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# Associations between Urinary Excretion of Cadmium and Proteins in a Nonsmoking Population: Renal Toxicity or Normal Physiology?

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**Short running title:** Associations between urinary cadmium and proteins

**Keywords:** Albumin, alpha-1-microglobulin, cadmium, cadmium toxicity, kidney effect, renal function, urinary excretion

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## Abbreviations and definitions

Cd	Cadmium
HMW	High molecular weight
LMW	Low molecular weight
LOD	Limit of detection
Mo	Molybdenum
MoO	Molybdenum oxide
Overnight sample	First morning sample
QC	Quality control
SD	Standard deviation
SG	Specific gravity
U-Alb	Urinary albumin concentration
U-AlbCrea	Urinary albumin concentration adjusted for creatinine concentration
U-Alb/h	Urinary excretion rate of albumin
U-AlbSG	Urinary albumin concentration adjusted for specific gravity
U-A1M	Urinary alpha-1-microglobulin concentration
U-A1MCrea	Urinary alpha-1-microglobulin concentration adjusted for creatinine concentration
U-A1M/h	Urinary excretion rate of alpha-1-microglobulin
U-A1MSG	Urinary alpha-1-microglobulin concentration adjusted for specific gravity
U-Cd	Urinary cadmium concentration
U-CdCrea	Urinary cadmium concentration adjusted for creatinine
U-Cd/h	Urinary excretion rate of cadmium
U-CdSG	Urinary cadmium concentration adjusted for specific gravity
U-Crea	Urinary creatinine concentration
U-Crea/h	Urinary excretion rate of creatinine
UF	Urinary flow rate

## **Abstract**

**Background:** Associations between cadmium and kidney function have been reported even at low levels of exposure in the general population. Recently, the causality of these associations has been questioned.

**Objectives:** To examine associations between urinary Cd (a biomarker of exposure) and urinary proteins used as biomarkers of kidney effects, based on repeated short-term sampling in healthy subjects.

**Methods:** Twenty-four hour urine samples were collected on two separate days at six fixed times from 30 healthy nonsmoking men and women (median age 39 years). The samples (N=354) were analysed for Cd (U-Cd) and two proteins used as kidney function biomarkers: albumin (U-Alb) and alpha-1-microglobulin (U-A1M). Concentrations were adjusted for creatinine concentration or specific gravity, and excretion rates (mass/hour) were calculated. Possible associations were assessed within each individual, and mean correlations and regressions were evaluated.

**Results:** There were clear positive mean associations within individuals between the excretion of U-Cd (mean 0.11 µg/g Creatinine, range 0.01-0.52 µg/g Creatinine) and both U-Alb and U-A1M. The associations were stronger for excretion rates and concentrations adjusted for specific gravity than for concentrations adjusted for creatinine. There were also significant positive associations of urinary flow with excretion of U-Cd, U-Alb and U-A1M.

**Conclusions:** Associations between short-term changes in U-Cd and markers of kidney function within individual nonsmoking study participants are unlikely to reflect effects of Cd toxicity. A more likely explanation is that these associations result from normal variation in renal function, including changes in urinary flow, that influence the urinary excretion of both Cd and proteins in the same direction. These effects of normal variability may result in overestimation of the effects of Cd on kidney function at low level Cd exposure.

## Introduction

Adverse effects of cadmium (Cd) on the kidney have been demonstrated in occupational settings with high Cd exposure since the late 1940s (Friberg 1950). For the general population, diet is the main source of Cd, except among smokers, for whom tobacco smoking is also an important route of exposure. In the last couple of decades, evidence of renal tubular damage, based on increased excretion of low molecular weight (LMW) proteins, has been reported in association with lower levels of Cd exposure in the general population (Akesson et al. 2005; EFSA 2009; Jarup et al. 1998; Jarup and Akesson 2009; Nordberg 2007). Cadmium excreted in urine (U-Cd) is widely used to assess exposure or body burden of Cd in the general population, and excretion of LMW proteins, such as beta-2-microglobulin, is used as a measure of effects on the kidney (EFSA 2009; Jarup et al. 1998). Another LMW protein in urine used for evaluation of effects on kidney function is alpha-1-microglobulin (U-A1M) (Akesson et al 2005; Bakoush et al. 2001; Penders and Delanghe 2004). Urinary excretion of high molecular weight (HMW) proteins such as urinary albumin (U-Alb) have also been reported to increase at high Cd exposure (Nordberg et al. 2009). The negative association between diuresis and concentrations of biomarkers in urine is well known, and therefore adjustment for dilution based on urine creatinine concentration (U-Crea) or specific gravity (SG) is used to avoid false positive associations when biomarkers are measured in individual spot urine samples (Berlin et al. 1985; Suwazono et al. 2005; Trevisan et al. 1994) . In contrast, when urine is collected over time, excretion rates can be calculated directly.

Recently the causality of associations between U-Cd and biomarkers of kidney effects in populations with low levels of exposure has been questioned due to possible confounding by smoking or by physiological sources of variability (Bernard 2008; Chaumont et al. 2010; Chaumont et al. 2012; Haddam et al. 2011). Several possible physiological mechanisms have been suggested; for example, LMW proteins and Cd bound to metallothionein may have

similar affinity for tubular binding sites, and excretion of both proteins and U-Cd may be influenced by normal variation in diuresis (urinary flow, UF) (Chaumont et al. 2012).

Smoking may be a confounder if it increases the excretion not only of U-Cd but also of urinary proteins (Bernard 2008; Chaumont et al. 2010; Haddam et al. 2011).

The possible influence of physiological factors on associations between U-Cd and biomarkers of kidney function is difficult to reveal in studies with only one spot urine sample per individual, since it is not possible to disentangle intra- and inter-individual variability.

Although average excretion of U-Cd and urinary proteins may be relatively stable over time within individuals, and may therefore accurately reflect long-term effects of Cd on kidney function, excretion of both biomarkers may vary over the day due to physiological factors such as diuresis, body position, and exercise. Then these short-term changes would not reflect biological effects of Cd toxicity. Therefore, if there are positive associations between excretion of U-Cd and urinary proteins over the day within individuals, such associations cannot be assumed to be due to Cd toxicity.

Our aim was to study the association between low level urinary excretion of Cd and two urinary proteins (U-Alb and U-A1M) within individuals, to assess whether associations reported between urinary excretion of Cd and proteins used as biomarkers of impaired kidney function may be due to temporary effects of physiological factors such as a change in UF on the biomarkers, rather than long-term toxic effects of Cd on kidney function.

## Materials and Methods

### *Study population*

Thirty nonsmoking healthy (no diabetes, hypertension or kidney disease) participants (15 men and 15 women) were recruited from the staff of our department and among students at the University of Gothenburg. Each participant filled out a questionnaire concerning age, weight, length, smoking habits, and diseases or medications. Median age and BMI of the participants was 39 years and 23.9 kg/m<sup>2</sup> (range 23-59 years, 19.1-28.7 kg/m<sup>2</sup>). The study was approved by the Ethics Committee of the University of Gothenburg and complied with all applicable requirements of international regulations. Participants gave written informed consent prior to the study.

### *Urine samples*

Timed samples (N=354) were collected and the volumes recorded on two separate days (mostly 4-6 days apart) at six fixed times over 24h; 09:30, 12:00, 14:30, 17:30, 22:00 and first morning urine (overnight samples). If urination was necessary between fixed times, the next container was used to ensure that all urine was collected over the 24h period. Individual sample volumes ranged between 20-1075 mL with a median volume of 245 mL, and 24h urine volume ranged between 465-4660 mL with a median volume of 1640 mL. There were no significant gender differences. Detailed instructions were given to ensure completeness of the 24h sampling. The collected samples were then transferred to Minisorb tubes (NUNC, Denmark) and kept at 4°C until analysis of proteins and creatinine (within 3 days of collection). Aliquots used for determination of U-Cd were frozen and analysed five years later. For one woman, samples were collected during one day only.

*Chemical analysis*

Cadmium concentrations in urine (U-Cd) corrected for molybdenum oxide (MoO) interference were determined at the Department of Occupational and Environmental Medicine, Lund University, by inductively coupled plasma mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK) in samples diluted ten times with an alkaline solution according to Barany et al. (1997). By addition of molybdenum (Mo) to blank urine, the formation of MoO calculated as Cd was evaluated. Addition of 500 µg Mo/L gave on average a contribution of 0.68 µg Cd/L. In all samples Mo was determined, thus a correction for the MoO interference was feasible by the known proportion Mo/MoO. Since the oxidation level could vary from day to day, blank urine with and without Mo addition (500 µg/L) was analysed among the urine samples in every series of analysis. All samples were prepared in duplicate, and the method imprecision (calculated as the coefficient of variation for duplicate preparations) was 9.5% and for samples with Cd content from Limit of detection (LOD) to 0.10 µg/L, the imprecision was 11%. LOD for U-Cd was 0.05 µg/L and in 92 out of 354 samples (26%) U-Cd was below the LOD. Three different quality control (QC) samples were used (Trace Elements Whole Blood, Seronorm AS, Billingstad, Norway, and Human Blood Reference Material, Le Centre de Toxicologie du Quebec, International Comparison Program, Canada) and the results versus recommended values ( $\pm$  SD) were  $0.26 \pm 0.03$  µg/L (N = 12) vs. 0.26-0.36 µg/L,  $0.93 \pm 0.04$  µg/L (N = 12) vs. 1.01  $\pm$  0.09 µg/L, and  $4.9 \pm 0.12$  µg/L (N = 12) vs.  $5.1 \pm 0.26$  µg/L, respectively.

Analyses of urinary albumin concentrations (U-Alb) were performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital in Gothenburg by an automated nephelometric immunochemical method using reagents and calibrator from Beckman Coulter (Fullerton, CA, USA). Internal reference samples were used in each analytical run, showing

satisfactory results. The LOD for U-Alb was 2.4 mg/L and in 113 out of 354 samples (32%) U-Alb was below the LOD.

Analyses of alpha-1-microglobulin (U-A1M) were performed at our department using the  $\alpha_1$ -microglobulin ELISA Kit K6710 (Immundiagnostik AG, Bensheim, Germany) as described elsewhere (Andersson et al. 2008). Calibrators, provided in each kit, with target values in the ranges 0.09-0.28 mg/L were always within the acceptable range. The LOD for U-A1M was 0.1 mg/L and one out of 354 samples (0.3%) was below the LOD.

Analyses of creatinine concentrations (U-Crea) were performed in fresh urine using the Jaffé method (Roche Diagnostics, Mannheim, Germany) with a LOD of 0.01 mmol/L. Specific gravity (SG) was measured in fresh urine with a Ceti Digit 012 refractometer (Medline, Oxfordshire, UK).

Excretion rates of cadmium, albumin, alpha-1-microglobulin and creatinine were calculated from urinary concentrations, volumes, and sampling times. Urinary flow rates (UF) were calculated from urinary volumes and sampling times. The concentrations of U-Cd, U-Alb and U-A1M were adjusted for U-Crea and SG to compensate for variation in dilution between the different samples.  $SG_{\text{Standard}}=1.015$  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, NC, USA). Associations between variables were assessed within individuals by calculations of Spearman correlation coefficients,  $r_s$ , (PROC CORR) or by separate linear regression models (PROC REG) for each participant. Since we hypothesized that physiological variation in protein excretions would affect Cd excretion rather than the opposite, Cd was the dependent variable in the regression models. Overall mean correlation coefficients and overall mean regression slopes (coefficients) were calculated by averaging the participant-specific sample

means, and were tested for significant deviation from zero using the Wilcoxon signed rank test (PROC UNIVARIATE). Differences in overall mean values between groups were tested using the Wilcoxon rank sum test (PROC NPAR1WAY). Statistical significance was determined at  $p < 0.05$ , and two-sided confidence intervals were used. For values below the LOD, the LOD divided by the square root of 2 was used in the statistical calculations (Hornung and Reed 1990).

## Results

Mean values of the individual mean concentrations and excretion rates of U-Cd, U-Alb and U-A1M across participants are shown in Table 1. The only significant difference between men and women was that women had lower U-A1M excretion rates (0.10 mg/h vs. 0.18 mg/h,  $p = 0.02$ ) than men.

### *Associations between urinary excretion of cadmium and albumin*

On average, U-Cd excretion was positively associated with U-Alb excretion within individual participants (see Figure 1 for an example of data for all samples from an individual participant and Figure 2 for the distributions of means across participants). The association was stronger for excretion rates (overall mean  $r_s = 0.44$ ,  $p < 0.001$ ) and concentrations adjusted for specific gravity (overall mean  $r_s = 0.37$ ,  $p < 0.001$ ) compared to concentrations adjusted for U-Crea (overall mean  $r_s = 0.26$ ,  $p = 0.001$ ). Exclusion of overnight samples ( $N = 59$ ), values below LOD ( $N = 113$ ), or very diluted or concentrated samples (U-Crea  $< 0.3$  g/L or  $> 3.0$  g/L ( $N = 28$ ), respectively, or SG  $< 1.010$  or  $> 1.030$  ( $N = 85$ )) did not change the overall results, and there were no significant gender differences in the association between U-Cd and U-Alb (data not shown). Overall means were similar when participants were stratified by age (above/below median 39 year) or BMI (above/below median  $23.9$  kg/m<sup>2</sup>) (data not shown).

*Associations between urinary excretion of cadmium and alpha-1-microglobulin*

On average, excretion of U-Cd was positively associated with U-A1M within individuals (Figures 1 and 2) with stronger overall associations for excretion rates (overall mean  $r_s=0.33$ ,  $p<0.001$ ) and concentrations adjusted for specific gravity (mean  $r_s=0.26$ ,  $p<0.001$ ) than for concentrations adjusted for U-Crea (mean  $r_s=0.21$ ,  $p=0.002$ ). As for U-Alb, overall means for associations between U-A1M and U-Cd were similar after excluding very diluted or very concentrated samples, overnight samples, or samples <LOD (N=92 for U-Cd versus U-A1M), and there were no significant differences when participants were stratified by age or BMI (data not shown).

Overall mean values of individual linear regression model coefficients were significantly  $>0$  for U-Alb and U-A1M when both were included in the same model as predictors of U-Cd, regardless of whether the dependent variable was U-Cd excretion rate or U-Cd concentration adjusted for creatinine or SG (data not shown).

*Associations between UF and U-Cd, urinary proteins and creatinine*

Regression analyses of data from individual participants indicated significant negative overall mean associations between UF and unadjusted concentrations of U-Cd, U-Alb and U-A1M, consistent with expectations (data not shown). However, mean values for associations between UF and urinary excretion rates across all participants were positive and significantly  $>0$  for Cd, albumin, and A1M (overall mean  $\beta=0.032\times 10^{-3}$ ,  $\beta=1.8\times 10^{-3}$ , and  $\beta=0.49\times 10^{-3}$ , respectively; Table 2). There was also a significant positive overall mean value for the association between UF and U-Crea excretion rate (overall mean  $r_s=0.25$ , overall mean  $\beta=0.25\times 10^{-3}$ ;  $p<0.001$ ). Overall mean values for associations between UF and SG-adjusted concentrations were negative, and were significantly  $<0$  for U-Cd and U-Alb. Overall mean values for associations between creatinine-adjusted concentrations and UF were not

significant although nearly so for U-Alb. Similar results were found for mean Spearman correlation coefficients across participants (data not shown). When UF was included in regression analyses with U-Cd as the dependent variable and protein excretion (U-Alb or U-A1M) as independent variables, overall mean regression coefficients for UF were significantly  $>0$  for U-Cd excretion and SG-adjusted U-Cd concentration, but not for creatinine-adjusted U-Cd concentration (Table 3). Overall mean values for associations with U-Alb and U-A1M (Table 3) were similar to overall means from models that were not adjusted for UF (Figure 2).

#### *Estimated effect of U-Crea or SG on U-Cd and proteins*

Overall mean values of within-individual correlation and regression coefficients indicated negative associations between urinary creatinine and creatinine-adjusted concentrations of urinary Cd, albumin, and A1M (Cd:  $r_s = -0.20$ ,  $p = 0.02$  and  $\beta = -0.02$ ,  $p = 0.07$ ; albumin:  $r_s = -0.31$ ,  $p < 0.001$  and  $\beta = -1.8$ ,  $p = 0.006$ ; and A1M:  $r_s = -0.15$ ,  $p = 0.03$  and  $\beta = -0.080$ ,  $p < 0.001$ , respectively) indicating that using creatinine to adjust for dilution was not perfect. In contrast, SG was not significantly associated with SG-adjusted concentrations of urinary Cd, albumin, or A1M (data not shown).

## **Discussion**

In this study, with multiple samples from each individual, we have estimated associations between urinary cadmium and urinary proteins used as biomarkers of kidney function. In this way we were able to evaluate whether associations may be caused by factors that vary within individuals, which cannot be differentiated from causal effects of Cd on renal function in studies based on a single spot urine sample from each subject. To the best of our knowledge, this is the first study to evaluate associations between urinary cadmium and proteins used as

biomarkers of kidney function within individuals in a group of environmentally exposed never-smokers.

Our results show clear positive mean associations between the urinary excretion of Cd and albumin and between urinary excretion of Cd and A1M at low levels of Cd exposure. Since Cd has a long biological half-time in the human body (EFSA 2009; Jarup and Akesson 2009; Nordberg 2007) the kidney Cd and whole body Cd are expected to be stable during the two sampling days, and urinary Cd should therefore be affected by normal physiological variation only. Thus, the associations between urinary Cd and protein excretion observed within individuals in our study are not consistent with an effect of Cd toxicity.

Chaumont et al. (2012) have suggested that protein excretion caused by Cd toxicity is unlikely at low levels of exposure. Instead, these investigators have proposed that Cd bound to metallothionein and certain LMW proteins share the same renal tubular binding site, and that physiological variation in tubular reabsorption will therefore affect excretion of Cd-metallothionein and LMW proteins in the same direction. Removal of proteins such as metallothionein, albumin, and A1M from the tubular fluid by endocytosis is mediated by two multiligand receptors, megalin and cubilin (Christensen et al. 2009). We showed clear associations between urinary flow and urinary cadmium, albumin, and A1M, which suggests that variation in urinary flow is an important determinant of variation in tubular reabsorption.

In a study of industrial workers, Haddam et al. (2011) found an association between urinary Cd and proteins, but concluded that the association was largely driven by smoking, diuresis and probably also by a coexcretion of Cd with the proteins. In our study urinary albumin and urinary A1M were significant predictors of urinary Cd excretion rate and urinary Cd concentration, regardless of which adjustment method for diuresis was used. However, we cannot determine if normal physiological variability in renal tubular reabsorption is the only

explanation. Cd in serum is bound not only to the LMW protein metallothionein, but also to albumin (Nordberg 1984). Although albumin is a large protein, it is partly filtered in the glomeruli, and can, by competitive inhibition of tubular reabsorption, affect also LMW proteins such as A1M (Bernard et al. 1987). However, serum Cd is mainly bound to metallothionein in individuals with low level Cd exposure through ingestion (Nordberg et al. 2007).

Investigating the effect of Cd toxicity on kidney function in a study population with low level exposure using markers of exposure and effect measured in the same urine sample is difficult, and factors that increase the variability of the biomarkers could either attenuate or overestimate an association. Our results indicate that caution is needed if urinary Cd is used as a biomarker of exposure when studying renal effects of low level Cd exposure. The fact that diuresis and other sources of normal physiological variability affect urinary Cd suggests that urinary Cd may be a poor marker of kidney Cd at very low exposure levels. However, although the creatinine-adjustment of Cd in this study was not perfect, creatinine adjusted Cd was less affected by normal physiological variations than Cd excretion rate or SG adjusted Cd concentration.

One limitation in this study is that the participants were recruited from university and hospital departments, but we believe that they are likely to be representative of the nonsmoking healthy general population with respect to normal intra-individual variability in Cd and protein excretion. Also, samples were from individuals with very low Cd exposures and it is not possible to assess relations between U-Cd and biomarkers of kidney function at medium or high levels of exposure.

## **Conclusions**

In conclusion, our study has shown clear positive overall mean associations between the urinary excretion of cadmium and urinary proteins in individuals with low-level exposure to cadmium. The overall mean associations were seen both for the low molecular weight protein A1M and the high molecular weight protein albumin. These associations are unlikely to be caused by Cd toxicity, but rather reflect temporary changes in urinary flow or other sources of normal physiological variability that affect the excretion of urinary Cd and urinary proteins in the same direction, resulting in an overestimation of the risk of renal toxicity from low level Cd exposure.

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## Tables

Table 1: Urinary cadmium, albumin and alpha-1-microglobulin

Biomarker	All (N=354, n=30)		Men (N=180, n=15)		Women (N=174 <sup>a</sup> , n=15)		p-value gender difference <sup>d</sup>
	Mean <sup>b</sup>	Range <sup>c</sup>	Mean <sup>b</sup>	Range <sup>c</sup>	Mean <sup>b</sup>	Range <sup>c</sup>	
<b>Cadmium</b>							
U-Cd (µg/L)	0.12	<LOD <sup>e</sup> -1.1	0.11	<LOD <sup>e</sup> -0.53	0.14	<LOD <sup>e</sup> -1.1	0.77
U-CdCrea (µg/gC) <sup>f</sup>	0.11	0.01-0.52	0.08	0.01-0.25	0.16	0.02-0.52	0.06
U-CdSG (µg/L) <sup>g</sup>	0.12	0.01-0.71	0.09	0.01-0.31	0.14	0.02-0.71	0.12
U-Cd/h (µg/h) <sup>h</sup>	0.007	0.0007-0.03	0.007	0.0009-0.02	0.008	0.0007-0.03	0.54
<b>Albumin</b>							
U-Alb (mg/L)	7.5	<LOD <sup>e</sup> -121	10.5	<LOD <sup>e</sup> -121	4.4	<LOD <sup>e</sup> -33	0.44
U-AlbCrea (mg/gC) <sup>f</sup>	6.3	1.1-78	7.4	1.2-42	5.3	1.1-78	0.37
U-AlbSG (mg/L) <sup>g</sup>	6.3	0.28-65	7.9	0.32-65	4.7	0.28-62	1.0
U-Alb/h (mg/h) <sup>h</sup>	0.47	0.02-4.9	0.64	0.08-4.5	0.31	0.02-4.9	0.26
<b>A1M</b>							
U-A1M (mg/L)	2.4	<LOD <sup>e</sup> -47	3.1	0.12-47	1.7	<LOD <sup>e</sup> -10	0.12
U-A1MCrea (mg/gC) <sup>f</sup>	2.0	0.11-31	2.2	0.11-31	1.7	0.22-7.6	0.35
U-A1MSG (mg/L) <sup>g</sup>	1.9	0.13-32	2.3	0.13-32	1.5	0.13-5.8	0.07
U-A1M/h (mg/h) <sup>h</sup>	0.14	0.002-1.8	0.18	0.01-1.8	0.10	0.002-0.54	0.02
Urinary flow rate (mL/h)	89.2	8.0-420	84.9	8.0-336	93.6	11-420	0.65

<sup>a</sup> One woman only provided samples over 1 day (6 samples)

<sup>b</sup> Mean values are calculated as mean of 30 individual means(n)

<sup>c</sup> Ranges are for all 354 urine samples (N)

<sup>d</sup> Test of gender difference are performed on 30 individual means (Wilcoxon rank sum test)

<sup>e</sup> Values below limit of detection (LOD) were replaced with LOD/ $\sqrt{2}$  in calculation.

<sup>f</sup> Concentrations adjusted for creatinine concentration (mean<sup>b</sup> U-Crea 1.2 g/L)

<sup>g</sup> Concentrations adjusted for specific gravity (mean<sup>b</sup> SG 1.017)

<sup>h</sup> Excretion rates

Table 2: Mean linear regression model coefficients for associations of urinary flow rate (independent variable mL/h) with urinary cadmium, albumin, or alpha-1-microglobulin (as dependent variables) based on separate within-individual models for 30 participants <sup>a</sup>.

Adjustment method <sup>b</sup>	Cd		Albumin		A1M	
	$\beta \times 10^{-3}$ (p-value) <sup>c</sup>	Intercept	$\beta \times 10^{-3}$ (p-value) <sup>c</sup>	Intercept	$\beta \times 10^{-3}$ (p-value) <sup>c</sup>	Intercept
Excretion rate	0.032 (<0.001)	0.0053	1.8 (<0.001)	0.32	0.49 (0.02)	0.11
Specific gravity adjusted	-0.58 (0.007)	0.14	-15 (0.005)	6.9	-9.0 (0.07)	2.5
Creatinine adjusted	-0.019 (0.49)	0.11	11 (0.05)	5.1	-1.4 (0.30)	2.0

<sup>a</sup> Individual regressions based on data for 6 samples per day over 2 days, 354 total samples (one participant had 6 samples only). For each individual participant,  $X = \beta(\text{urinary flow rate}) + \text{intercept}$ , where  $X = \text{Cd, albumin, or A1M, respectively}$ .

<sup>b</sup> urinary excretion rate in  $\mu\text{g/h}$  for Cd,  $\text{mg/h}$  for albumin and A1M, specific-gravity adjusted concentrations in  $\mu\text{g/L}$  for Cd and  $\text{mg/L}$  for albumin and A1M, creatinine-adjusted concentrations in  $\mu\text{g/g}$  Creatinine for Cd and  $\text{mg/g}$  Creatinine for albumin and A1M

<sup>c</sup> p-value for significant deviation of the mean regression slope across individuals based on Wilcoxon signed rank test

Table 3: Mean regression coefficients for the association of urinary Cd (dependent variable) with urinary flow (mL/h) and albumin (Model 1) or urinary flow and alpha-1-microglobulin (Model 2), based on separate within-individual models for 30 participants <sup>a</sup>.

Adjustment method <sup>b</sup>	Model 1			Model 2		
	$\beta_1$ (p-value) <sup>c</sup>	$\beta_2 \times 10^{-3}$ (p-value) <sup>c</sup>	Intercept	$\beta_1$ (p-value) <sup>c</sup>	$\beta_2 \times 10^{-3}$ (p-value) <sup>c</sup>	Intercept
Excretion rate	0.013 (<0.001)	0.016 (0.004)	0.0035	0.025 (<0.001)	0.011 (0.007)	0.0042
Specific gravity adjusted	0.014 (<0.001)	-0.45 (0.02)	0.093	0.019 (<0.001)	-0.56 (0.02)	0.11
Creatinine adjusted	0.007 (0.007)	-0.096 (0.90)	0.098	0.014 (<0.001)	-0.089 (0.99)	0.094

<sup>a</sup> Individual regressions based on data for 6 samples per day over 2 days, 354 total samples (one participant had 6 samples only). For each individual participant, Model 1: U-Cd =  $\beta_1$ (urinary flow rate) +  $\beta_2$  (U-Alb) + intercept; Model 2: U-Cd =  $\beta_1$ (urinary flow rate) +  $\beta_2$  (U-A1M) + intercept.

<sup>b</sup> urinary excretion rate in  $\mu\text{g/h}$  for Cd,  $\text{mg/h}$  for albumin and A1M, specific-gravity adjusted concentrations in  $\mu\text{g/L}$  for Cd and  $\text{mg/L}$  for albumin and A1M, creatinine-adjusted concentrations in  $\mu\text{g/g}$  Creatinine for Cd and  $\text{mg/g}$  Creatinine for albumin and A1M

<sup>c</sup> p-value for significant deviation of the mean regression slope across individuals based on Wilcoxon signed rank test

### Figure legends

Figure 1: Example showing the association, for one of 30 individuals, between excretion rates, concentrations adjusted for specific gravity, and concentrations adjusted for creatinine concentration, of cadmium and albumin, or cadmium and alpha-1-microglobulin, respectively.

Figure 2: Distributions of individual Spearman correlation coefficients ( $r_s$ ) and individual regression slopes ( $\beta$ ) for associations between urinary cadmium and albumin (left), and urinary cadmium and alpha-1-microglobulin (right), calculated for 30 individuals [individual values based on 6 samples per day over 2 days for each participant, 354 total samples (one participant had 6 samples only)]. Boxplot, indicating 10<sup>th</sup>, 25<sup>th</sup>, mean (dotted line), median, 75<sup>th</sup> and 90<sup>th</sup> percentiles across all participants, with dots indicating outliers. All measures deviate significantly from zero ( $p < 0.05$ ). Urinary excretion rates in  $\mu\text{g/h}$  for Cd,  $\text{mg/h}$  for albumin and A1M; specific-gravity adjusted concentrations in  $\mu\text{g/L}$  for Cd,  $\text{mg/L}$  for albumin and A1M; creatinine-adjusted concentrations in  $\mu\text{g/g}$  Creatinine for Cd,  $\text{mg/g}$  Creatinine for albumin and A1M.

Figure 1

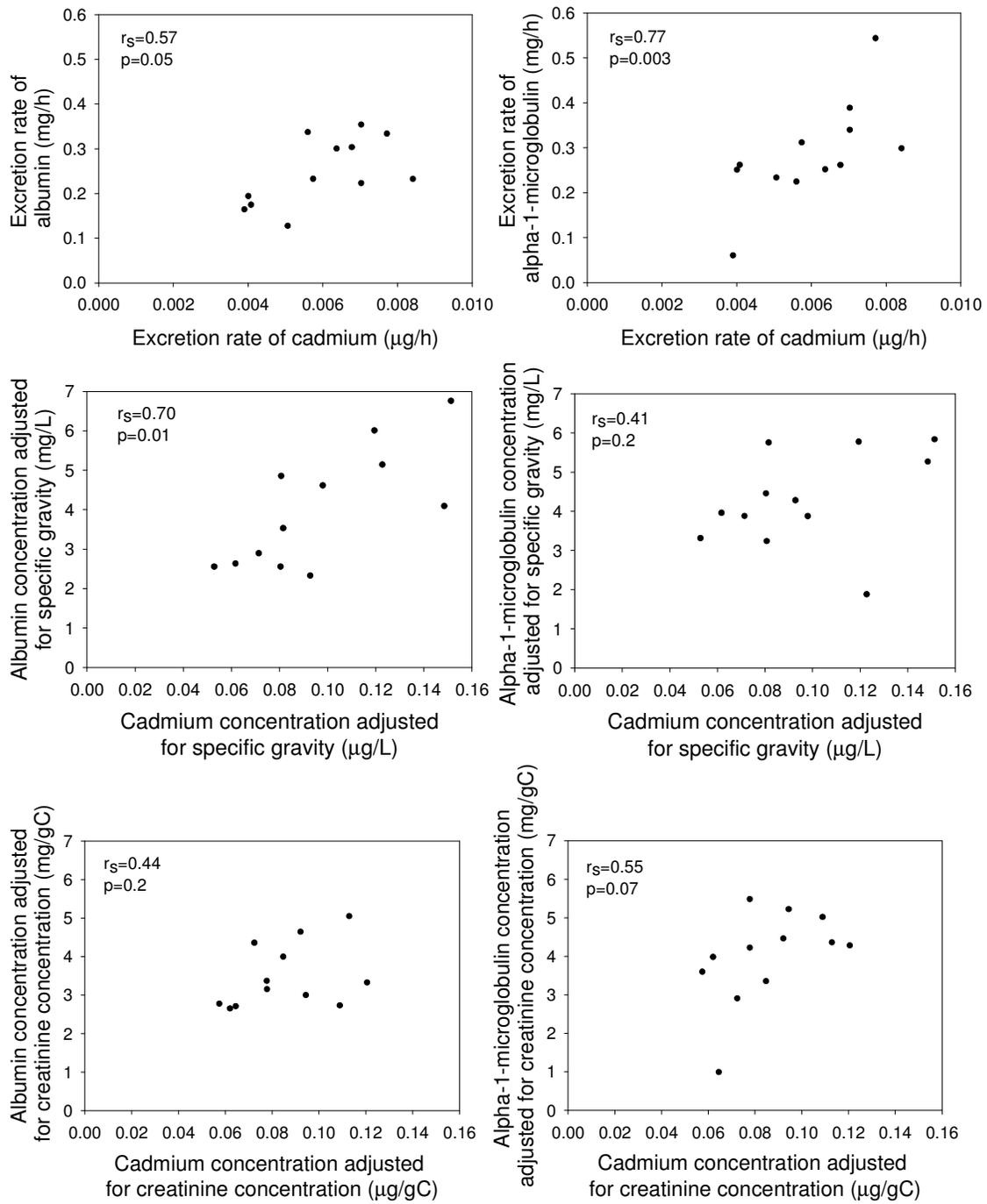


Figure 2

