Int Arch Occup Environ Health (2008) 81:263–271
DOI 10.1007/s00420-007-0206-z

ORIGINAL ARTICLE

Biological variations in cadmium, α_1-microglobulin, β_2-microglobulin and *N*-acetyl-β-D-glucosaminidase in adult women in a non-polluted area

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Received: 23 October 2006 / Accepted: 7 May 2007 / Published online: 18 October 2007

Abstract
Objectives This study was initiated to investigate the extents of biological variations in cadmium and three common tubular dysfunction marker levels in blood and urine through repeated sampling.
Methods A 12-month survey and a 10-week survey were conducted in an area with no known cadmium pollution. In the 12-month survey, five adult women offered urine samples once every month and blood samples once in every season, respectively. In the 10-week survey, 17 adult women gave urine samples once every week. Blood and urine samples were analyzed for cadmium (Cd-B and Cd-U) by graphite-furnace atomic absorption spectrometry, and urine samples were analyzed also for α_1-microglobulin (α_1-MG-U), β_2-microglobulin (β_2-MG-U) and *N*-acetyl-β-D-glucosaminidase (NAG-U) by conventional methods, all under strict quality control. The results were subjected to statistical analysis to examine the extents of biological variations through-out the study periods.
Results Variations in geometric means (GMs) for Cd-B, Cd-U, α_1-MG-U, β_2-MG-U, and NAG-U were all small; the ratio of the largest GM over the lowest GM was 1.1 for Cd-B, 2 for Cd-U and 2 to 3 for α_1-MG-U, β_2-MG-U, and NAG-U in the 12-month survey, and 1.7 at largest for all parameters in the 10-week survey. The within-subject variations during the 12-month or 10-week periods were however large, i.e., more than 4–5-fold difference between the smallest and the largest values obtained for the same subject. Effects of the correction for urine density to reduce the variations were limited. In contrast, within-subject variation in Cd-B was small with a ratio of 1.3.
Conclusions Variations in GM values for Cd-U, α_1-MG-U, β_2-MG-U, and NAG-U at different time of sampling are small so that single measurement would be acceptable as far as the evaluation on a group basis is the study objective. Within-subject variations are wide however, the ratio of the largest value over the smallest value being 4–5 or more, irrespective of correction for urine density. Therefore, care should be practiced when evaluation on an individual basis is intended. Very low within-subject variation in Cd-B may suggest the advantage of Cd-B over Cd-U for individual evaluation among general populations if blood sampling is accepted.

Keywords *N*-Aetyl-β-D-glucosaminidase · Adult women · Blood · Cadmium · α_1-Microglobulin · β_2-Microglobulin · Monthly variation · Urine · Weekly variation

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Introduction

Cadmium (Cd) in biological materials, typically urine, has been used as an exposure marker not only for biological

monitoring of working populations but even more commonly for general population monitoring. Urine is a preferred material as sufficient data for comparative evaluation are available and sampling is not invasive by nature (International Programme on Chemical Safety 1992a, b). Because Cd once absorbed will be excreted with a long biological half-life, e.g., in the order of 10 years (International Programme on Chemical Safety 1992a, b; Schaller 1996), it has been expected that Cd levels in blood and urine from the same subject may not vary substantially, when the intensity of exposure to Cd is rather constant as is the case of general population exposure, for whom the major exposure source is foods (e.g. rice as the staple food for Japanese populations; Watanabe et al. 2000; Tsukahara et al. 2003). Accordingly, common practice in general population monitoring is based on single (un-repeated) urine sampling, especially when the size of the target population is large (e.g., Sartor et al. 1992; Černá et al. 1997; dell'Omo et al. 1999; Hoffmann et al. 2000; Ikeda et al. 2000; Paschal et al. 2000; Noonan et al. 2002; Ezaki et al. 2003; Horiguchi et al. 2004).

Nevertheless, biological variation of urinary Cd as a Cd exposure marker, separately from possible analytical problems (such as instrument-dependent bias; Zhang et al. 1997), has been calling attention of several researchers (Arisawa et al. 1997; Mason et al. 1998; Ikeda et al. 2005). This study group also has reported wide variation in Cd-U (Yamagami et al. 2006), assumedly due to seasonal changes in types of foods consumed (Kruzynski 2004; Bragigand et al. 2004).

The present survey was initiated to examine if Cd in blood and urine (Cd-B and Cd-U, respectively) are biologically stable within the same subject among general populations. Biological variations in the urinary levels of three commonly used tubular dysfunction markers, i.e.,α_1-microglobulin (e.g., Tohyama et al. 1986; Moriguchi et al. 2004, 2005), β_2-microglobulin (e.g. Iwata et al. 1993; Arisawa et al. 1997; Moriguchi et al. 2003) and *N*-Acetyl-β-D-glucosaminidase (e.g., Kawada 1995; Moriguchi et al. 2003), were also examined.

Subjects and methods

Study subjects and biological materials

The participants were technical staff of an occupational health service institution. A 12-month survey to examine between-month variation was initiated in December, 2004 and continued till November, 2005. The participants were recruited from the subjects who showed relatively high Cd-U levels in a previous survey (Yamagami et al. 2006). The study was started with seven participating women, of whom five women completed the participation by offering peripheral blood samples in the middle of January, April, July and December (thus, four times) and 2nd morning urine samples in the middle of each month (12 times). A 10-week survey to study between-week variation was from the fourth week of February to the fourth week in April, 2006. Of 20 women who expressed their will to participate, 17 women succeeded to complete the participation by offering 2nd morning urine samples in the middle of each week (thus ten times).

Due flexibility in sampling time was considered to avoid menstruation periods in both surveys. Care was taken to minimize possible contamination of samples (e.g., dusts) in sample collection process, as well as decomposition of the markers (β_2-microglobulin in particular) during transportation and storage, following the procedures previously described (Ezaki et al. 2003).

Ethical issue

The Ethics Committee of Kyoto Industrial Health Association approved the study protocol. Each of the participants in the 12-month survey and the 10-week survey provided her informed consent.

Analytical methods and quality controls

Cadmium in blood and urine samples (Cd-B and Cd-U) was analyzed by graphite furnace atomic absorption spectrometry (Ezaki et al. 2003). α_1-Microglobulin (α_1-MG-U), β_2-microglobulin (β_2-MG-U), *N*-acetyl-β-D-glucosaminidase (NAG-U), and creatinine (CR or cr) in urine, and urine specific gravity (SG or sg) were measured by latex agglutination immunoassay (Yamagami et al. 2006), RIA (Ezaki et al. 2003), ELISA (Moriguchi et al. 2003), colorimetry (Ezaki et al. 2003) and refractometry (Ezaki et al. 2003), respectively. In some instances, Cd-U ,α_1-MG-U, β_2-MG-U and NAG-U concentrations were corrected for CR (Jackson 1966) or SG (taking 1.016 as a standard: Buchwald 1964; Rainsford and Lloyd Davies 1965) and expressed as e.g. Cd-U_{cr} and Cd-U_{sg}, respectively. Non-corrected observed values were expressed as e.g. Cd-U_{ob}. SG was expressed in terms of factor G (Levine and Fahy 1945) which is defined as factor $G = (SG - 1.000) \times 1{,}000$. α_1-MG-U, β_2-MG-U, NAG-U, CR and SG were measured as soon after sample collection as possible, whereas Cd was measured when all samples were available as to be described below; the blood and urine samples for Cd analyses were stored at –30°C until analyzed.

The quality of the measurement of Cd-U was approved by External Intercomparison Programme 36 and 37 (occupational and environmental levels; 2006), and that of creatinine by Japan Medical Association (2004–2006). As the possible within-subject variation (between months, as well

as between weeks) was one of the foci of attention in the present study on Cd-B and Cd-U, samples from the same subject were set in a series and analyzed as one batch to minimize batch-to-batch difference. Such design could not be applied in cases of α_1-MG-U, β_2-MG-U and NAG-U analyses, because of possible instability during storage. Accordingly, strict internal quality control was applied for these analyses. On daily analyses, the three markers mostly stayed well within the ±2SD ranges in the control charts and never ran out of the ±3SD ranges; the SD ranges were calculated by repeated (20 times) analyses of the reference materials. The monthly means were 97–101%, 99–106% and 99–104% of the target concentrations in cases of α_1-MG-U, β_2-MG-U and NAG-U, respectively, and the CV was less than 2% for the three markers.

Reagents for internal quality control of α_1-MG-U, β_2-MG-U and NAG-U analyses

A reference material for both α_1-MG-U and β_2-MG-U determinations, QC-LX-3 'Eiken', was purchased from Eiken Chemical (Tokyo, Japan), and that for NAG determination, 'NAG Test Shionogi control', was a product of Shionogi (Osaka, Japan).

Statistical analysis

Cd-B, Cd-U, α_1-MG-U, β_2-MG-U and NAG-U were distributed log-normally (Shimbo et al. 2000; Ezaki et al. 2003), and CR, SG (in terms of *G*), age and hematological parameters were distributed normally (Ezaki et al. 2003; Tsukahara et al. 2003). Accordingly, geometric means (GMs) coupled with geometric standard deviations (GSDs), and arithmetic means (AMs) ± arithmetic standard deviations (ASDs) were taken as representative distribution parameters of the former and the latter group, respectively.

Results

Basic characteristics of participating women in the two surveys

The results of urinalyses and blood analyses (including Cd in blood) at the times of the first sample collection are summarized in Table 1, together with the ages of the subjects; blood samples were not collected in the 10-week survey.

The average age of the five participants in the 12-month survey was 48.6 years (in a range of 44–56 years) at the beginning of the survey. In reflection of the recruitment process (for details, see "Subjects and methods" section), both GM Cd-U_{ob} and GM Cd-U_{cr} were in excess of 2.5 μg/l or μg/g cr, respectively. The dysfunction marker levels were not remarkable, e.g., the GM β_2-MG-U_{ob} of 79 μg/l or β_2-MG-U_{cr} of 110 μg/g cr (Table 1) was the level commonly observed in previous studies (Ezaki et al. 2003; Moriguchi et al. 2003). Cd-B, 3.1 μg/l as GM (Table 1), was however somewhat higher than the commonly observed level of 1.8 μg/l among Japanese populations (Shimbo et al. 2000). None of the five participants had apparent anemia, although one had low serum ferritin of 4.9 ng/ml.

The average age of the 17 participants in the 10-week survey was about 40 years. Although the highest Cd-U_{cr} (4.5 μg/g cr) was slightly higher than the level in the 12-month survey group (3.9 μg/g cr), the GM Cd-U_{cr} remained at 1.5 μg/g cr, the level usually observed among Japanese women in the area surveyed (Ezaki et al. 2003; Yamagami et al. 2006). The levels of the three tubular dysfunction markers were not remarkable (Ezaki et al. 2003; Moriguchi et al. 2003).

Between-month variation in Cd-U, Cd-B and urinary markers of tubular dysfunction

The results of the 12-month follow-up of the five subjects are summarized in Table 2 in terms of Cd in blood and urine, and the three dysfunction markers in urine. The variation was evaluated on a group basis (the left half in Table 2) and also on an individual basis (the right half). To make comparison on a group basis, the GM values were calculated for each month, and the smallest, average and the largest values among the 12 GM values (one GM per month, for 12 months). Further calculation of the ratio of the largest over the smallest GM values showed that the ratios were around 2–3, irrespective of the parameters studied. In other words, the group GM ($n = 5$) may vary by two to three times within the 12-month period. The results for Cd-U_{ob} are presented in Fig. 1 as an example for visual understanding of variation in Cd-U, both on a group basis (time-dependent variation in GM values shown by solid circles) as well as the variations on an individual bases (a polygonal line for each case). It should also be noted that the ratio was smaller for Cd-U when corrected either for CR or for SG (1.6 or 1.7, as compared with 2.2 for Cd-U_{ob}), and smaller variations after urine density correction were also common among the cases of α_1-MG-U, β_2-MG-U or NAG-U.

In order to make evaluation of variation on an individual basis, the smallest and the largest values were identified among the 12 measurements for each of the five individuals, and the ratio of the largest/the smallest (L/S ratio) was calculated. The smallest and the largest among the five ratios were then identified to show the range of variations in the ratios. Because the number of cases available was rather limited (i.e., $n = 5$), the second largest among the five ratios was also identified to avoid by-chance observation of the

Table 1 Basic characteristics of the participating women in the 12-month survey and the 10-week survey

Parameter	(Unit)	12-month survey (5 subjects)[a]				10-week survey (17 subjects)[b]			
		AM/GM		Lowest	Highest	AM/GM		Lowest	Highest
Age	(years)	AM	48.6	44	56	AM	39.5	27	57
Urinalysis									
Creatinine (CR, cr)	(g/l)	AM	0.92	0.31	1.97	AM	0.85	0.12	1.69
Specific gravity (SG, sg)	(G)	AM	16.2	9	27	AM	18.1	4	29
Cd-U_{ob}	(μg/l)	GM	2.54	0.7	5.3	GM	1.06	0.3	3.2
Cd-U_{cr}	(μg/g cr)	GM	2.71	2.0	3.9	GM	1.45	0.4	4.5
Cd-U_{sg}	(μg/l)	GM	2.11	1.2	3.1	GM	1.03	0.3	2.8
α_1-MG-U_{ob}	(mg/l)	GM	1.52	0.5	5.6	GM	1.75	0.2	5.5
α_1-MG-U_{cr}	(mg/g cr)	GM	2.11	1.0	6.3	GM	2.40	1.2	4.4
α_1-MG-U_{sg}	(mg/l)	GM	1.64	0.9	3.6	GM	1.70	0.8	3.5
β_2-MG-U_{ob}	(μg/l)	GM	78.9	27	203	GM	84.5	20	251
β_2-MG-U_{cr}	(μg/g cr)	GM	109.9	64	258	GM	115.7	60	228
β_2-MG-U_{sg}	(μg/l)	GM	85.4	49	147	GM	82.0	41	201
NAG-U_{ob}	(Unit/l)	GM	2.10	0.7	6.5	GM	1.9	0.4	8.1
NAG-U_{sr}	(Unit/g cr)	GM	2.93	2.3	3.4	GM	2.61	1.0	7.5
NAG-U_{sg}	(Unit/l)	GM	2.28	1.2	3.9	GM	1.85	0.7	5.2
Cd-B	(μg/l)	GM	3.14	2.5	5.1				
Hematology									
Hemoglobin	(g/100 ml)	AM	13.0	11.2	14.3				
Erythrocytes	(10^4 cells/mm^3)	AM	458	435	527				
Hematocrit	(%)	AM	39.7	36.5	43.4				
Serum iron	(μg/100 ml)	AM	67.8	27	120				
Serum ferritin	(ng/ml)	AM	29.0	4.9	68.7				

[a] Results at the first examination (Urinalysis; December 2004: Heamtology including Cd-B; January 2005)

[b] Results at the first examination (February 2005); three women participated also in the 12-month survey

large ratio. The average ratios were presented in addition to show the general trends.

It was apparent that the variation on an individual basis (in terms of the average L/S ratio) was always greater than the variation on a group basis (i.e., the L/S ratios for GM values) ($P < 0.01$ by Wilcoxon test when the pairs of the 13 items were compared; P value was also <0.01 when 12 pairs excluding Cd-B were compared). The largest L/S ratios for individual observed (i.e., non-corrected) values were in excess of 10 (the second largest were also around 10) for all urinary parameters, and the ratios were smaller when corrected either for CR or for SG; the reducing effects appeared to be most marked for Cd-U. In contrast, the average of the individual variations for Cd-B was as small as 1.2, and accordingly, the variation in GMs were very small (1.1; Table 2, Fig. 2).

Between-week variation in Cd and tubular dysfunction markers in urine

In the 10-week survey, similar analyses were conducted with ten determinations each of 17 women to examine the variations in a shorter term of weeks. As described above, the Cd-U levels in the 10-week survey subjects were generally lower than that of the 12-month subjects, and 17 subjects were studied in the former survey in contrast to five subjects in the latter survey. Nevertheless, the results in the 10-week survey were similar to the findings in the 12-month survey in the sense that variations on a group basis was smaller than the variations on an individual basis ($P < 0.01$ by Wilcoxon test), and that correction for urine density usually reduced variations with one exception of variation in GM of α_1-MG-U (Table 3). The largest H/L ratio as an indicator of variation on the individual basis was around 5 for Cd-U even after correction for urine density, and similar ratios were observed also for the three dysfunction markers even after correction for urine density (Fig. 3).

Variation following the sequence in time

It was expected that, if the parameters were reproducible, the value measured on one occasion should correlate very closely with the value measured on the immediately previous occasion. To examine this possibility, the correlation of

Table 2 Variation of parameters in monthly measurements in the 12-month survey of five cases

Parameter	(Unit)	GM[a]				H/L ratio[c] for individuals			
		Lowest	Average	Highest	H/L ratio[b]	Smallest[d]	Average[d]	Largest[d]	95%UL[e]
Cd-U_{ob}	(μg/l)	1.9	2.6	4.1	2.2	2.4	6.7	12.0	14.7
Cd-U_{cr}	(μg/g cr)	2.3	2.9	3.6	1.6	1.4	2.2	2.6	3.2
Cd-U_{sg}	(μg/l)	1.7	2.3	3.0	1.7	1.8	3.3	5.0	5.8
α_1-MG-U_{ob}	(mg/l)	1.2	2.5	4.2	3.5	5.8	9.1	14.4	16.5
α_1-MG-U_{cr}	(mg/g cr)	1.5	2.9	4.3	2.8	3.6	5.4	9.2	10.2
α_1-MG-U_{sg}	(mg/l)	1.1	2.2	3.0	2.7	3.8	5.3	7.1	7.7
β_2-MG-U_{ob}	(μg/l)	58	106	184	3.2	4.4	8.8	14.8	17.0
β_2-MG-U_{cr}	(μg/g cr)	73	119	166	2.3	2.8	5.6	7.5	9.4
β_2-MG-U_{sg}	(μg/l)	53	92	136	2.6	3.2	5.0	9.1	9.8
NAG-U_{ob}	(Unit/l)	2.1	3.2	5.5	2.6	3.1	7.9	15.4	17.8
NAG-U_{cr}	(Unit/g cr)	2.9	3.6	4.8	1.6	2.3	3.3	4.1	4.7
NAG-U_{sg}	(Unit/l)	2.1	2.8	3.9	1.9	3.3	4.4	6.5	7.0
Cd-B[f]	(μg/l)	2.9	3.1	3.3	1.1	1.1	1.2	1.3	1.3

[a] Geometric mean of the five cases were calculated on each of the 12 occasions of the measurements

[b] The lowest and the highest values were identified among the 12 GMs, and the ratio of the highest/the lowest (H/L) was calculated

[c] The lowest and the highest values among the 12 meaurements were identified for each of the five cases, and the ratio of the highest/the lowest (H/L) was calculated

[d] The smallest, the average and the largest among the five cases were identified

[e] Calculated as Average + 2×ASD

[f] Four measurements, i.e., once every three months

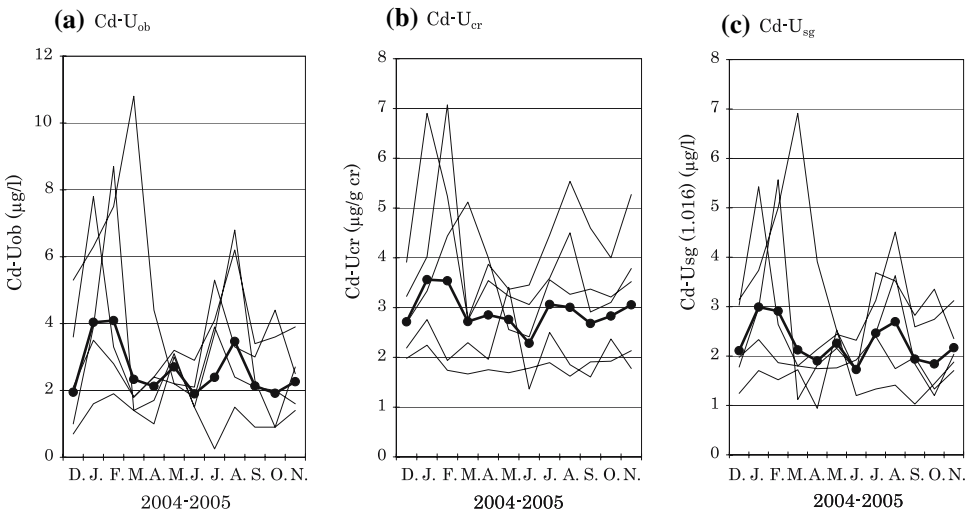

Fig. 1 Variations in Cd-U in the 12-month survey. The *thick lines* connecting *solid circles* show variations in GM values. *Polygonal lines* show five individual cases. Capitals on the horizontal axis stand for the months of a year, from D. for December to N. for November

the values in one particular month (or week) with the values in the immediately previous month (or week) was examined for each parameter. Thus, correlations between 11 pairs in the 12-month survey for each of the five subjects, and between 9 pairs in the 10 week survey for each of the 17 subjects were examined. The correlation analyses were conducted with the values without any conversion and also with the values after logarithmic conversion. The results (without conversion) from the 12-month survey were summarized in the left half in Table 4, and those from 10-week survey in the right half, in terms of the smallest, average and the largest coefficients among the five and 17 cases, respectively.

With regard to the urinary parameters in the 12-month survey, the largest coefficients among the five cases were mostly significant (with P between 0.01 and 0.05) irrespective of the parameters, but the average values were generally insignificant ($P > 0.05$). Logarithmic conversion did not improve the correlation (data not shown). In contrast, the coefficients for Cd-B were very large, being >0.98 in all cases, indicating high reproducibility in Cd-B.

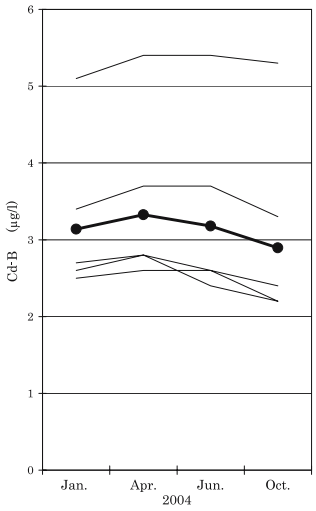

Fig. 2 Variations in Cd-B in the 12-month survey. The *thick lines* connecting solid circles show variations in GM values. *Polygonal lines* show five individual cases. Letters on the horizontal axis stand for four months of a year at a three-month interval, from Jan. for January to Oct. for October

The results were essentially the same for the 10-week survey. The largest coefficients were statistically significant ($P < 0.01$) for all parameters studied, irrespective of logarithmic conversion, and the average values were significant ($P < 0.05$) in majority of cases probably due to the large number of cases studied (i.e., $n = 17$), although the smallest coefficients were mostly insignificant ($P > 0.05$).

Discussion

Occupational and environmental epidemiology studies on Cd exposure usually depend on un-repeated determination of parameters in urine especially when working with large populations (e.g., Sartor et al. 1992; Černá et al. 1997; dell'Omo et al. 1999; Hoffmann et al. 2000; Ikeda et al. 2000; Paschal et al. 2000; Noonan et al. 2002; Ezaki et al. 2003; Horiguchi et al. 2004). Accordingly, possible biological variation in Cd-exposure related parameters such as Cd-B, Cd-U, α_1-MG-U, β_2-MG-U, and NAG-U is a matter of concern with regard to the reliability of the observation (Fraser and Harris 1989; Manson et al. 1998), separate from variation in analytical chemistry.

The present surveys revealed that the variations in GMs for Cd-B, Cd-U, α_1-MG-U, β_2-MG-U and NAG-U were generally small (in the left half in Tables 2 and 3). The surveys, however, made it clear further that the within-subject variations were large (i.e., the difference being 4–5-folds or even larger between the smallest and the largest values from the same subject), and that the correction for urine density did not always reduce the variations (in the right half in Tables 2 and 3). The former observation is in accordance with the general trend that individual values converge towards the mean so that mean values are better stabilized. In a sharp contrast, within-subject variation in Cd-B was small (in the right half in Table 2).

Table 3 Variation of parameters in weekly measurements in the 10-week survey of 17 cases

Parameter	(Unit)	GM[a]				H/L ratio[c] for individuals			
		Lowest	Average	Highest	H/L ratio[b]	Smallest[d]	Average[d]	Largest[d]	95%UL[e]
Cd-U_{ob}	(μg/l)	0.88	0.96	1.22	1.4	1.0	3.6	6.8	6.3
Cd-U_{cr}	(μg/g cr)	1.24	1.37	1.49	1.2	1.2	2.4	4.4	4.6
Cd-U_{sg}	(μg/l)	0.92	1.03	1.12	1.2	1.5	2.7	6.9	5.5
α_1-MG-U_{ob}	(mg/l)	1.28	1.53	1.78	1.4	2.1	5.2	16.5	11.7
α_1-MG-U_{cr}	(mg/g cr)	1.63	2.20	2.73	1.7	1.6	2.9	5.4	4.8
α_1-MG-U_{sg}	(mg/l)	1.44	1.66	1.99	1.4	1.5	2.6	4.3	4.1
β_2-MG-U_{ob}	(μg/l)	70.6	78.6	99.7	1.4	2.0	4.2	10.3	8.6
β_2-MG-U_{cr}	(μg/g cr)	103.1	112.0	125.2	1.2	1.2	2.2	8.2	3.8
β_2-MG-U_{sg}	(μg/l)	79.8	84.8	92.8	1.2	1.5	2.6	4.8	5.8
NAG-U_{ob}	(μg/l)	1.30	1.70	2.14	1.6	2.0	4.6	13.0	10.3
NAG-U_{cr}	(μg/g cr)	2.11	2.46	2.72	1.3	1.4	2.3	7.8	5.2
NAG-U_{sg}	(μg/l)	1.58	1.85	2.05	1.3	1.5	2.4	4.5	3.9

[a] Geometric mean of the 17 cases were calculated on each of the ten occasions of the measurements. In case the value was <LOD, it was assumed as if it were LOD/2

[b] The lowest and the highest values were identified among the 10 GMs, and the ratio of the highest/the lowest (H/L) was calculated

[c] The lowest and the highest values among the 10 meaurements were identified for each of the 17 cases, and the ratio of the highest/the lowest was calculated

[d] The smallest, the average and the largest among the 17 cases were identified

[e] Calculated as Average + 2×(standard deviation)

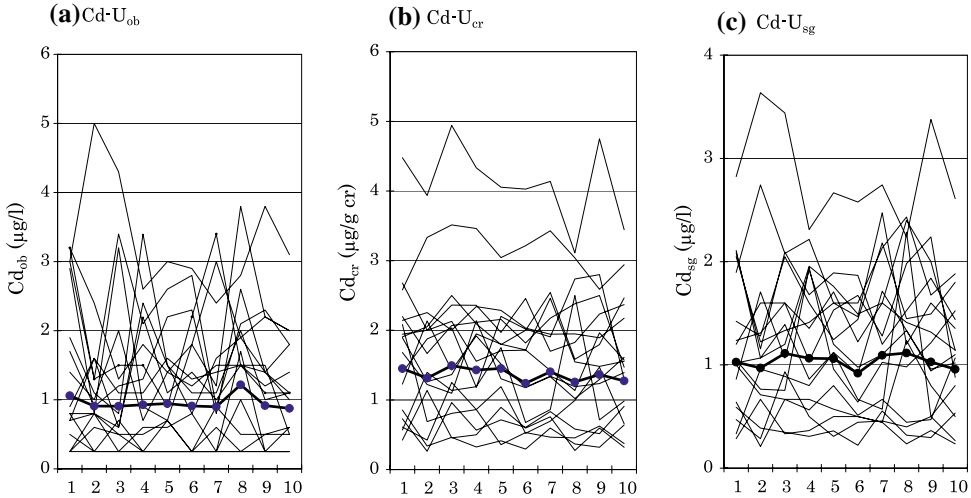

Fig. 3 Variations in Cd-U in the 10-week survey. The *thick lines* connecting *solid circles* show variations in GM values. *Polygonal lines* show 17 individual cases. The numbers on the horizontal axis show a flow in time of 10 weeks

Table 4 Time-dependent correlation of cadmium in blood and urine, and the three dysfunction markers in urine

Parameter	Correlation coefficient[a]					
	The 12-month survey			The 10-week survey		
	Smallest	Avergae	Largest	Smallest	Avergae	Largest
Cd-U$_{ob}$	−0.671	0.440	0.908	0.373	0.629	0.830
Cd-U$_{cr}$	0.365	0.734	0.957	0.783	0.865	0.948
Cd-U$_{sg}$	−0.274	0.551	0.905	0.568	0.767	0.951
α_1-MG$_{ob}$	−0.400	0.397	0.913	0.134	0.435	0.721
α_1-MG$_{cr}$	−0.498	0.420	0.948	0.392	0.610	0.781
α_1-MG$_{sg}$	−0.076	0.492	0.966	0.303	0.646	0.868
β_2-MG$_{ob}$	−0.535	0.085	0.699	−0.065	0.464	0.862
β_2-MG$_{cr}$	−0.904	0.331	0.954	0.509	0.706	0.847
β_2-MG$_{sg}$	−0.536	0.299	0.954	0.355	0.697	0.954
NAG$_{ob}$	−0.059	0.533	0.885	0.230	0.459	0.748
NAG$_{cr}$	−0.286	0.232	0.902	0.159	0.580	0.724
NAG$_{sg}$	−0.150	0.547	0.928	0.355	0.582	0.841
Cd-B	0.992	0.995	0.998			

[a] Coefficients of the correlation between the values under consideration with the values obtained in the immediately previous measurement (both without logarithmic conversion). 12 and 10 measurements with 5 and 17 women were made in the 12-month and 10-week surveys, respectively. The smallest, the average, and the largest coeffcients were calculated out of 11 and 9 coefficients, respectively. It was out of three coeffcients in case of Cd-B, because only four measurements were made. $P < 0.01$ and 0.05 for $r > 0.959$ and 0.878 in the 12-month survey ($n = 5$), respectively, and for $r > 0.606$ and 0.482 in the 10-week survey ($n = 17$)

The small time-dependent variation in group means (such as GMs) among general populations is in good agreement with previous observations.

Findings on group bases after repeated urine sampling are available in Cd exposure monitoring studies in environmental as well as occupational fields.

Namely, Kido et al. (1988) studied 74 residents (men and women combined, aged 50 years or more) in a Cd-polluted area twice at a 5-year interval, the first survey immediately after the removal of Cd-polluted soil from rice paddy and the second survey 5 years later. A few subjects showed substantial reduction in Cd-U (e.g., from about 50 μg/g cr to about 20 μg/g cr) in the 5-year period, but the reduction on a group basis was small (i.e., from 10.0μg/g cr to 9.6 μg/g cr in GM) with no statistical significance ($P > 0.05$) for the difference. The β_2-MG-U$_{cr}$ level tended to be further elevated during the 5-year period among those whose β_2-MG-U$_{cr}$ values were already above 1,000 μg/g cr in the first survey. The β_2-MG-U$_{cr}$ levels stayed below 1,000 μg/g cr in the second survey, however, in most of those whose β_2-MG-U$_{cr}$ levels were less than 1,000 μg/g cr in the first survey. It is interesting to note that the β_2-MG-U$_{cr}$ levels showed substantial variations among the 35 subjects with <1,000 μg/g crβ_2-MG-U, with more than 10-fold

differences between the two determinations (not only decrease but also increase, possibly suggesting recovery and aggravation, respectively) in many cases.

In a 3-year monitoring of residents who had been exposed environmentally to Cd, Arisawa et al. (1997) measured Cd-U twice (three years apart) in 48 residents (men and women combined) and β_2-MG-U four times (every year) in 47 residents five or more years after restoration of the polluted rice paddy. There was a significant ($P < 0.01$) reduction in Cd-U in the 3-year period (e.g., from 8.4 μg/g cr to 6.7 μg/g cr). When correlation coefficients were examined for observed and CR-corrected values after logarithmic conversion, the coefficients between the initial and last measurements were 0.38 and 0.88, respectively, for Cd-U, and 0.91 and 0.93 forβ_2-MG-U ($P < 0.01$ for all correlation coefficients). In case of log β_2-MG-U, the coefficient between the measurement at the initial occasion and that one year later was 0.93 and 0.94. Based on these findings, the authors concluded that the single measurement of the two parameters can reliably estimate Cd-U andβ_2-MG-U average levels at least for a 5-year period.

Ikeda et al. (2005) collected paired urine samples from 195 apparently healthy never-smoking adult women on two occasions at an interval of about 10 months. Comparison on a group basis showed that the correlation between the pairs of Cd-U concentrations and also that of α_1-MG-U concentrations were close with coefficients of 0.4–0.6. Although the coefficient was smaller (0.3) for β_2-MG-U_{ob}, the correlation was improved to 0.4 when Cd-U was corrected for urine density. It was thus concluded that single (unrepeated) determination may be acceptable for Cd-U and α_1-MG-U, and probably for β_2-MG-U also to estimate the Cd burden or possible effects of Cd exposure on tubular function.

In occupational settings, Roels et al. (1989) made a 5-year follow-up on 23 men who retired from Cd exposure-associated work 6–11 years before the initiation of the study. It was not appropriate, however, to estimate between- as well as within-subject variation in this study, because tubular functions of the subjects were most likely still affected by Cd even after their retirements.

Observations on within-subject variations are limited. In a study with five workers (four men and one woman) who were occupationally exposed to Cd (Cd exposure estimates such as Cd in air or Cd in biological materials were not given), Ormos et al. (1985) collected urine samples three times with an interval of two weeks and analyzed for β_2-MG-U together with retinol binding protein in urine. Considerable variations were observed in both parameters, and it was by almost one order of magnitude in some cases. Correction for CR was not effective in reducing within-subject variation. As stated above, the observation by Kido et al. (1988) on wide variation inβ_2-MG-U_{cr} among those who had <1,000 μg β_2-MG/g cr may also be taken as examples to show that within-subject variation is wide in β_2-MG-U.

In over-all evaluation therefore, it is prudent to conclude that time-dependent variations in GM values for Cd-U, α_1-MG-U, β_2-MG-U, and NAG-U were small irrespective of correction for urine density so that single un-repeated measurement would be acceptable as far as the evaluation on a group basis is the objective of the study. Within-subject variations were however wide, the ratio of the largest value over the smallest value being 4–5 even after correction for urine density. Thus, care should be practiced when evaluation on an individual basis is intended. In this respect, high stability in Cd-B may suggest that Cd-B is superior to Cd-U for individual evaluation, as far as the exposure is expected to be rather constant and the population under study accepts blood sampling.

Acknowledgments The authors are grateful to the administration and staff in Hokuriku Health Service Association, Toyama 930-0177, Japan, and those in Kyoto Industrial Health Association, Kyoto, 604-8472, Japan.

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