) found an estimated RCd value in the range of 0.08–0.19 ppm in men, and 0.13–0.18 ppm in women. Among 50-year-old subjects, Watanabe et al. found values of 0.13–0.15 and 0.17–0.10 ppm for men and women, respectively. The values were calculated by substitution of the rates of abnormal urinary findings in the controls into the regression formula for subjects in both studies. In the present study, using a BMD approach, we found a threshold RCd level that differed slightly from those in the previous studies. With model B, the estimated threshold level for proteinuria with glucosuria was 0.20 ppm in women non-users and

Table 3 BMD/BMDL values of cadmium concentration in rice according to model and gender

Model	Urinary findings	Explanatory variable	Men			Women		
			BMD/BMDL of RCd (ppm)			BMD/BMDL of RCd (ppm)		
			BMR		$p(0)$	BMR		$p(0)$
			5%	10%		5%	10%	
A		Age only						
	Proteinuria		0.14/0.13	0.25/0.23	12.2%	0.11/0.09	0.20/0.17	16.5%
	Glucosuria		0.28/0.22	0.53/0.42	20.7%	0.16/0.13	0.30/0.23	14.0%
	Proteinuria with glucosuria		0.30/0.27	0.47/0.43	4.0%	0.22/0.18	0.35/0.29	5.4%
B		Age and						
	Proteinuria	RW (+)	0.13/0.12	0.23/0.21	14.9%	0.10/0.08	0.18/0.15	21.5%
		RW (−)	0.16/0.14	0.28/0.25	10.9%	0.13/0.10	0.23/0.19	14.0%
	Glucosuria	RW (+)	[a]	[a]	[a]	0.15/0.11	0.28/0.21	17.2%
		RW (−)	[a]	[a]	[a]	0.19/0.14	0.33/0.26	12.5%
	Proteinuria with glucosuria	RW (+)	[a]	[a]	[a]	0.18/0.15	0.31/0.25	7.3%
		RW (−)	[a]	[a]	[a]	0.25/0.20	0.40/0.32	4.6%

Model A, Factor of drinking and/or cooking river water (RW) is not contained in explanatory variable of the multiple logistic regression analysis; Model B, Factor of drinking and/or cooking river water (RW) is contained in explanatory variable; RW (+), Positive reply by all subjects to either "Have you used Jinzu River water for drinking?" or "Have you used Jinzu River water for cooking?" RW (−), Negative reply by all subjects to both questions; Proteinuria $\geq$ 10 mg/dL. Glucosuria $\geq$ 1/32%; RCd, Cadmium concentration in rice; A benchmark dose (BMD) approach was used to estimate the threshold level of rice Cd. The BMD low (BMDL) was calculated using the profile likelihood method. BMR, Benchmark response; p (0), Probability of positive finding at zero exposure; BMD/BMDL are calculated assuming mean age (62.5 years in males and 62.1 years in females). [a] Since factor of drinking and/or cooking river water (RW) is not significant in the multiple logistic regression analysis, BMD/BMDL value is the same as in model A

0.15 ppm in women users. As an index of renal tubular dysfunction, proteinuria with glucosuria is less sensitive than measures of a low molecular weight protein such as β_2-microglobulin (β_2-MG); hence the value in the present study may be higher than the value calculated for β_2-MG-uria. Moreover, we interpret the difference of 0.05 ppm between women non-users and users (0.20 and 0.15 ppm, respectively) to represent the influence of drinking and/or cooking with Jinzu River water. For a daily mean rice intake of 333.5 g [determined in duplicate samples of the meals consumed (Ishikawa Prefecture Department of Health 1976)], daily Cd consumption from rice with 0.05 ppm Cd would be 16.7 µg. Assuming a daily water intake of 2 L, the Cd concentration of the river water was estimated to be about 0.008 ppm. This value exceeded the WHO guideline for Cd in drinking-water (0.003 ppm) based on an allocation of 10% of the provisional tolerable weekly intake (PTWI, 7 µg/kg of body weight/week). It is therefore plausible that drinking and/or cooking with Jinzu River water, in addition to eating Cd-polluted rice, made a significant contribution to the occurrence of renal tubular dysfunction. After the 1960's, Cd concentrations in Jinzu River and well water fell to almost undetectable levels (<0.001 ppm; Study group for Itai-itai disease 1968). The maximum Cd concentration in Jinzu River water samples collected in July, 1967 was 0.009 ppm (Study group for Itai-itai disease 1968). Before the 1960's, Cd concentrations in river and well water in the endemic region were completely unknown, making it very difficult to quantify Cd intake from Jinzu River water used for drinking and/or cooking. Our use of a multiple logistic model to estimate the influence of Cd consumption from the Jinzu River before the 1960's thus provides significant new knowledge.

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