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ORIGINAL ARTICLE

Sampling of urinary cadmium: differences between 24-h urine and overnight spot urine sampling, and impact of adjustment for dilution

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Abstract
Purpose Urinary cadmium (U-Cd) sampling can be done either by 24-h urine or spot urine sampling, and adjustment for dilution is usually needed. The choice of sampling period and adjustment technique could, however, potentially induce bias. The aim of the study was to compare 24-h urine and spot urine sampling and two dilution adjustment techniques, when assessing U-Cd.
Methods Separate 24-h urine (U24) and timed overnight spot urine (UON) samples were collected from 152 healthy kidney donors. U-Cd, creatinine concentration (U-Crea) and specific gravity (SG) were analysed. Differences between U24 and UON samples were tested using paired *t* test, and the effect of urinary flow rate (UF) was assessed by linear regression.
Results The cadmium excretion rate (U-Cd/h) was lower in the UON than in U24 samples (mean 0.017 µg/h vs. 0.021 µg/h; $p < 0.001$). This decrease was found also for the creatinine-adjusted U-Cd (U-CdCrea) (mean 0.36 µg/gC and 0.41 µg/gC; $p < 0.001$). For U-Cd adjusted for specific gravity (U-CdSG), the difference was reversed, but not statistically significant. The creatinine excretion rate (U-Crea/h) decreased at low UF, especially in the UON.
Conclusions Since U-Cd/h was lower in UON than in U24 samples, the former will underestimate the true Cd excretion. This was seen for U-CdCrea but not for U-CdSG. However, it may be an advantage that the U-CdSG is similar, irrespective of sampling strategy. At low UF, U-CdCrea will be biased upwards. Whether U24 or UON samples adjusted for U-Crea or SG best reflect kidney-Cd is still unknown.

Keywords Urinary cadmium · Creatinine · Specific gravity · 24-h urine · Spot urine

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Introduction

Cadmium (Cd) is a nephrotoxic metal that accumulates in the kidney. The two major sources of Cd exposure in the general population are diet and tobacco smoking (EFSA 2009). Urinary Cd (U-Cd) sampling is widely used to assess exposure or body burden of Cd in the general population, since the method is easy and noninvasive (Jarup et al. 1998). U-Cd sampling can be done either by 24-h urine collection, which is often the case in hospitalized patients, or by spot urine sampling, which is the standard method used in epidemiologic studies when a large number of samples are needed and 24-h sampling is complicated or might cause contamination problems. Although spot urine sampling is more feasible, 24-h urine samples give a better estimation of the true Cd excretion since it is the mean value over a 24-h time period and hence less influenced by physiological and other factors. U-Cd is usually adjusted for variations in dilution due to high or low urinary flow (UF), especially if the results are compared with those of other studies or with guideline values. Adjustment is performed using the creatinine concentration (U-Crea) or specific gravity (SG) (Berlin et al. 1985; Suwazono et al. 2005; Trevisan et al. 1994).

The choice of sampling period and adjustment technique could potentially induce bias. The literature regarding differences between sampling periods and adjustment techniques for U-Cd, or the effect of UF on Cd excretion, is limited. Some studies have examined the use of U-Crea or SG adjustment of U-Cd (U-CdCrea and U-CdSG, respectively) (Berlin et al. 1985; Suwazono et al. 2005; Trevisan et al. 1994) and for xenobiotics in general (Carrieri et al. 2001; Elkins et al. 1974; Moriguchi et al. 2005; Pearson et al. 2009). Berlin et al. (1985) found a close correlation between 24-h urine samples and spot urine samples for unadjusted U-Cd, U-CdCrea and U-CdSG. Trevisan et al. (1994) found higher U-Cd adjusted for U-Crea in daytime samples than in night-time samples. Sata et al. (1995) found no statistically significant correlation between U-CdCrea and UF. All three studies were performed on occupationally exposed workers.

The aim of the present study was to compare two commonly used sampling periods and two dilution adjustment techniques when assessing U-Cd at low-level Cd exposure. Three main aspects were examined in a study population of healthy kidney donors from the general population:

1. Differences, in excretion rates, and unadjusted and adjusted concentrations, between 24-h urine and overnight spot urine samples
2. Effects of urinary flow rate on the excretion rates of cadmium and creatinine and on the concentrations of cadmium
3. Effect modification of gender, age or smoking

Subjects and methods

Study population

Between 1999 and 2005, 152 healthy kidney donors, 65 men and 87 women, were recruited as has been described previously (Barregard et al. 2010). The median age among the study subjects was 50 years (range 24–70), and 91 out of 152 (60%) were current or former smokers. Two men had been occupationally exposed to cadmium when working in the metal refining industry; otherwise, only minor differences in background factors between men and women were found (Barregard et al. 2010). Background data of study subjects are presented in Table 1. The study was approved by the Ethics Committee of the University of Gothenburg.

Urine samples

The donors were admitted 1–2 days before the transplantation, and separate 24-h urine (U24) samples and timed overnight spot urine (UON) samples were collected, when possible. Collection times were recorded, and the total urine volumes were measured. A U24 sample was missing for 16 subjects, and for another 5 subjects, a UON sample was missing, due to time restraint before surgery. Five U24 samples and one UON sample were excluded, due to incomplete urine sampling. In total, 131 complete U24 samples (56 men and 75 women) and 146 complete UON samples (63 men and 83 women) were obtained. The median urine volume and collection time for U24 samples were 1,776 mL (range 444–5,790 mL) and 23.3 h (range 20.5–26.6 h), and for UON samples, 388 mL (range 95–1,050 mL) and 8.6 h (range 6–14.8 h). Cadmium concentrations in urine samples (U24-Cd and UON-Cd) were determined in four different batches at the Department of Occupational and Environmental Medicine, Lund University, by inductively coupled plasma mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK) (Barany et al. 1997). Limits of detection for U-Cd were between 0.01 and 0.02 μg/L. External quality control (QC) samples analysed together with the urine samples showed satisfactory results. Two different QC samples for blood were used (Trace Elements Whole Blood, SERO AS, Billingstad, Norway, and Human Blood Reference

Table 1 Background factors in study subjects

	All	Men	Women
Subjects (*N*)	152	65	87
Age: median (range)	50 (24–70)	51 (30–70)	49 (24–64)
Smoking (*N*)[a]			
Never smokers	60	27	33
Ever smokers	91	38	53
Pack-years: median (range)	13 (0.39–51)	12 (0.39–51)	13 (1.2–36)
Current smokers	36	17	19
Former smokers	55	21	34

[a] One women did not answer the question

Material, Le Centre de Toxicologie du Quebec, International Comparison Program, Canada). Results versus recommended values were 4.74 μg/L ($N = 2$, SD = 0.08) versus 5 μg/L, 4.69 μg/L ($N = 4$, SD = 0.07) versus 5 μg/L, 0.31 μg/L ($N = 3$, SD = 0.02) versus 0.37–0.41 μg/L, 4.5 μg/L ($N = 3$, SD = 0.05) versus 4.8–5.3 μg/L, and 1.59 μg/L ($N = 3$, SD = 0.08) versus 1.69 ± 0.10 μg/L.

Urinary creatinine concentrations (U24-Crea and UON-Crea) were determined using the Jaffé method (Roche Diagnostics) in the first three batches, and in the last batch, using an enzymatic method (Modular P and CREAplus R1, R2, Roche/Hitachi, Roche Diagnostics, GmbH, Mannheim, Germany) at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg. Specific gravity (U24-SG and UON-SG) was measured with a refractometer (Medline, Ceti, Digit 012, Oxfordshire, UK). Excretion rates of cadmium and creatinine (U24-Cd/h, UON-Cd/h, U24-Crea/h, and UON-Crea/h) were calculated from urinary concentration, volume and sampling time. Urinary flow rates (U24-UF and UON-UF) were calculated from urinary volume and sampling time. U-Cd was adjusted for creatinine (U24-CdCrea and UON-CdCrea) and SG (U24-CdSG and UON-CdSG) to compensate for variation in dilution between the different samples. $SG_{Standard} = 1.015$ was used in SG adjustment calculations (Suwazono et al. 2005).

Statistics

Data analyses were performed on untransformed data using SAS software 9.1 (SAS Institute, Cary, NC, USA). Differences between U24 samples and UON samples were tested using paired *t* test (PROC UNIVARIATE) for a total of 125 paired U24 and UON samples (54 men and 71 women). The effect of UF was assessed by linear regression (PROC REG). Differences between groups were tested using Wilcoxon rank sum test (PROC NPAR1WAY). Pearson correlation coefficients were calculated (PROC CORR). Statistical significance was determined at $p < 0.05$, and two-sided confidence intervals were used.

Results

Data on age and smoking habits of the 152 study subjects are shown in Table 1. Excretion rates (U-Cd/h and U-Crea/h) and concentrations of cadmium and creatinine (U-Cd and U-Crea), together with excreted mass of U-Cd/24 h, urinary flow rates (UF) and specific gravity (SG) for 24-h urine sample and overnight spot urine sample (U24 and UON), are shown in Table 2.

Differences, in excretion rates, and unadjusted and adjusted concentrations, between 24-h urine and overnight spot urine samples

Excretion rates, U-Cd/h and U-Crea/h, were both significantly lower in UON compared to U24 (0.017 μg/h vs. 0.021 μg/h, $p < 0.001$ and 52.0 mg/h vs. 55.0 mg/h, $p = 0.005$, respectively) (Fig. 1). As could be expected, UF was also significantly lower (39%) in UON compared to U24 samples ($p < 0.001$), and consequently, both U-Crea and SG were significantly higher in UON than in U24 ($p < 0.001$) (Table 2). When U-Cd was adjusted for dilution, creatinine-adjusted U-Cd (U-CdCrea) was significantly lower (12%) in UON compared to U24 ($p < 0.001$) in contrast to specific gravity-adjusted U-Cd (U-CdSG), for which no significant difference was found ($p = 0.09$) (Table 2). The difference between UON-CdCrea and U24-CdCrea was most marked in the upper range of U-Cd (Fig. 2). There were high correlations between UON-CdCrea and U24-CdCrea ($r_p = 0.77$, $p < 0.001$), and UON-CdSG and U24-CdSG ($r_p = 0.70$, $p < 0.001$) (Fig. 2). Excluding very diluted or concentrated samples (U-Crea < 0.3 g/L or >3.0 g/L or SG < 1.010 or >1.030) did not change the correlation for U-CdCrea, but the correlation coefficient for U-CdSG became somewhat higher.

Effects of urinary flow rate on excretion rates of cadmium and creatinine and on the concentrations of cadmium

In regression analyses, no effect of UF was seen on U-Cd/h ($p = 0.5$ in U24, $p = 0.1$ in UON) (Fig. 3), which otherwise could have explained the lower U-Cd/h in UON samples compared to U24 samples. Overnight creatinine excretion rate, UON-Crea/h, was found to increase with increased UON-UF ($\beta = 0.13$, $p = 0.01$), while U24-Crea/h did not (Fig. 4). After indications, in the exploratory analysis (scatterplot, UON-Crea/h versus UON-UF), of a positive association for lower UF and no association for higher UF, a change point in data for UON was determined by fitting regression models (no intercept) that allowed different change points, and then comparing their fit, using R^2. A change point at 42 mL/h was calculated among the UON samples, and the UON samples were consequently divided into two subgroups with UON-UF < 42 mL/h and ≥42 mL/h. In the subgroup with low UON-UF, UON-Crea/h increased with increased UON-UF ($\beta = 1.1$, $p < 0.001$), while no association was seen in the group with UON-UF ≥ 42 mL/h ($\beta = 0.03$, $p = 0.7$). There was no significant effect of UF on U24-CdCrea, UON-CdCrea or U24-CdSG. However, UON-CdSG decreased with increasing UF ($\beta = -0.0015$, $p = 0.02$).

Table 2 Excretion rates and concentrations for cadmium (unadjusted and adjusted) and creatinine, and urinary flow rates in 24-h and overnight spot urine samples. Differences between 24-h and overnight spot urine samples were calculated for 125 paired samples (54 men and 71 women)

	All				Men				Women				*p* value gender difference
	N	Mean	Range	*p* value difference 24 h-overnight	*N*	Mean	Range	*p* value difference 24 h-overnight	*N*	Mean	Range	*p* value difference 24 h-overnight	
Cadmium excretion rate													
U24-Cd/h (μg/h)	131	0.021	0.003–0.066	<0.001	56	0.022	0.003–0.066	0.03	75	0.020	0.003–0.060	<0.001	0.51
UON-Cd/h (μg/h)	146	0.017	0.003–0.049		63	0.019	0.003–0.049		83	0.016	0.003–0.047		0.28
Amount U-Cd/24 h (μg/24 h)	131	0.51	0.07–1.6		56	0.54	0.07–1.6		75	0.49	0.07–1.4		0.51
Cadmium concentration													
U24-Cd (μg/L)	131	0.30	0.03–1.0	<0.001	56	0.32	0.03–1.0	0.002	75	0.28	0.04–1.0	<0.001	0.53
UON-Cd (μg/L)	146	0.43	0.06–2.0		63	0.44	0.07–2.0		83	0.42	0.06–1.4		0.90
U24-CdCrea (μg/gC)	131	0.41	0.04–1.3	<0.001	56	0.33	0.04–1.2	0.09	75	0.47	0.08–1.3	<0.001	0.001
UON-CdCrea (μg/gC)	146	0.36	0.06–1.3		63	0.30	0.06–1.1		83	0.40	0.09–1.3		0.001
U24-CdSG (μg/L)	131	0.29	0.04–1.0	0.09	56	0.27	0.04–0.73	0.30	75	0.30	0.06–1.0	0.18	0.13
UON-CdSG (μg/L)	146	0.31	0.05–1.1		63	0.30	0.05–1.1		83	0.33	0.07–0.92		0.07
Creatinine concentration													
U24-Crea (g/L)	131	0.77	0.18–3.0	<0.001	56	0.99	0.41–3.0	<0.001	75	0.61	0.18–1.6	<0.001	<0.001
UON-Crea (g/L)	146	1.3	0.33–2.5		63	1.5	0.43–2.5		83	1.1	0.33–2.5		<0.001
Specific gravity													
U24-SG	131	1.016	1.005–1.038	<0.001	56	1.018	1.008–1.038	<0.001	75	1.014	1.005–1.037	<0.001	<0.001
UON-SG	146	1.021	1.006–1.040		63	1.023	1.006–1.040		83	1.019	1.006–1.037		0.007
Urinary flow rate													
U24-UF (mL/h)	131	81.1	20.4–246	<0.001	56	77.2	29.8–171	<0.001	75	84.1	20.4–246	<0.001	0.76
UON-UF (mL/h)	146	49.5	11.5–150		63	50.7	19.2–150		83	48.6	11.5–105		0.63
Creatinine excretion rate													
U24-Crea/h (mg/h)	131	55.0	22.2–103	0.005	56	69.3	39.2–103	0.15	75	44.4	22.2–67.7	0.01	<0.001
UON-Crea/h (mg/h)	146	52.0	14.5–92.9		63	64.9	14.5–92.9		83	42.3	20.4–69.1		<0.001

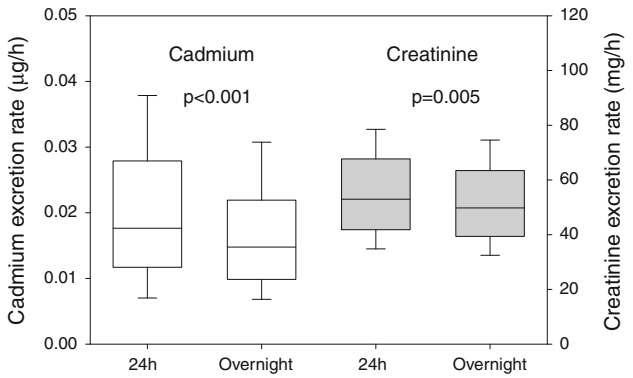

Fig. 1 Excretion rates of cadmium (*unfilled*) and creatinine (*filled*) in 24-h urine samples and overnight spot urine samples. Medians and 10th, 25th, 75th and 90th percentiles are presented in the figure

Effect modification of gender, age or smoking

As described above (Fig. 3), no effect of UF on U-Cd/h was found in the total study group. However, when the study subjects were divided into subgroups of men and women, or ever, never and current smokers, a statistically significant increase ($p < 0.05$) of UON-Cd/h with increased UON-UF was seen for never smokers, and a trend ($p = 0.07$) towards an association was seen for women (Table 3). When the two groups (never smokers and women) were divided into two subgroups of age ($<$ and $\geq$ median age, respectively), statistically significant associations were found for both never smokers ($p = 0.01$) and women ($p = 0.03$) in the groups below median age (data not shown).

There was no significant difference in UF or U-Cd/h between men and women (Table 2), except for currently smoking men who had significantly higher U-Cd/h than smoking women (data not shown). U-Crea/h, U-Crea and SG were significantly higher among men than women (Table 2). U-CdCrea was significantly higher among women than men for both U24 and UON, except for the group of current smokers (data not shown).

As expected, ever smokers had significantly higher U-CdCrea than never smokers in the total material, for men as well as for women, separately (data not shown). For U-CdSG, however, significant differences between never and ever smokers were only found for UON samples in the total material and for men (data not shown). Ever smokers, both men and women, also had significantly higher U-Cd/h

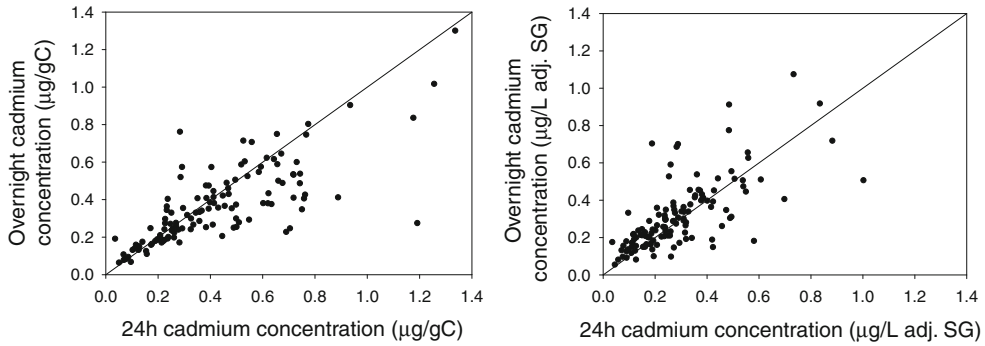

Fig. 2 Overnight spot urine cadmium versus 24-h urine cadmium adjusted for creatinine (µg/gC) or specific gravity (µg/L adj. SG) together with the 1:1 lines. Regression equations for cadmium concentrations adjusted for creatinine concentration or specific gravity were UON-CdCrea = 0.098 + 0.63 × U24-CdCrea and UON-CdSG = 0.096 + 0.75 × U24-CdSG

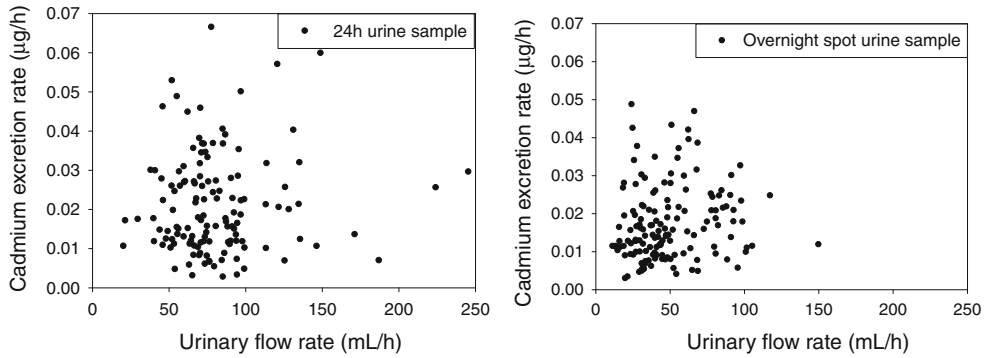

Fig. 3 Cadmium excretion rate versus urinary flow rate for 24-h urine and overnight spot urine samples

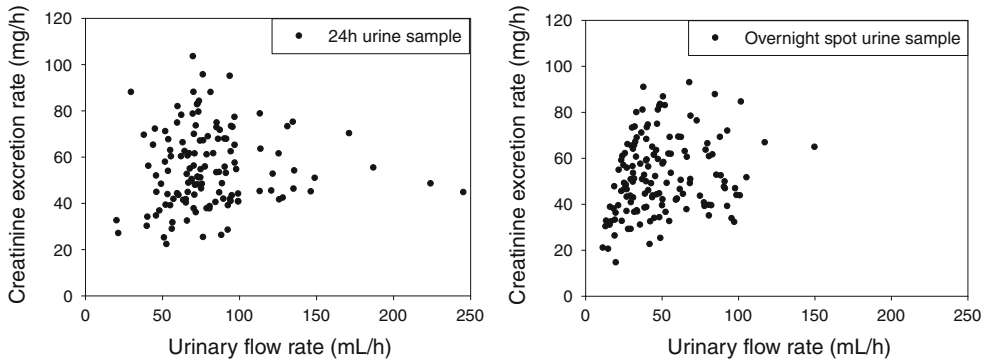

Fig. 4 Creatinine excretion rate versus urinary flow rate for 24-h urine and overnight spot urine samples

Table 3 Results of regression analysis for urinary cadmium excretion rate (U-Cd/h, μg/h) versus urinary flow rate (UF, mL/h) for the total group and subgroups

	U24 $\beta \times 10^{-5}$	UON $\beta \times 10^{-5}$
All	2	5
Women	5	7[a]
Men	−4	−3
Ever smokers	−2	1
Never smokers	4	10[b]
Current smokers	−10	3

[a] $p = 0.07$; [b] $p < 0.05$

compared to never smokers (0.024 μg/h vs. 0.017 μg/h, $p = 0.004$ for U24 and 0.019 μg/h vs. 0.014 μg/h, $p = 0.002$ for UON). In the total material, statistically significant correlations were found between the cumulative cigarette consumption (measured as pack-years) and all U-Cd measures; $r_p = 0.25$, $p = 0.004$ for U24-Cd, $r_p = 0.36$, $p < 0.001$ for UON-Cd, $r_p = 0.35$, $p < 0.001$ for U24-CdCrea, $r_p = 0.49$, $p < 0.001$ for UON-CdCrea, $r_p = 0.27$, $p = 0.002$ for U24-CdSG, and $r_p = 0.49$, $p < 0.001$ for UON-CdSG.

Discussion

Cadmium excretion rate

We found that cadmium excretion rate (U-Cd/h) was lower in overnight spot urine (UON) samples than in 24-h urine (U24) samples. Lower U-Cd/h in UON samples has also been seen by others (Yokoyama et al. 2000). One reason for this could be an increased excretion rate of Cd when urinary flow rate (UF) increases, since UF is higher in daytime than overnight. For women, there was such a trend towards increased U-Cd/h with increasing UF in UON samples, but the association could not be seen for men. We have also noted that U-Cd/h increased significantly with UF in never smokers. Furthermore, the association between U-Cd/h and UF became significant in young women (<50 years of age) and in young never smokers (<50 years of age). When the variability in different groups was examined, we noted a somewhat larger relative standard deviation in U24 samples than in UON samples, and also in ever smokers compared to never smokers (data not shown). This might explain why an association between U-Cd/h and UF was mainly seen in UON samples from never smokers.

Creatinine excretion rate

The creatinine excretion rate (U-Crea/h) was also significantly lower in UON samples than U24 samples. In UON samples, UON-Crea/h decreased with decreased UON-UF, but the effect was mainly seen at low UF. Urinary flow rate was significantly lower in UON samples than U24 samples, which could explain why the association for U-Crea/h was primarily seen in UON samples. For U24 samples, the association was only significant for women. An association between U-Crea/h and UF has been described earlier in the literature (Trachtenberg et al. 2010; Greenberg and Levine 1989). Urinary creatinine concentration (U-Crea) was, as expected, significantly lower for women compared to men, and the magnitude of the difference is in agreement with findings in other studies (Carrieri et al. 2001; Suwazono et al. 2005). There was a high correlation between U-Crea and specific gravity (SG), as has been reported previously (Carrieri et al. 2001; Moriguchi et al. 2005).

Cadmium concentrations adjusted for creatinine concentration or specific gravity

Since the U-Cd/h was lower in UON samples than in U24 samples, the former will underestimate the true cadmium

excretion if a U24 sample is regarded as the 'gold standard'. This was also found for U-Cd adjusted for U-Crea (U-CdCrea), which has been described earlier (Trevisan et al. 1994), but not for U-Cd adjusted for SG (U-CdSG). Although U-CdSG did not show the true difference between U24-Cd and UON-Cd, it may be an advantage that the U-CdSG is similar, irrespective of sampling strategy. The effect of UF on U-Crea/h was most clear at low UF. Therefore, in cases with low UF (resulting in low U-Crea/h), the U-CdCrea will be biased upwards. Men have significantly higher U-Crea/h than women, in contrast to U-Cd/h. Therefore, the U-CdCrea is significantly higher for women compared to men, which has been reported previously (Jarup et al. 1998). No gender difference was seen for U-CdSG. For U-CdSG, it was surprising that significant differences between never and ever smokers were found only for UON samples in the total material and for men, despite the difference between ever and never smokers in kidney-Cd concentration (Barregard et al. 2010). Creatinine-adjusted U-Cd showed the expected difference between smokers and nonsmokers.

High correlations were found between UON-CdCrea and U24-CdCrea, and UON-CdSG and U24-CdSG, as previously reported (Berlin et al. 1985). No association was found between U-CdCrea and UF, which also has been reported previously (Sata et al. 1995). However, UON-CdSG decreased with increasing UF.

Whether U24 or UON samples adjusted for U-Crea or SG best reflect kidney-Cd concentration is, however, still unknown. Even if U24 samples better reflect the true average Cd excretion, it is possible that the association between kidney-Cd and UON-Cd is stronger than for U24-Cd samples, due to more stable activity conditions overnight. The same argument could be used for U-CdCrea, which reflects the true difference in Cd excretion between U24 and UON samples, while U-CdSG does not. The associations between cumulative smoking (pack-years) and UON-Cd were stronger than those between smoking and U24-Cd, while associations between U-CdCrea and cumulative smoking, and U-CdSG and cumulative smoking were equally strong. Thus, UON-Cd seems to be at least as valid as U24-Cd. The choice of adjustment technique should also take into account the fact that U-CdCrea tends to be higher among women (who on average have lower muscle mass) than in men, even if the Cd exposure is the same. On the other hand, the fact that U-Crea adjustment therefore also includes some adjustment for body and kidney size may be an advantage. The same long-term daily Cd exposure will cause higher average U-CdCrea in women, but this reflects the fact that kidney-Cd will be higher in women, who have on average about 15–20% lower kidney weight (Barregard et al. 2010).

Strengths and limitations

This is a study of kidney donors from the general population with a low-level Cd exposure, who should be considered representative of the healthy part of the Swedish general population. Two study subjects had a history of occupational Cd exposure, but the exposures occurred a long time ago and were only moderate. We therefore believe that these two subjects also were in a steady-state condition. The analyses of Cd in urine were carried out by an experienced laboratory, and quality control samples analysed at the same time as the samples showed acceptable results, especially for the low-level control samples.

A strength of this study is the fact that separate UON samples and U24 samples were taken; the UON sample is not included in the U24 sample. The samples were collected when the study subjects were hospitalized, which reduced the risk of contamination. Only incomplete urine samples have been excluded from the material. It is often recommended that very concentrated (U-Crea > 3 g/L) or dilute (U-Crea < 0.3 g/L) samples should be excluded, since the validity of such samples could be questioned. However, in the present study, such urine samples were included, since one of the aims was to study the impact of UF on U-Cd excretion.

In conclusion, the present study indicates that Cd excretion is lower in the night than during daytime. Therefore, UON samples may underestimate the true Cd excretion. On the other hand, the correlation between UON-Cd and U24-Cd is high, for U-CdCrea as well as for U-CdSG, and UON-Cd seems to predict the additional Cd exposure from smoking better than U24-Cd. Overnight samples have the advantage of more stable activity and physiology compared to daytime samples.

Conflict of interest The authors declare that they have no conflicts of interest.

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