# Biomonitoring of Cadmium

## – Relationship between Cadmium in Kidney, Blood and Urine, Interpretation of Urinary Cadmium, and Implications for Study Design

Magnus Åkerström

Department of Occupational and Environmental Medicine

Institute of Medicine

Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

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To my family

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# ABSTRACT

Cadmium is an environmental contaminant which accumulates in the kidney and can potentially affect human health at relatively low concentrations. Biomarkers such as cadmium in urine or blood are normally used to assess the body burden of cadmium. We studied the relationship between cadmium in urine, blood, and kidney by using 109 healthy environmentally exposed kidney donors. The variability in urinary cadmium excretion, its interpretation, and effects on the study design were further examined using repeated urinary samples from 30 non-smoking healthy men and women. The results showed a strong association between cadmium in urine and kidney ($r_p$=0.7), with an excretion corresponding to a biological half-time of about 30 years. A kidney cadmium of 25 µg/g corresponded to a urinary cadmium of 0.42 µg/g creatinine (i.e. a urine to kidney ratio of 1:60). Previous estimates of the urine to kidney cadmium ratio (1:20) may thus underestimate the kidney cadmium at low urinary cadmium excretion. On average, 70% of the urinary cadmium excretion could be explained by kidney cadmium. Urinary cadmium excretion was also affected by cadmium in blood and urinary albumin excretion. There was a circadian rhythm in the urinary cadmium excretion over 24h, affecting both the interpretation of urinary cadmium measures and the appropriate study design. There was an association between urinary cadmium and urinary proteins within individuals. Hence, when urinary cadmium is used as a biomarker for cadmium body burden, normal short-term variability in renal function may result in an overestimation of the nephrotoxicity of cadmium.

# SAMMANFATTNING PÅ SVENSKA

Kadmium finns i vår miljö främst till följd av industriella utsläpp och från användning av kadmiuminnehållande konstgödsel och avloppsslam. Kadmium tas upp från kosten och från tobaksrök, har en lång halveringstid i kroppen (decennier) och ansamlas främst i njuren. Traditionellt har man sett skador på njurarna som den främsta risken vid en långvarig låg kadmiumexponering. Kadmium i urin har ansetts vara ett bra mått på halten av kadmium i njure men det har inte funnits tillräckligt med studier från levande människor med låg kadmiumexponering. I avhandlingen har sambandet mellan kadmium i njure, urin och blod studerats med hjälp av prover insamlade från 109 levande njurdonatorer och halveringstiden för kadmium i njure har beräknats. Dessutom har variationen i kadmium- och proteinutsöndring i urin studerats hos 30 individer som lämnat upprepade urinprov under två dygn. Faktorer som påverkar tolkningen av urinkadmium samt lämplig studiedesign har även studerats.

Resultaten visar att det finns ett starkt samband ($r_p$=0,7) mellan kadmium i njure och urin, och utifrån urinutsöndringen kan halveringstiden för kadmium i njure skattas till cirka 30 år. Ett njurkadmium på 25 µg/g motsvarar ett urinkadmium på 0,42 µg/g kreatinin, vilket ger en lägre kvot mellan urin- och njurkadmium (1:60) jämfört med tidigare utförda vetenskapliga studier (1:20). Detta betyder att den tidigare bedömningen av sambandet mellan urin- och njurkadmium underskattar njurkadmium vid låga urinkadmiumnivåer.

Studien av individerna med upprepade urinprover visar en tydlig dygnsvariation i kadmiumutsöndring, vilket man bör ta hänsyn till när man planerar en studie. Det fanns även ett tydligt samband mellan urinkadmium och urinproteiner inom individer (t.ex. över ett dygn) vilket talar för att de samband mellan urinkadmium och urinprotein vid låga kadmiumnivåer, som tidigare tolkats som en skadlig effekt på njuren av kadmium, istället kan bero på naturliga variationer i utsöndring av kadmium och proteiner.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I.   Akerstrom M, Barregard L, Lundh T, Sallsten G. The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. *Toxicology and Applied Pharmacology. 2013 May 1;268(3):286-93*

II.  Akerstrom M, Sallsten G, Lundh T, Barregard L. Associations between Urinary Excretion of Cadmium and Proteins in a Nonsmoking Population: Renal Toxicity or Normal Physiology? *Environmental Health Perspectives. 2013 Feb;121(2):187-91.*

III. Akerstrom M, Lundh T, Barregard L, Sallsten G. Effect of molybdenum oxide interference on urinary cadmium analyses. *International Archives of Occupational and Environmental Health. 2013 Jul;86(5):615-7*

IV.  Akerstrom M, Barregard L, Lundh T, Sallsten G. Variability of urinary cadmium excretion in spot urine samples, first morning voids, and 24 h urine in a healthy non-smoking population: Implications for study design. *Journal of Exposure Science and Environmental Epidemiology. 2014 Mar;24(2):171-9*

# ABBREVIATIONS

B-Cd          Blood cadmium

BMI           Body mass index

Cd            Cadmium

CI            Confidence interval

Crea          Urinary creatinine

GFR           Glomerular filtration rate

ICC           Intraclass correlation

ICP-MS        Inductively coupled plasma mass spectrometry

Mo            Molybdenum

$r_p$         Pearson's correlation coefficient

$r_s$         Spearman's correlation coefficient

SG             Specific gravity

U-A1M          Urinary alpha-1-microglobulin

U-Alb          Urinary albumin

U-Cd           Urinary cadmium

UF             Urinary flow rate

# CONTENT

# 1  INTRODUCTION

As with most environmental contaminants, information on the health effects of cadmium was first derived from settings with occupational or high environmental exposures to cadmium. Early reports of kidney damage from zinc production (probably caused by cadmium exposure) date back to the 19th century (Seiffert 1897), and adverse effects of cadmium on the human kidney have been demonstrated in occupational settings with high cadmium exposure since the late 1940s (Friberg 1950).

One of the earlier reports of health effects in environmentally exposed populations was the itai-itai disease, a severe disease manifested by tubular and glomerular dysfunction and bone injury consisting of a combination of osteomalacia and osteoporosis. The itai-itai disease was found to be caused by intake of rice, locally grown on fields in Japan with high levels of cadmium pollution from nearby zinc mines (Hagino and Kono 1955). Since then, a large number of studies have found health effects of cadmium exposure in environmentally exposed populations, without specific industrial exposures and at relatively low cadmium exposures (Åkesson et al. 2005; Chen et al. 2006; de Burbure et al. 2006; Hong et al. 2004; Järup et al. 2000; Olsson et al. 2002).

In contrast to many other contaminants in our environment, cadmium exposure may not be decreasing (EFSA 2009; Järup et al. 1998; Vahter 1982; WHO 2007), except in some highly contaminated areas. Much attention has therefore been drawn to studies of cadmium exposure in the environmentally exposed population during the last couple of decades.

This thesis focuses on the application and interpretation of biomarkers of cadmium exposure, especially in studies of kidney effects, in the environmentally exposed population with low-level cadmium exposure.

## 1.1 Environmental exposure to cadmium

The diet is the main source of cadmium exposure in the environmentally exposed population, though for smokers, tobacco consumption is also an important route of exposure (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007; WHO 2011). The soil on agricultural farm fields is contaminated with cadmium through airborne deposition from industrial activities and from the use of sewage sludge or cadmium-containing fertilizers, and cadmium is accumulated in the crops (EFSA 2009; Järup et al. 1998; Nawrot et al. 2010; Nordberg et al. 2007; Prozialeck and Edwards 2010; Satarug et al. 2010; WHO 2007). Cadmium is generally present in all foods but the concentration varies to a great extent, depending on the type of food and level of environmental contamination. High levels of cadmium are found in shellfish, offal such as liver and kidney, and certain seeds. Food from plants generally contains more cadmium compared to meat, eggs, and dairy products; and plants such as rice, wheat, potatoes, root vegetables, and green leafy vegetables contain more cadmium than other plants (EFSA 2009; Järup and Åkesson 2009; Sand and Becker 2012; WHO 2011).

### Uptake, distributions and excretion of cadmium in humans

In humans, the gastrointestinal cadmium absorption after ingestion is 3-5%, while cadmium absorption from inhalation is 10-50% (EFSA 2009; Nawrot et al. 2010; Nordberg et al. 2007; Prozialeck and Edwards 2010; Satarug et al. 2010; WHO 2007). The absorption of cadmium is related to iron status, and is generally higher in women than in men (Berglund et al. 1994; Flanagan et al. 1978; Julin et al. 2011; Vahter et al. 1996). After uptake, cadmium in blood is bound to albumin and metallothionein. The small cadmium-metallothionein complex is filtered in the renal glomerulus, reabsorbed in the tubular cells, and accumulated in the kidney with a biological half-time ranging from 10 to 30 years (EFSA 2009; Järup and Åkesson 2009;

Nawrot et al. 2010; Nordberg et al. 2007; WHO 2007; Åkesson et al. 2014). Approximately 50% of the total cadmium body burden is accumulated in the kidney, and so kidney cadmium and cadmium body burden are often referred to each other (EFSA 2009; Järup and Åkesson 2009; Nawrot et al. 2010; Nordberg et al. 2007; Åkesson et al. 2014). Absorbed cadmium is excreted in urine and faeces. Thus, urinary cadmium and blood cadmium are widely used as biomarkers to assess the body burden of cadmium in the environmentally exposed population (Järup et al. 1998; Nordberg et al. 2007).

## 1.2   Human health effects from environmental cadmium exposure

Until now, the main adverse effect of cadmium exposure in the environmentally exposed population has been considered to be renal tubular damage, measured as an increased urinary excretion of low molecular weight proteins such as beta-2-microglobulin, alpha-1-microglobulin, and retinol-binding protein (EFSA 2009; Järup and Åkesson 2009; Järup et al. 1998; Nawrot et al. 2010; Nordberg et al. 2007; Prozialeck and Edwards 2010; Satarug et al. 2010; WHO 2007; Åkesson et al. 2014). However, other cadmium-related effects, mainly bone effects but also an increased risk of lung cancer and oestrogen-dependent cancers, have been seen in recent years (Åkesson et al. 2008; EFSA 2009; Engström et al. 2011; Engström et al. 2012; Järup and Åkesson 2009; Julin et al. 2012; Nawrot et al. 2010; Satarug et al. 2010; Thomas et al. 2011; Åkesson et al. 2014).

Current health risk assessments, based on kidney effects, have shown an impaired renal tubular reabsorptive function when urinary cadmium concentration exceeds 4 µg/g creatinine (EFSA 2009; Järup and Åkesson 2009; Nordberg et al. 2007; WHO 2011; Åkesson et al. 2014). However, these adverse effects have recently been seen even at lower levels of cadmium exposure, including those occurring in most environmentally exposed populations (Åkesson et al. 2005; Chen et al.

2006; de Burbure et al. 2006; Hong et al. 2004; Järup et al. 2000; Olsson et al. 2002). Still, the causality of these associations between urinary cadmium and urinary protein excretions has been questioned, both before and after the studies included in this thesis were carried out, because of possible confounding by smoking or physiological sources of variability (Bernard 2008; Chaumont et al. 2010; Chaumont et al. 2012; Chaumont et al. 2013; Haddam et al. 2011; Åkesson et al. 2014). Thus, it is still a point of discussion whether or not kidney effects are the most critical effect of cadmium in the environmentally exposed population, and if effects on the kidney (i.e. an increased excretion of low molecular weight proteins in urine) may occur at very low levels of cadmium exposure.

## 1.3   Biomonitoring of environmental cadmium exposure

Assessing the cadmium body burden in humans requires the use of biomarkers of exposure. An ideal biomarker should be non-invasive, easily accessible, and well documented in terms of the relationship between the biomarker and the body burden and factors which might affect the interpretation of the biomarker (Nordberg et al. 2007). Cadmium in urine is widely used as a biomarker for long-term cadmium exposure, while blood generally is used for assessment of short-term exposure (EFSA 2009; Järup et al. 1998; Nawrot et al. 2010; Nordberg et al. 2007; Åkesson et al. 2014). However, for environmentally exposed populations, with only small variations in their cadmium exposure, cadmium in blood will also represent the long-term cadmium exposure (Järup et al. 1998).

Ideally, the 24h urinary excretion should be used (Nawrot et al. 2010; Nordberg et al. 2007), but the collection of 24h urine samples is laborious and the risk of incomplete or contaminated samples is great. Spot urine samples are more feasible, and studies have shown a close relationship between urinary 24h cadmium excretion and cadmium

measured in spot urine samples (Berlin et al. 1985). However, as for all biomarkers in spot urine, the urinary cadmium concentration needs to be adjusted for dilution. This is often done by using the creatinine concentration in the urine sample (cadmium concentration to creatinine concentration ratio), as creatinine is assumed to be excreted at a constant rate, or by using the specific gravity of the urine sample (Berlin et al. 1985; Suwazono et al. 2005; Trevisan et al. 1994). If the excretion time and total volume of the urine sample are known, the cadmium excretion rate can also be used (Nordberg et al. 2007). The different methods for adjusting urinary cadmium for dilution all have their qualities, but the most suitable adjustment method for urinary cadmium concentrations in spot urine samples needs to be evaluated depending on the aim of the study.

## 1.4  Variability in cadmium biomarkers and implications for study design

When cadmium biomarkers are used in epidemiological studies, another important factor is the variability between and within individuals (Li et al. 2010; Lin et al. 2005; Rappaport and Kupper 2008). The biomarker should reflect the individual exposure and body burden. A preferred biomarker has a large variation between individuals with different mean levels (i.e. there is a close relation between the biomarker and the body burden). It is also important to have biomarkers with low variation within individuals (i.e. stable levels between repeated samples from the same individual), since most assessments are based on single measurements.

The biological half-time of a biomarker in the human body has been shown to affect the total variability, with slower elimination leading to a decreased variability in the biomarker (Lin et al. 2005). However, a biomarker with a long biological half-time and a stable long-term level may still be affected by different sources of variability which will induce short-term variability (variability within a day or a week). This

short-term variability will affect the interpretation of the measured biomarker in a study.

Potential sources of short-term variation in repeated measurements of cadmium biomarkers in urine include natural physiological variations (e.g. variations in urinary flow rate, excretion rate, or absorption rate), the choice of sampling time (time of the day), and the method used to adjust the urinary cadmium concentration for diuresis. Another potential source of variability is the analytical method, which will be included in the within-individual variance. The analytical variability is generally small (Mason et al. 1998), but the analytical method needs to be validated. Factors affecting the analysis, such as molybdenum oxide-based interference (Jarrett et al. 2008; Suzuki et al. 2008), may have a large impact and need to be taken into consideration and corrected for.

The relationship between the within- and between-individual variability for a given biomarker might attenuate the result of an epidemiological study (Rappaport and Kupper 2008), and so sources of variation need to be investigated and controlled for in the study design.

## 1.5 Relationship between cadmium in kidney and cadmium in urine and blood

One of the most important criteria in evaluating the optimal biomarker of cadmium body burden is the relationship between body burden and the biomarker. Close relationships between cadmium in kidney, urine, and blood are anticipated at steady state. Attempts have been made to quantify the relationship using *in vivo* measurements such as X-ray fluorescence (Börjesson et al. 1997; Börjesson et al. 2001; Christoffersson et al. 1987; Nilsson et al. 2000; Nilsson et al. 1995) and neutron activation (Ellis et al. 1979; Mason et al. 1999; Roels et al. 1981) or by autopsy studies (Orlowski et al. 1998; Satarug et al. 2002). However, the current *in vivo* techniques are not sensitive enough to

investigate the relationship between kidney cadmium and cadmium in blood and urine at low-level cadmium exposures (Nilsson et al. 2000; Nilsson et al. 1995). Thus, most of the current information originates from studies of occupationally exposed workers, which may not be representative for environmentally exposed individuals with a lower level of cadmium body burden and a different exposure pattern. In addition, autopsy studies of environmentally exposed individuals may not be representative of the healthy part of the population, and cadmium levels in urine and blood may change post mortem. Moreover, data concerning diet and smoking habits may be uncertain in these studies.

Reviews of the existing data (occupational and environmental exposure) show that a urinary cadmium concentration of 2.5 µg/g creatinine corresponds to a kidney cadmium concentration of about 50 µg/g; that is, a urine to kidney cadmium ratio of about 1:20, if a linear relationship between kidney cadmium and cadmium in urine is assumed (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007). However, there is still a need for more information regarding the relationship between kidney cadmium and cadmium in urine at the lower cadmium levels which occur in environmentally exposed populations without any industrial sources of exposure.

# 2 AIMS OF THIS THESIS

The overall aim of this thesis was to improve the existing methods for biomonitoring and the interpretation of urinary cadmium levels in studies of environmentally exposed individuals, with a special reference to studies of kidney effects.

The specific aims were:

To study the association between cadmium in kidney, urine, and blood by:
- determining the relationship between cadmium in kidney and cadmium in urine and blood (*Paper I*)
- determining factors affecting the relationship between cadmium in kidney, urine, and blood (*Paper I*)
- determining the excretion rate and thereby the biological half-time of cadmium in kidney (*Paper I*)

To improve the interpretation of cadmium measured in urine by:
- studying the causality of an association between urinary cadmium and urinary protein excretion at low cadmium levels (*Paper II*)
- studying the effect of molybdenum oxide-based interference on urinary cadmium analysis when certain inductively coupled plasma mass spectrometry (ICP-MS) methods are used (*Paper III*)
- studying short-term variations in urinary cadmium excretion during the day (*Paper IV*)

To evaluate how these results should affect the study design by:
- assessing how variability in urinary cadmium excretion should affects sample sizes in studies (*Paper IV*)
- assessing which measure best represents cadmium in kidney/body burden (*Papers I-IV*)

# 3 MATERIALS AND METHODS

## 3.1 Study populations and study designs

Two different study populations, both environmentally exposed to cadmium, were used in this thesis. Background data for the study participants in each study population are shown in Table 1. Different exclusion criteria were used in the different papers, depending on the aim of the study, as indicated below. When a subpopulation of the total study population was used (*Papers I & IV*), no substantial difference were seen between the subgroup and their corresponding total study population. For detailed information, see *Papers I-IV*.

Informed consent was obtained from all study participants, and the studies were approved by the Ethics Committee of the University of Gothenburg.

*Table 1. Background data for study populations 1 & 2*

| Variables | Study population 1 (*Papers I & III*) | Study population 2 (*Papers II & IV*) |
|---|---|---|
| Study participants, N[a] | 152 | 30 |
|     Women | 87 | 15 |
|     Men | 65 | 15 |
| Median age, years (range) | 50 (24-70) | 39 (23-59) |
|     Women | 49 (24-64) | 39 (23-56) |
|     Men | 51 (30-70) | 35 (23-59) |
| Ever smokers, N[a] (Current smokers, %) | 91[b] (40) | 0 (0) |
|     Women | 53[b] (36) | 0 (0) |
|     Men | 38[b] (45) | 0 (0) |
| Median BMI, kg/m$^2$ (range) | 25 (18-32) | 24 (19-29) |
|     Women | 25 (18-32) | 23 (19-29) |
|     Men | 25 (22-32) | 25 (21-29) |

[a]*N=number of samples,* [b]*Median pack-years (range): 13 (0.4-51) for all smokers, 13 (1.2-36) for women, and 12 (0.4-51) for men*

## Study population 1 (*Papers I & III*)

Living healthy kidney donors were recruited between 1999 and 2005 to a study of heavy metal concentrations in kidney, blood, and urine (the TINA study) (Barregard et al. 2010). Of the 167 eligible donors admitted at the Department of Transplantation and Liver Surgery at Sahlgrenska University Hospital for kidney transplantation, 152 (81%) participated after giving their informed consent. Occupational history and smoking habits were collected through a structured interview.

All donors had been examined with routine tests less than one year before the transplantation, in order to be accepted as a kidney donor. The donors were admitted to the hospital 1-2 days before the transplantation. Blood samples were taken in 10 mL Venoject II tubes (Terumo Europe, Leuven, Belgium), and separate 24h and timed overnight urine samples were collected in pre-washed polypropene bottles and transferred to 14 mL polypropene tubes (Sarstedt, Nümbrecht, Germany). A small part of a reference kidney cortex biopsy, taken as a routine procedure during the transplantation, was available for metal analysis and handled as described in Barregard et al (2010). The numbers of complete samples are shown in Figure 1.
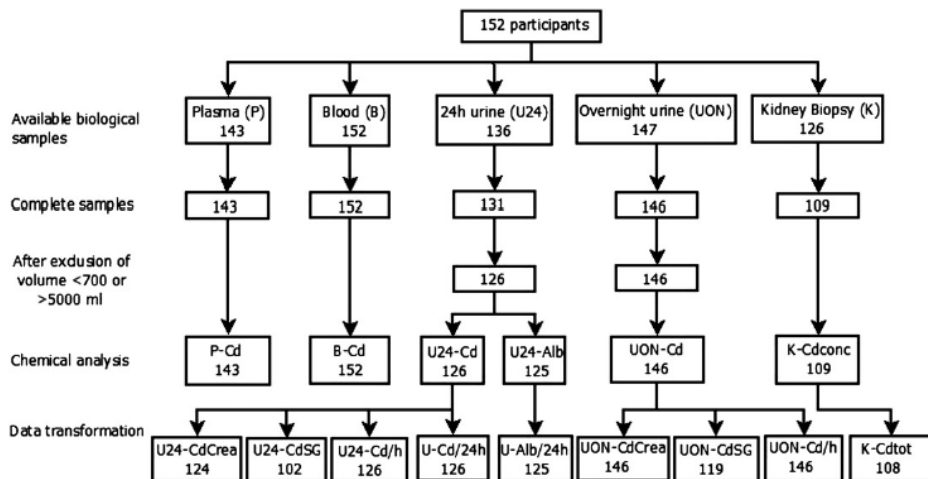


*Figure 1. Number of collected, missing, excluded, and remaining kidney biopsies, and urine and blood samples in study population 1 (Paper I, Figure 1)*

In *Paper I*, calculations were performed both for all data and for the subgroup with a kidney biopsy, while *Paper III* was based on the urine samples only. Urine samples with a 24h urine volume above 5000 mL or below 700mL were regarded as not representative, and were excluded from both studies. In addition, very diluted or concentrated samples (urinary creatinine <0.3 g/L or >3.0 g/L and specific gravity <1.010 or >1.030) were excluded in *Paper I* in order to assess the association between kidney cadmium and cadmium in urine under normal conditions. These samples were however used in *Paper III*, since we wished to study the effect of molybdenum oxide-based interference over the entire range of data.

## Study population 2 (*Papers II & IV*)

Thirty non-smoking healthy participants (15 men and 15 women, all free from diabetes, hypertension, and kidney disease) were recruited for a study of short-term variability in urinary protein excretions (Andersson et al. 2008). The study participants were recruited in 2006 among the staff at the Department of Occupational and Environmental Medicine at Sahlgrenska University Hospital and among students at the University of Gothenburg. Each participant filled out a questionnaire concerning age, weight, height, diseases, and medications.

Participants provided timed urine samples during two separate days, mostly within one week. Each day, they were asked to provide urine samples at six fixed time points: 09:30 (second morning sample), 12:00, 14:30, 17:30, 22:00, and overnight (first morning sample). Detailed information was given to ensure complete 24h urine sampling. All study participants provided 12 urine samples each, except one woman, who only provided samples during one day (six samples).

The samples were transferred to Minisorb tubes (NUNC, Roskilde, Denmark) and kept at 4°C until analysis of proteins (albumin and alpha-1-microglobulin) and creatinine within three days of collection.

Aliquots used to determine urinary cadmium were frozen (-20°C) and analysed five years later.

In *Paper II*, all data were used in the statistical analyses to study the association between urinary cadmium and protein excretions. In *Paper IV*, when the variability in urinary cadmium excretion was studied, six study participants were excluded from the statistical analysis: the woman with samples from only one day, another woman with repeatedly very low 24h urine excretion (around 500 mL), and four participants (1 man and 3 women) for whom more than 50% of the 12 urinary samples had cadmium levels below the limit of detection.

## 3.2 Chemical analyses and data transformations

A short summary of the chemical analyses and data transformations follows below; more details can be found in *Papers I-IV*.

All samples were analysed together with external quality control samples, showing satisfactory results, and all sampling material had been demonstrated free from cadmium prior to the studies. Samples below the limit of detection were assigned the value $\frac{limit\ of\ detection}{2}$ or $\frac{limit\ of\ detection}{\sqrt{2}}$ depending on the distribution of data (Hornung and Reed 1990). Since all urine samples in these studies were timed and the volumes were measured, it was possible to calculate urinary flow rate, 24h excretions, and urinary excretion rates of cadmium and proteins. Urinary concentrations of cadmium and proteins were adjusted for dilution by urinary creatinine concentration or specific gravity (reference =1.015) (Suwazono et al. 2005).

### Cadmium concentrations in kidney, urine and blood
Cadmium concentrations in kidney, urine, and blood samples were analysed by ICP-MS (Thermo X7, Thermo Elemental, Winsford, UK) , in samples diluted ten times (Barany et al. 1997). The analyses were

carried out at the Department of Occupational and Environmental Medicine, Lund University Hospital. The procedure for analysis of cadmium in kidney biopsies has been described elsewhere (Barregard et al. 2010).

The dry weight cadmium concentration of the kidney cortex was transformed to wet weight concentration by multiplying by 0.18 (Barregard et al. 1999; Elinder et al. 1990). The kidney weight for each participant was estimated from the body surface area (Kasiske and Umen 1986). The total amount of cadmium in the kidney was calculated by multiplying the estimated kidney weight by the concentration of cadmium in the kidney cortex and then dividing by 1.25 to adjust for the higher cadmium concentration in the cortex compared to the rest of the kidney (Svartengren et al. 1986).

The urinary cadmium concentrations were corrected for molybdenum oxide-based interference, since molybdenum oxide formed during the analysis from the molybdenum naturally present in urine might interfere with cadmium isotopes [111]Cd and [114]Cd (Jarrett et al. 2008; Suzuki et al. 2008). The correction for molybdenum oxide-based interference was made by adding molybdenum (500 µg/L) to blank urine samples several times in each analytical run, and evaluating the formation of molybdenum oxide. Molybdenum was determined in all samples, and thus a correction for the molybdenum oxide-based interference could be made via the known proportion of molybdenum to molybdenum oxide.

All urine samples in study population 1 were reanalysed in one batch 2012 since only half of the urine samples had been corrected for molybdenum oxide-based interference in the initial analysis. However, after reanalysing all the samples from this population (131 24h samples and 146 overnight urine samples) it was possible to calculate the effect of molybdenum oxide-based interference on the urinary cadmium analysis (*Paper III*).

Repeated measurements of specific gravity, in connection with the reanalysis, in 20 randomly chosen samples showed no evidence of dehydration of the urine samples after being stored in the freezer (-20 °C) for up to 13 years, when compared to the measurements that were carried out in fresh urine.

## Urinary protein and creatinine concentrations, and specific gravity

Two urinary proteins were analysed as a measure of adverse effects on the kidney: albumin (Ferraro et al. 2010; Nordberg et al. 2012; Nordberg et al. 2009; Åkesson et al. 2014) and the low molecular weight protein alpha-1-microglobulin (Åkesson et al. 2005; Bakoush et al. 2001; Penders and Delanghe 2004). Urinary albumin (used in *Papers I, II, & IV*) was analysed in both study populations using a nephelometric immunochemical method with reagents and calibrators from Beckman Coulter (Fullerton, CA, USA). Urinary alpha-1-microglobulin was analysed in study population 2 and used in *Papers II & IV*. The analysis was performed using the $\alpha_1$-microglobulin ELISA kit K6710 (Immundiagnostik AG, Bensheim, Germany).

Urinary creatinine concentration and specific gravity were analysed in order to allow adjustment for dilution in cadmium and protein concentrations from spot urine samples. Urinary creatinine concentrations were analysed in fresh urine using two different methods. All samples in study population 2 and three out of four batches in study population 1 were analysed using the Jaffé method (Roche Diagnostics, Mannheim, Germany). The remaining samples in study population 1 were analysed with an enzymatic method (Modular P and CREAplus R1, R2, Roche/Hitachi, Roche Diagnostics, GmbH, Mannheim, Germany). Specific gravity was determined in fresh urine with a Ceti Digit 012 refractometer (Medline, Oxfordshire, UK).

# 3.3 Statistical methods

Data analyses were performed using version 9.1 of the SAS software package (SAS Institute, Cary, NC, USA). Statistical significance was determined at $p < 0.05$, and two-sided confidence intervals were used if not stated otherwise.

**Mean values of groups, individuals, and sampling times, and tests of significant differences (*Papers I-IV*)**

The mean levels of different biomarkers were not a focus point in this thesis, but mostly merely used to describe the study populations. Average levels in groups were generally described using arithmetic means and ranges on untransformed data.

In *Papers II & IV*, data consisted of repeated measurements from 30 study participants. In *Paper II*, the mean values of urinary cadmium and protein excretions were calculated as arithmetic means of the 30 individual arithmetical means. The mean cadmium excretion was also calculated in *Paper IV* using mixed-effect models (see below) to fully account for the repeated sampling. In the graphical presentation of the circadian rhythms of urinary cadmium, urinary protein excretion, and urinary flow rate in *Paper IV*, geometric means of individual arithmetic means for each time point of sampling were used.

Significant differences between paired samples were tested using a paired t-test. Differences between groups were tested for significance using the Wilcoxon rank-sum test, since the data were not generally normally distributed.

**Correlations, linear regression models, and estimates of the biological half-time of cadmium in kidney (*Papers I-IV*)**

Associations between variables were assessed with correlation coefficients (*Papers I & II*) and linear regression models (*Papers I-IV*). In *Paper I*, Pearson's correlation coefficients ($r_p$) were calculated to study the linear relationship between kidney cadmium and cadmium biomarkers in blood and urine. For the repeated samples in *Paper II*,

Spearman's correlation coefficients ($r_s$) were calculated within each study participant, as data were skewed. The overall mean correlation coefficients were calculated by averaging the participant-specific correlation coefficients, and tested for significant deviation from zero using the Wilcoxon signed-rank test.

In the regression models in *Papers I-III*, untransformed data were used except for urinary protein excretions which were naturally log-transformed due to a highly skewed distribution in combination with a small spread in data. Differences between regression slopes were investigated using the F-test. When backward stepwise elimination was used to derive the final models in multiple regression models, p<0.1 was used as the inclusion criterion if not stated otherwise.

Most of the regression models in *Paper I* were weighted, since the data were not fully homoscedastic, using the weight function $\frac{1}{(estimated\ U-Cd/24h)^2}$ when the association between kidney cadmium and urinary cadmium was studied and when determinants for urinary cadmium were calculated using multiple regression models.

Studying the association between urinary cadmium, urinary protein excretion, and urinary flow rate in *Paper II*, we hypothesized that physiological changes in urinary protein excretions and urinary flow rate would affect urinary cadmium excretion (rather than the opposite), and hence chose urinary cadmium as the dependent variable. The overall mean regression coefficients for these associations were calculated and tested for deviation from zero in the same way as the correlation coefficients mentioned above.

In *Paper I*, the biological half-time ($t_{1/2}$) of cadmium in kidney was estimated using Equation 1, assuming a one-compartment model.

$$Cd\ mass\left(t_{\frac{1}{2}}\right) = Cd\ mass\ (t_0) \times \frac{1}{2} = Cd\ mass(t_0) \times e^{-k \times t_{\frac{1}{2}}}$$

$$\rightarrow\ e^{-k \times t_{\frac{1}{2}}} = \frac{1}{2}$$

$$\rightarrow\ Biological\ half-time\ (t_{\frac{1}{2}}) = \frac{\ln(2)}{elimination\ constant\ (k)} \qquad (1)$$

The elimination constant (k) was determined from the association between the 24h cadmium excretion and the total amount of cadmium in kidney.

## Mixed-effect models (*Paper IV*)

Mixed-effect models were used on data from study population 2 *(Paper IV)*, to estimate the between- and within-individual variability in the urinary cadmium excretion and to determine factors affecting the urinary cadmium excretion (Rappaport and Kupper 2008). Before inclusion in the models, the normality of each cadmium measure in spot urine, overnight urine, and 24h urine was tested using the Shapiro-Wilks test to determine whether untransformed or log-transformed data should be used in the analyses. Natural log-transformed data were used in the mixed-effect models (Equation 2) for all measures, since the data were skewed.

$$Y_{ij} = \ln(X_{ij}) = \mu_Y + \sum_{u=1}^{U} \partial_u C_{ui} + b_i + e_{ij} \qquad (2)$$

$X_{ij}$ is the exposure level for the ith person in the jth measurement, $\mu_Y$ is the fixed mean (log-transformed) exposure level for the population, $b_i$ is the random effect of the ith person, and $e_{ij}$ is the random error for the jth measurement of the ith person. The model also contains additional fixed effects for U covariates (determinants and interaction terms) $C_1$, $C_2$, …, $C_u$; $\partial_u$ are regression coefficients representing the U covariates. The random effects, $b_i$ and $e_{ij}$, are assumed to be mutually independent and normally distributed with means of zero.

Before applying the mixed-effects model to each measure of cadmium in urine, a likelihood test was used to assess whether a common fixed mean exposure level and common variances could be used for men and women. The between- and within-individual variance components ($\sigma_{bY}^2$ and $\sigma_{wY}^2$) were calculated for each measure using a model (Equation 2) which contained only random effects, a global mean, and gender (Model 1A, null model). The natural-scale mean exposure level was estimated as $\mu_X = e^{(\mu_Y + 0.5\sigma_Y^2)}$ where $\sigma_Y^2 = \sigma_{bY}^2 + \sigma_{wY}^2$. Estimates of 95% between- and within-individual fold ranges ($_bR_{0.95}$ and $_wR_{0.95}$) were determined for each measure of urinary cadmium excretion using Model 1A, where $_bR_{0.95} = e^{3.92\sigma_{bY}}$ and $_wR_{0.95} = e^{3.92\sigma_{wY}}$. The estimated ratio of the between-individual biomarker variance to total observed variance (the intraclass correlation, ICC) was calculated as $ICC = \frac{\sigma_{bY}^2}{\sigma_Y^2}$ (Rappaport and Kupper 2008).

Determinants of urinary cadmium excretion were investigated in study population 2 (*Paper IV*) using Model 1B for spot urine samples and Model 1C for overnight urine and 24h urine samples. These models were constructed by adding more covariates to model 1A: sampling time, age, body mass index (BMI), urinary flow rate, and urinary protein excretions (as well as their interaction terms with gender). Covariates with a Spearman correlation coefficient above 0.5 were not included in the models simultaneously in order to avoid multicollinearity. The final models were achieved by removing non-significant determinants by backwards stepwise elimination.

## Calculation of group sizes (*Paper IV*)

The effect of the variability in urinary cadmium excretion on the appropriate study design was investigated in study population 2 (*Paper IV*) by calculating the number of samples required to achieve a certain accuracy for two different types of study: epidemiological individually-based studies of log-transformed exposure to log-

transformed response relationships, and studies of differences in the mean level of cadmium exposure between groups.

For the first type of study, the degree of attenuation (i.e. the ratio between the regression coefficient estimated in the study, $\beta_{est}$, and the true regression coefficient, $\beta_{true}$) for a given measure of urinary cadmium excretion was determined from estimated variance components in Model 1A (Equation 2) using the equation:

$$b = \frac{\beta_{est}}{\beta_{true}} = (1 + \frac{\lambda}{n})^{-1} \tag{3}$$

where the bias is 1-b, $\lambda = \frac{\sigma_{wY}^2}{\sigma_{bY}^2}$, and n is the number of repeated measurements per individual (Brunekeef et al. 1987; Heederik et al. 1991).

For the second type of study, the number of samples per group (m) required in order to detect a statistically significant (p<0.05) difference of 10%, 25%, 50%, or 100% respectively in the geometric mean values with a statistical power (P) of 80% was calculated using the total variance and the ICC derived from Equation 2, with just a global mean, and the following formula (Li et al. 2010):

$$m = 2(Z_\alpha + Z_\beta)^2 \sigma_Y^2 [1 + (n-1)ICC]/(nd^2) \tag{4}$$

where $Z_\alpha$ and $Z_\beta$ are the $\alpha$[th] and $\beta$[th] percentiles of a standard Gaussian distribution (one-tailed), $\alpha$ is the desired type I error ($\alpha$ =0.05), $\beta$ is the desired type II error ($\beta$ =1-P), n is the number of samples per individual (1, 2 or 3), and d is the difference in means of log-transformed concentration between the two groups (Li et al. 2010).

# 4 RESULTS

This section is a summary of the main results; for further details the reader is referred to the separate papers (*Papers I-IV*). Some additional results which do not appear in the papers have also been included.

## 4.1 Mean cadmium concentrations in kidney, urine, and blood in the two study populations (*Papers I-IV*)

The mean kidney cortex cadmium concentration was 15.0 µg/g and the mean total amount of cadmium in the kidney was 3860 µg in the total group of study population 1. Mean kidney cortex cadmium concentrations were 17.9 µg/g and 10.4 µg/g for ever-smokers and never-smokers respectively and 17.1 µg/g and 12.5 µg/g for women and men respectively, as described previously (Barregard et al. 2010).

The measures of cadmium in urine and blood in study population 1 and 2 are summarized in Table 2 (*Paper II*, Table 1 and *Paper III*, Table 1). The urinary cadmium concentrations among participants with a kidney biopsy did not differ substantially from those in the total study population (*Paper I*, Table 2). Using a mixed-effect model on the repeated measurements of study population 2 showed a urinary cadmium concentration in overnight samples (first morning samples) of 0.08 µg/g creatinine for men and 0.17 µg/g creatinine for women (p=0.009) and a 24h urinary cadmium excretion of 0.18 µg for both men and women (*Paper IV*, Table 1).

*Table 2. Mean concentrations and ranges of cadmium in urine and blood for study populations 1 & 2*

| Measure | Study population 1 (*Papers I & III*) | | | Study population 2 (*Papers II & IV*) | | |
|---|---|---|---|---|---|---|
| | N | Mean | Range | $N^a$/n | $Mean^b$ | $Range^c$ |
| Spot urine samples[d] | | | | | | |
| U-Cd (µg/L) | 146 | 0.34 | 0.02-1.9 | 354/30 | 0.12 | <LOD[e]-1.1 |
| U-CdCrea (µg/gC)[f] | 146 | 0.28 | 0.04-1.1 | 354/30 | 0.11 | 0.01-0.52 |
| U-CdSG (µg/L)[g] | 146 | 0.25 | 0.04-1.0 | 354/30 | 0.12 | 0.01-0.71 |
| U-Cd/h (µg/h)[h] | 146 | 0.013 | 0.002-0.047 | 354/30 | 0.007 | 0.001-0.03 |
| 24h urine samples | | | | | | |
| U-Cd (µg/L) | 131 | 0.18 | 0.02-0.77 | - | - | - |
| U-CdCrea (µg/gC)[f] | 131 | 0.25 | 0.03-1.0 | - | - | - |
| U-CdSG (µg/L)[g] | 131 | 0.18 | 0.03-0.66 | - | - | - |
| U-Cd/h (µg/h)[h] | 131 | 0.012 | 0.002-0.037 | 30 | 0.007 | 0.003-0.02 |
| U-Cd/24h (µg) | 131 | 0.30 | 0.04-0.89 | 30 | 0.17 | 0.06-0.36 |
| Blood samples | | | | | | |
| B-Cd (µg/L) | 109 | 0.51 | <LOD[e]-2.9 | - | - | - |

*N=number of samples, n=number of individuals*
[a]*One woman only provided samples over 1 day (6 samples)*
[b]*Mean values are calculated as mean of 30 individual means (n)*
[c]*Ranges are for all 354 urine samples (N)*
[d]*Overnight urine samples for population 1 and six spot urine samples per day for population 2*
[e]*Values below limit of detection (LOD) were replaced with LOD/√2 or LOD/2 in calculations according to the distribution of data.*
[f]*Concentrations adjusted for creatinine concentration*
[g]*Concentrations adjusted for specific gravity*
[h]*Excretion rates*

## 4.2 Associations between cadmium in kidney and cadmium in urine and blood (*Paper I*)

Positive and highly significant correlations were seen between the concentration of cadmium in kidney cortex and the concentrations of cadmium in urine and blood samples in study population 1 (*Paper 1*, Table 2). The correlation was generally stronger for the measures of cadmium in urine ($r_p$= 0.44-0.70, p<0.001 respectively) than for

cadmium in blood ($r_p$= 0.44, p<0.001). The correlation coefficient increased when urinary cadmium was adjusted for dilution, and somewhat higher correlations were seen for 24h urine samples compared to overnight urine samples. There was also a relatively strong association ($r_p$= 0.63, p<0.001) between the 24h urinary cadmium excretion and the total amount of cadmium in the kidney (Figure 2).
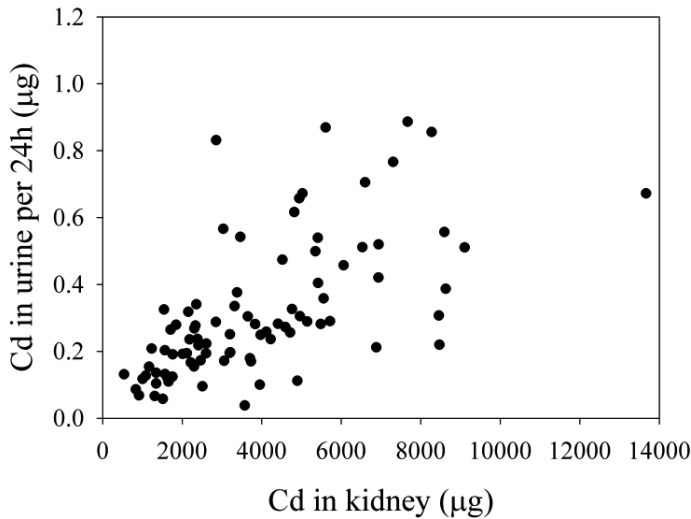


*Figure 2. Association between the 24h urinary cadmium excretion and the total amount of cadmium in the kidney.*

The overnight urinary cadmium concentration adjusted for creatinine concentration explained more of the total variability ($R^2$=0.44) compared to the other measures of cadmium in overnight urine samples ($R^2$=0.31-0.19; Figure 3). According to the linear regression model, kidney cortex cadmium levels of 10 and 25 µg/g would correspond to an overnight urinary cadmium concentration adjusted for creatinine concentration of 0.21 and 0.42 µg/g creatinine, respectively, giving a urinary cadmium to kidney cadmium ratio of about 1:60.
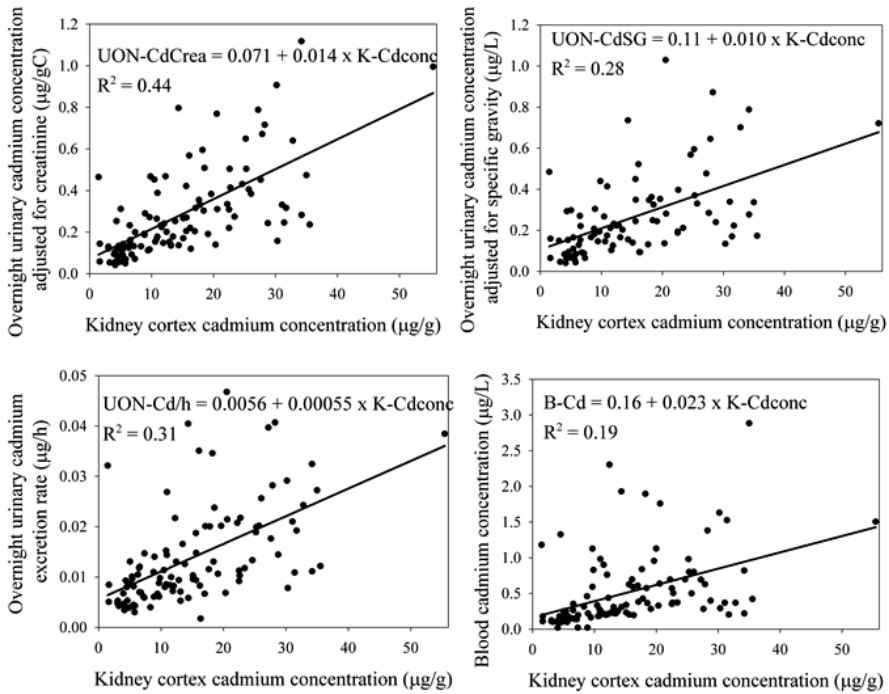
*Figure 3. Associations between kidney cortex cadmium (wet weight) and overnight urine and blood cadmium concentrations. Results from linear (unweighted) regression models (Paper I, Figure 2)*

A nonlinear relationship was seen between the total amount of urinary cadmium excreted in 24h and the total amount of cadmium in kidney (*Paper I*). There was also a significant difference ($p<0.001$) between the slopes for low versus high kidney cadmium concentrations (< 15 µg/g compared to $\geq$ 15 µg/g) when weighted regression models without intercept were used (Figure 4). The ratio of urinary cadmium to kidney cadmium also differed significantly between these two groups.
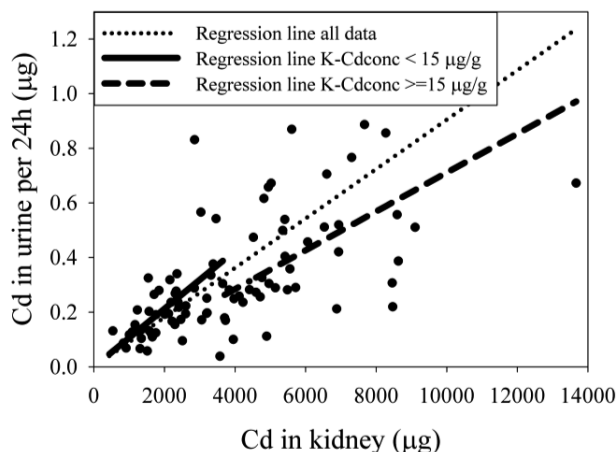
*Figure 4. Cadmium in urine per 24 h versus total cadmium in kidney using weighted regression without intercept for a linear model. Regression lines are included for the total dataset and for the subgroups with kidney cadmium below or above 15 μg/g (Paper I, Figure 3).*

## 4.3 Excretion rate and biological half-time of cadmium in kidney (*Paper I*)

Elimination constants for different models were estimated in study population 1 from the association between urinary cadmium per 24h and the total amount of cadmium in the kidney using weighted regression models (*Paper I*).

If a straight line equation without intercept was assumed, the estimated elimination constant was $0.09\times10^{-3}$; that is, the fraction of the total amount of kidney cadmium excreted in urine per 24h was 0.009%. According to Equation 1, the estimated biological half-time for cadmium in kidney was 21.0 years (95% confidence interval [95% CI]: 18.9-23.6 years). Separate calculations for kidney cadmium concentrations <15 μg/g and ≥ 15 μg/g showed elimination constants of $0.11\times10^{-3}$ and $0.07\times10^{-3}$, and biological half-times of 17.9 years (95% CI: 15.7-20.8 years) and 26.7 years (95% CI: 23.4-31.1 years), respectively. If a straight line equation with an intercept was assumed for the total study population, the elimination constant was $0.06\times10^{-3}$

and the biological half-time was 30.4 years (95% CI: 24.3-40.5 years) with an intercept of 0.073 µg/24h.

Since the relationship between urinary cadmium per 24h and the total amount of cadmium in the kidney was found to be nonlinear, a polynomial model without intercept (U-Cd/24h= $0.11 \times 10^{-3}$ × K-Cdtot – $5.8 \times 10^{-9}$ × K-Cdtot$^2$) was used to calculate the biological half-time for different kidney cadmium levels, using Equation 1. The estimated half-times for kidney cadmium levels of 2000 µg, 4000 µg, and 6000 µg (approximately corresponding to kidney cadmium concentrations of 8 µg/g, 15 µg/g, and 23 µg/g, respectively) were 21.2 years, 28.5 years, and 43.5 years, respectively.

## 4.4 Determinants for urinary cadmium excretion (*Papers I & IV*)

Determinants for the 24h urinary cadmium excretion were investigated in study population 1 using weighted regressions for three different linear regression models: one for all participants, one using different equations for never- and ever-smokers, and one using different equations for low and high kidney cortex cadmium concentrations (< or ≥ 15 µg/g) (*Paper I*). The final models explained about half of the total variability using determinants as the total amount of cadmium in the kidney, blood cadmium, log-transformed 24h urinary albumin excretion, gender, and smoking (Table 3).

Kidney cadmium was a significant determinant for all models. The log-transformed 24h urinary albumin excretion was a significant determinant for all participants combined, for never-smokers, and for those with a kidney cortex cadmium <15 µg/g. Conversely, blood cadmium was a significant determinant for ever-smokers and for those with a kidney cortex cadmium ≥ 15 µg/g.

*Table 3. Effects of kidney cadmium, blood cadmium, urinary albumin, gender, and smoking on 24h urinary cadmium excretion (Paper I, Table 3).*

| Model[a] | Dependent variable[b] | Independent variables[c] | Estimates (p-value) | $R^2$ | Correlation[d] (p-value) |
|---|---|---|---|---|---|
| Model 1 | | | | | 0.71 (<0.001) |
| All (N=86) | U-Cd/24h | Intercept | -0.029 (0.4) | 0.60 | |
| | | K-Cdtot | 0.000056 (<0.001) | | |
| | | log(U-Alb/24h) | 0.045 (0.02) | | |
| | | Smoking | 0.061 (0.008) | | |
| Model 2 | | | | | 0.73 (<0.001) |
| Never-smokers (N=31) | U-Cd/24h | Intercept | -0.0038 (0.09) | 0.48 | |
| | | K-Cdtot | 0.000052 (<0.001) | | |
| | | log(U-Alb/24h) | 0.034 (0.09) | | |
| Ever-smokers (N=55) | U-Cd/24h | Intercept | -0.010 (0.9) | 0.42 | |
| | | K-Cdtot | 0.000041 (<0.001) | | |
| | | B-Cd | 0.081 (0.06) | | |
| | | Sex | -0.070 (0.06) | | |
| | | Age | 0.0036 (0.05) | | |
| Model 3 | | | | | 0.72 (<0.001) |
| K-Cdconc <15 µg/g (N=47) | U-Cd/24h | Intercept | -0.031 (0.03) | 0.52 | |
| | | K-Cdtot | 0.000062 (<0.001) | | |
| | | log(U-Alb/24h) | 0.040 (0.01) | | |
| | | Smoking | 0.065 (0.006) | | |
| K-Cdconc ≥15 µg/g (N=39) | U-Cd/24h | Intercept | -0.012 (0.9) | 0.29 | |
| | | K-Cdtot | 0.000058 (0.002) | | |
| | | B-Cd | 0.12 (0.07) | | |

[a]*Models derived by weighted regressions using backwards elimination from the full model U-Cd/24h=intercept+K-Cdtot+B-Cd+log(U-Alb/24h)+age+sex+UF+GFR+smoking*
[b]*Unit is µg/24h*
[c]*Units as follows: K-Cdtot (µg), U-Alb/24h (mg/24h), smoking (never=0, ever=1), B-Cd (µg/L), sex (men=0, women=1), and age (years)*
[d]*Pearson correlation between U-Cd/24h and estimated U-Cd/24h per model*
*N=number of samples*

Determinants for urinary cadmium excretion were also investigated for spot urine, 24h urine, and overnight urine samples in study population 2, using two mixed-effects models: Model 1B and Model 1C (Equation 2, *Paper IV*). Gender, time point of sampling (for spot urine samples), age, urinary flow rate, and protein excretion (mainly urinary albumin) were significant determinants for most measures of urinary cadmium excretion (*Paper IV*, Tables 2-3). The covariates explained on average 41% of the total variance for spot urine samples and 33% for 24h urine and overnight urine samples. When time point of sampling was removed from model 1B, the total variance increased by an average of 11% (average difference -4% for between-individual variance and +40% for within-individual variance).

## 4.5  Association between urinary cadmium and urinary proteins within individuals (*Paper II*)

On average, urinary cadmium excretion was positively associated ($p<0.001$) with both urinary albumin and alpha-1-microglobulin within the study participants of study population 2 (Figure 5).

The associations between urinary cadmium excretion and urinary protein excretion were stronger when expressed as urinary excretion rates (overall mean $r_s=0.44$ for association with urinary albumin and $r_s=0.33$ for urinary alpha-1-microglobulin) and urinary concentrations adjusted for specific gravity (overall mean $r_s=0.37$ for urinary albumin and $r_s=0.26$ for urinary alpha-1-microglobulin), compared to when the urinary excretions were expressed as urinary concentrations adjusted for creatinine concentrations (overall mean $r_s=0.26$ for urinary albumin and $r_s=0.21$ for urinary alpha-1-microglobulin) (Figure 5).

When both urinary albumin and urinary alpha-1-microglobulin were included in the regression models for each study participant, both proteins were on average found to significantly predict the urinary excretion of cadmium.
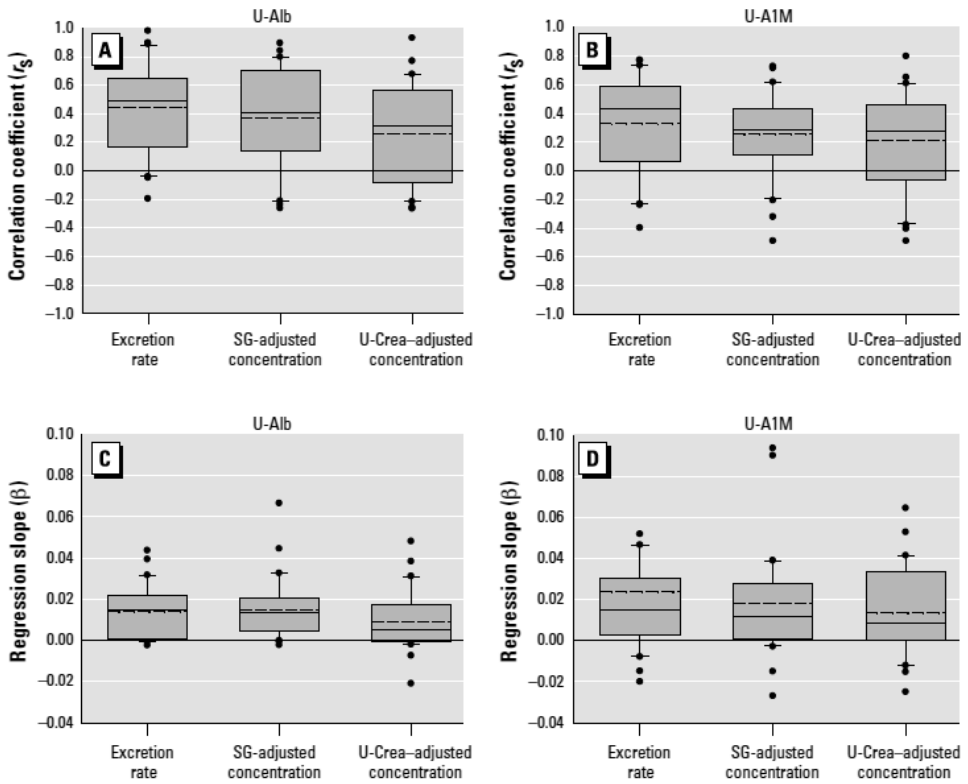
*Figure 5. Distributions of individual Spearman correlation coefficients ($r_S$; A, B) and individual regression coefficients ($\beta$; C, D) for associations between U-Cd and U-Alb (A, C), and U-Cd and U-A1M (B, D), calculated for 30 individual participants. Individual values are based on 6 samples per day over 2 days for each participant, with 354 total samples (1 participant had 6 samples only). Boxes indicate 10th and 25th percentiles; dotted line, mean; solid line, median; and whiskers, 75th and 90th percentiles across all participants, with dots indicating outliers (Paper II, Figure 2).*

When the urinary flow rate was included in the linear regression models between urinary cadmium (dependent variable) and urinary proteins (albumin or alpha-1-microglobulin), the overall mean regression coefficients for urinary flow rate were significantly larger than zero both when expressed as urinary excretion rates and when expressed as urinary concentrations adjusted for specific gravity, but not when expressed as urinary concentrations adjusted for creatinine concentrations (*Paper II*, Table 3). Similar overall mean regression

28

coefficients for urinary albumin and alpha-1-microglobulin were seen in the models with and without inclusion of urinary flow rate.

## 4.6  Effect of molybdenum oxide-based interference on urinary cadmium analysis (*Paper III*)

The median reduction of the urinary cadmium concentration was 55% in the 24h urine samples and 38% in the overnight urine samples after correcting for the molybdenum oxide-based interference (*Paper III*, Figure 1). The reanalysis showed good agreement with initial levels for the samples that had already been corrected in the initial analysis.

## 4.7  Short-term variations in urinary cadmium excretion during the day and implications for study design (*Paper IV*)

The short-term variability in urinary cadmium excretion was investigated in study population 2 (*Paper IV*) using Model 1A (Equation 2). The total variance ranged between 0.20 and 0.62, and the between-individual variance dominated the total variability for most urinary measures of cadmium. The ICC increased when the urinary cadmium concentration was adjusted for dilution and when a specific time point of sampling was used (overnight or 24h urine samples compared with spot urine samples) (*Paper IV*, Table 1).

Expressed as fold ranges, 95% of the urinary cadmium excretions for a given individual were roughly within a 2- to 4-fold range for the 24h excretion and overnight samples and within a 4- to 10-fold range for all spot urine samples. Meanwhile, 95% of the participants had a urinary cadmium excretion roughly within a 4- to 10-fold range; that is, highly exposed individuals had 4-10 times higher exposure compared to low-exposed individuals.

Since time point of sampling was an important determinant for urinary cadmium excretions in the spot urine samples (see section 4.4), mean urinary cadmium excretions were calculated as geometric means of arithmetic means per individual for the six sampling times (Figure 6) (*Paper IV*).
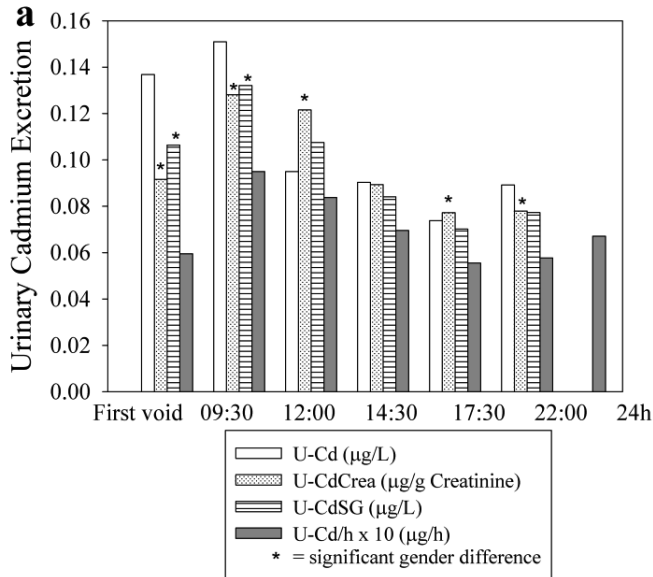


*Figure 6. Urinary cadmium concentrations (unadjusted and adjusted for diuresis) and excretion rates for different sampling times. Geometric means of individual arithmetic means (of two days) for 24 participants and 48 samples per time point (Paper IV, Figure 1).*

All measures of urinary cadmium excretion showed a circadian rhythm, with higher excretion during the daytime (especially in the second morning sample) compared to overnight. Similar results were also seen for the urinary flow rate, urinary albumin, and urinary alpha-1-microglobulin, although albumin had a more stable excretion during the daytime (*Paper IV*, Figure 1). Time point of sampling was still a significant factor ($p < 0.001$), for all measures of cadmium in urine, when other determinants such as gender, age, urinary flow rate, and urinary protein excretion were included in the mixed-effect models (Model 1B, Equation 2). Compared to the overnight sample, urinary

cadmium excretion was generally significantly higher in the second morning sample (09:30) and significantly lower in the afternoon and evening samples (14:30, 17:30, and 22:00) (*Paper IV*, Table 2).

### Calculations of group sizes (*Paper IV*)

The results showed that at least two repeated samples per individual were required in an individually-based study of exposure-response relationship when using measures of urinary cadmium excretion in spot urine samples (Equation 3). Alternatively, one sample per individual was sufficient when measures of urinary cadmium excretion in 24h or overnight samples were used (*Paper IV*, Figure 3).

For studies aimed at comparing cadmium levels between groups, 24h urinary cadmium excretion required the smallest group sizes (Equation 4), but apart from this there were no substantial differences between the measures in spot urine or overnight urine samples (*Paper IV*, Table 4). The number of individuals per group depended strongly on the size of the difference, with about 10 individuals per group needed to detect a difference of 100% and about 500 individuals for a difference of 10%. Separate calculations on men and women showed that the number of individuals needed per group was lower for men than for women (*Paper IV*, Supplementary Information). The number of repeated samples per individual in the groups had only a small effect on the group sizes (*Paper IV*, Table 4).

## 4.8 Associations between 24h urine cadmium excretion and concentrations in spot urine and blood samples (*Paper I*)

There were significant correlations ($p < 0.001$) between the 24h urinary cadmium excretion and all measures of urinary cadmium excretion in overnight samples among study participants with a kidney biopsy in study population 1 (urinary cadmium excretion rate: $r_p = 0.84$, urinary cadmium concentration adjusted for creatinine: $r_p = 0.75$, and urinary

cadmium adjusted for specific gravity: $r_p = 0.74$). The 24h urinary cadmium excretion was also associated with blood cadmium ($r_p = 0.48$, p<0.001), but not among never-smokers (*Paper I*).

## 4.9   Effects of urinary flow rate, urinary creatinine excretion, and specific gravity on cadmium measures in urine (*Papers II-IV*).

For study population 2, urinary flow rate was a significant determinant of most urinary cadmium measures in mixed-effect models (Equation 2) but not for 24h urinary cadmium excretion (*Paper IV*, Tables 2-3). No effect of urinary flow rate on the urinary cadmium excretion rate was found in 24h urine and overnight urine samples in study population 1 (*Paper III*).

The overall mean values for the association between urinary flow rate and urinary cadmium concentration adjusted for creatinine concentration were not significant, when investigated within study participants in study population 2 (*Paper II*). However, a significant positive association was seen between urinary flow rate and urinary cadmium concentration adjusted for specific gravity. There was also a significant positive overall mean value for the association between urinary flow rate and urinary creatinine excretion rate (overall mean $r_s = 0.25$, overall mean regression coefficient= $0.25 \times 10^{-3}$, p<0.001). Nevertheless, exclusion of very diluted or concentrated samples in *Paper II* did not change the overall result.

A positive association was seen between urinary flow rate and the urinary creatinine excretion rate in overnight urine samples in study population 1, as reported in a publication, not included in this thesis (Akerstrom et al. 2012). The association was mainly seen at low urinary flow rates (regression coefficient=1.1, p<0.001 for urinary

flow rates < 42 mL/min and regression coefficient=0.03, p=0.7 for urinary flow rates ≥ 42 mL/min) but not in the 24h urine samples.

When the association between the urinary cadmium concentration adjusted for creatinine concentration and the creatinine concentration was investigated within study participants of study population 2 (*Paper II*), a negative association was found (overall mean $r_s$= -0.20, p=0.02 and overall mean regression coefficient=-0.02, p=0.07). In contrast, specific gravity was not significantly associated with specific gravity-adjusted urinary cadmium concentrations within the study participants.

# 5  DISCUSSION

This thesis focuses on the application and interpretation of biomarkers of cadmium exposure in populations with environmental low-level cadmium exposure, with a special reference to studies of kidney effects. The importance of this lies in the fact that many studies are performed among populations with low-level exposures, rather than the high-exposure groups on which most current knowledge is based.

Our studies are the first to provide empirical data on the relationship between cadmium in kidney and cadmium in urine and blood, and on the biological half-time of cadmium in the human kidney, using data from healthy living individuals with low-level cadmium exposure. Our data make it possible to study factors affecting the relationship between cadmium in kidney, urine, and blood, and factors affecting the excretion of urinary cadmium. We also offer one of only a few studies focusing on the effect of within- and between-individual variance on the study design when biomarkers of cadmium exposure are used.

## 5.1  Cadmium in kidney, urine, and blood for individuals with a low-level environmental exposure

When biomarkers are used in epidemiologic studies and for risk assessments, information on excretion of the biomarker and the association between the biomarker and the target organ/body burden is needed for both high and low levels of exposure. In an epidemiological study, it is also important to control for potential confounders; that is, factors that will affect both the measured biomarker and the studied outcome. Knowledge of factors that will affect the excretion of the biomarker (i.e. determinants) is therefore important.

## Relationship between kidney cadmium and cadmium in urine and blood

The kidney cadmium concentration was compared to the measures of cadmium in urine and blood (study population 1, *Paper I*), since this is what most risk assessments are based on. The strength of this relationship also gives a measure of the performance of these measures as biomarkers for cadmium body burden. The results showed relatively close relationships between kidney cortex cadmium and cadmium both in urine and blood, and these associations were highly significant ($r_p$=0.44-0.70 for urine, $r_p$=0.44 for blood; *Paper I*, Table 2). This indicates that all studied measures of cadmium in urine and blood represent the kidney cadmium or the body burden of cadmium relatively well at these low levels of cadmium exposure.

Similar results on environmentally exposed individuals have been shown for kidney cadmium and urinary cadmium in two different autopsy studies ($r_p$=0.85 and $r_p$=0.58) (Orlowski et al. 1998; Satarug et al. 2002), and for kidney cadmium and blood cadmium in two different *in vivo* studies ($r_s$=0.49 and $r_s$=0.33) using X-ray fluorescence (Nilsson et al. 2000; Nilsson et al. 1995). However, *in vivo* measurements using X-ray fluorescence or neutron activation have historically failed to show a significant association between kidney cadmium and urinary cadmium in environmentally exposed individuals (Nilsson et al. 2000; Nilsson et al. 1995). This might be explained by a high limit of detection for kidney cadmium in these analytical techniques. The minimum detectable concentration for the X-ray fluorescence measurements was about 15 µg/g at a typical kidney depth, which is similar to the median kidney cadmium level in our study. Similarly, the neutron activation measurements had a minimum detectable amount of 2500 µg as compared with the median kidney cadmium amount of 3400 µg in our study.

Risk assessments for kidney effects of cadmium, based on other biomarkers of cadmium exposure than kidney cadmium, require

knowledge of the quantitative relationships between kidney cadmium and these biomarkers. Previous to our study, researchers have estimated that a kidney cortex cadmium concentration of 25 µg/g corresponds to an overnight urinary cadmium concentration adjusted for creatinine of 1.25 µg/g creatinine (i.e. a urinary cadmium to kidney cadmium ratio of 1:20) (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007). Our estimate based on study population 1 (*Paper I*), with a median kidney cortex cadmium level of 15 µg/g, was considerably lower. A urinary cadmium concentration of 0.42 µg/g creatinine corresponded to a kidney cortex cadmium concentration of 25 µg/g, giving a urinary cadmium to kidney cadmium ratio of 1:60. Consequently, the current risk assessment underestimates the kidney cadmium level for populations with a low-level cadmium exposure.

A possible reason for the much higher urinary to kidney cadmium ratio in the literature (1:20) might be that it is based on individuals with kidney cadmium levels high enough to cause an increased "leakage" of urinary cadmium due to renal cadmium toxicity. Another possible explanation is that the urinary cadmium levels in those studies were overestimated due to analytical limitations or post-mortem changes. There is also some previous evidence of dose-dependence in the kinetics of cadmium (Engström and Nordberg 1979; Järup et al. 1998). However, even results from the *in vivo* X-ray fluorescence studies on environmentally exposed individuals indicate a much lower urine to kidney cadmium ratio than 1:20, in agreement with our findings, if all participants with kidney cadmium below the minimum detectable concentration are taken into account (Nilsson et al. 2000; Nilsson et al. 1995).

The shape of the relationship between kidney cadmium and urinary cadmium was investigated in study population 1 (*Paper I*) using the total amount of cadmium in the kidney and the 24h urinary cadmium excretion, as these measures most likely represent the long-term cadmium burden more reliably than the kidney cortex concentration

and the cadmium concentration in spot urine samples (Nawrot et al. 2010; Nordberg et al. 2007).

The results showed a nonlinear relationship, with a relatively higher excretion of urinary cadmium at lower kidney cadmium concentrations compared to higher kidney cadmium concentrations. It might be explained by the dose-dependent cadmium kinetics mentioned above (Engström and Nordberg 1979; Järup et al. 1998), with a longer cadmium retention at higher kidney cadmium levels. This nonlinearity could also be an effect of factors other than kidney cadmium that may affect the urinary cadmium excretion (urinary proteins and recent cadmium exposure, discussed more in detail below), which will have a relatively stronger effect at lower cadmium levels. A nonlinear relationship might have an effect on pharmacokinetic modelling (often used in risk assessments), calculations of the biological half-time, and the interpretation of very low urinary cadmium levels.

## Elimination and biological half-time of cadmium in kidney at environmental low-level exposures

The elimination constant of cadmium in kidney was estimated in study population 1 (*Paper I*) as the regression coefficient between the amount of cadmium in the kidney and the 24h urinary cadmium excretion. The fraction of the total amount of cadmium in the kidney excreted in urine over 24h was estimated at 0.009% using a model without an intercept; and 0.006% using a model with an intercept, in agreement with previous findings (EFSA 2009; Nordberg et al. 2007). When the data were stratified for kidney cadmium concentration, to account for the nonlinear relationship between kidney cadmium and urinary cadmium, the estimates were 0.011% for low kidney cadmium levels (<15 µg/g) and 0.007% for higher levels.

The elimination coefficients were used to estimate the biological half-time of cadmium in kidney, assuming a one-compartment model (Equation 1). We estimated the half-time to be about 30 years at a typical kidney cortex concentration of 15 µg/g. Using different models

gave half-times between 18 and 30 years when linear models were used, while a nonlinear model gave biological half-times between 21 and 44 years in our range of data. Our estimate of a 30-year biological half-time of cadmium in kidney is in the higher range compared to previous estimates (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007).

## Determinants for urinary cadmium excretion

We studied several different determinants for urinary cadmium excretion in both study population 1 (*Paper 1*) and study population 2 (*Paper IV*).

In addition to kidney cadmium, determinants such as blood cadmium, urinary protein excretions, age, and gender were included in a multivariate model for the 24h urinary cadmium excretion in study population 1 (*Paper I*). The final model, derived by step-wise backwards elimination, showed that about 70% of the urinary cadmium excretion could be explained by kidney cadmium for an average individual with a kidney cadmium concentration of 15 µg/g and a 24h urinary cadmium excretion of 0.31 µg/24h. The remaining 30% was explained by blood cadmium levels, urinary protein excretion, and other determinants.

Study population 1 (*Paper I*) included some smokers. As expected, smoking status (ever- vs. never-smokers) was a significant determinant for urinary cadmium excretion, as reported by other researchers (Adams et al. 2011; EFSA 2009; Ikeda et al. 2005; Järup et al. 1998; Nordberg et al. 2007; WHO 2011). Blood cadmium was also a significant determinant for urinary cadmium, especially for smokers and for participants with a kidney cadmium concentration above 15 µg/g. However, the additional impact of blood cadmium on the urinary cadmium excretion still represents renal clearance of cadmium. Blood cadmium is assumed to increase more than kidney cadmium after the short-term cadmium exposure which occurs in smokers. In these cases,

blood cadmium might also equilibrate with compartments with faster turnover of cadmium than the kidney compartment. Thus, when blood cadmium is high and a steady state of kidney cadmium has not been reached (which may be the case in smokers below 50 years of age), a fraction of the cadmium filtered in the glomeruli may be excreted rather than reabsorbed. In agreement with our findings, this impact of blood cadmium on urinary cadmium excretion, over and above the impact of kidney cadmium, has been predicted in metabolic models of cadmium in humans (Kjellström and Nordberg 1978).

An effect of urinary protein excretion (mainly urinary albumin) on urinary cadmium excretion was found in both study populations (*Papers I & IV*), a result also reported by others both prior to and after our studies (Chaumont et al. 2012; Chaumont et al. 2013; Haddam et al. 2011). In study population 1 (*Paper I*) the effect was mainly seen for never-smokers and individuals with a kidney cortex cadmium concentration below 15 µg/g, but in study population 2 (*Paper IV*) the effect was seen for most urinary measures of cadmium exposure in overnight and spot urine samples. This association probably represents normal variability in renal physiology, resulting in temporarily increased or decreased cadmium excretion independent of kidney cadmium (Bernard 2008; Chaumont et al. 2012; Chaumont et al. 2013; Haddam et al. 2011; Åkesson et al. 2014). The excretion of cadmium and proteins is assumed to change in the same direction, since cadmium bound to metallothionein and proteins such as albumin share the same tubular binding site (Christensen et al. 2009); this results in an association between the two during temporary changes in the renal physiology.

Gender and age were significant determinants for urinary cadmium for most cadmium measures in urine in study population 2 (*Paper IV*). This is as expected, since women generally have a higher cadmium absorption because of low iron stores (Berglund et al. 1994; Flanagan et al. 1978; Julin et al. 2011; Vahter et al. 1996), and cadmium

accumulates with age because of the long half-time of cadmium in the human body (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007; WHO 2007). In study population 1 (*Paper I*), where other determinants such as kidney and blood cadmium were included in the multivariate models, the effects of gender and age were mainly seen among smokers.

We also found an effect of urinary flow rate and time point of sampling on urinary cadmium excretion, especially in spot urine samples, among the repeated samples from study population 2 (*Paper IV*). An effect of urinary flow rate indicates that the adjustment for diuresis was imperfect, as discussed by others (Berlin et al. 1985; Chaumont et al. 2013; Elkins et al. 1974; Greenberg and Levine 1989; Suwazono et al. 2005). An effect of the time point of sampling indicates a circadian rhythm in the urinary cadmium excretion which will be discussed below.

## 5.2 The interpretation of cadmium measured in urine and effects on current risk assessments for kidney effects

When choosing a biomarker for cadmium exposure, it is important to consider not only information regarding the association between the biomarker and the target organ/body burden, but also information concerning the interpretation of a biomarker and factors affecting the interpretation under certain circumstances. For studies of kidney effects, measured as an increased urinary excretion of proteins, such information is vital since both the cadmium exposure and kidney effect are measured in the same urine sample. Thus, factors affecting the urinary cadmium excretion might also affect the urinary protein excretion.

# Urinary cadmium as a biomarker in studies of kidney effects

The interpretation of urinary cadmium as a biomarker for cadmium exposure in epidemiological studies of kidney effects, at a low level cadmium exposure, was investigated by studying the association between urinary cadmium and urinary proteins within study participants in study population 2 (*Paper II*). Urinary protein excretion was also included as a determinant for the urinary cadmium excretion in *Paper I*.

Within the participants of study population 2 (*Paper II*), an association between urinary cadmium and urinary proteins was seen for all urinary measures. The association remained for most urinary measures even after the inclusion of more determinants (*Papers I & IV*). Because cadmium has a long biological half-time in the human body (EFSA 2009; Järup et al. 1998; Nordberg et al. 2007), the cadmium body burden is expected to be stable over two sampling days (mostly within one week), and urinary cadmium should only be affected by normal physiological variations. Thus, the association observed within participants in our study (*Paper II*) is not consistent with an effect of cadmium toxicity on the kidney.

Such an association has also been observed at low-level cadmium exposures in other study populations previously (Chaumont et al. 2012; Haddam et al. 2011) and later (Chaumont et al. 2013), and has been associated with temporal changes in renal physiology, since urinary cadmium bound to metallothionein and urinary proteins such as albumin share the same binding site (Chaumont et al. 2012). The association has been proposed to be driven largely by smoking (Haddam et al. 2011). However, we found an association among never-smokers, regardless of how the spot urine samples were adjusted for diuresis, and thus the association could not be explained by smoking only. We also found an effect of urinary flow rate for most urinary

measures of cadmium, suggesting that urinary flow is an important determinant for the variation in tubular reabsorption (*Paper II*).

These results indicate that caution is needed if urinary cadmium is used as a biomarker of exposure when studying renal effects of cadmium toxicity at low-level cadmium exposures. Studies that have used urinary cadmium as a biomarker for cadmium exposure, when studying kidney effects, might have overestimated the risk of kidney effects from cadmium toxicity at low cadmium exposures.

## Short-term variability within and between individuals and effects on the interpretation of urinary cadmium

Using mixed-effects models on the repeated samples from study population 2 (*Paper IV*), the short-term variability within and between individuals was studied for the available measures of cadmium in urine. This variability may lead to a biased prediction if urinary cadmium is used as a biomarker for cadmium exposure in epidemiologic studies or studies comparing exposure levels between groups.

The between-individual variability dominated the total variability for most measures of cadmium in urine (ICCs between 0.39 and 0.89), indicating a close relationship between the biomarker of exposure and differences in exposure level between individuals (Rappaport and Kupper 2008). A review on biomonitoring of occupational exposures drew a similar conclusion regarding biomarkers with long biological half-times (Symanski and Greeson 2002). The ratio between the within- and between-individual variability decreased (on average a decrease of about 50%) when urinary cadmium was adjusted for dilution and when overnight and 24h urine samples were chosen before spot urine samples (on average about 70%). This indicates a larger reproducibility for these biomarkers, which demonstrates the need for adjusting spot urine samples for dilution and for the use of time-specific sampling such as the collection of overnight urine samples in

studies. The latter point was also stressed by the finding of a circadian rhythm in urinary cadmium excretion (*Paper IV*).

Previous studies have reported a reduction of the variance when adjusting urinary cadmium concentrations for dilution (Arisawa et al. 1997; Mason et al. 1998; Yamagami et al. 2008). In our study, this was the case for urinary cadmium concentrations adjusted for creatinine in overnight samples, but not for the cadmium concentrations adjusted for specific gravity. However, adjustment for dilution increased the ICC for all measures (*Paper IV*).

Only a few studies have investigated the short-term within-individual variability of urinary cadmium, and most of them had longer time intervals between the collected urine samples compared to our study. A similar within-individual variation in urinary cadmium concentrations adjusted for dilution was seen in second morning samples taken over 10 sequential weeks among 17 environmentally exposed women in Japan (Yamagami et al. 2008). However, a study with repeated urine samples taken three months apart found a substantially lower ICC for urinary cadmium compared to our study (Gunier et al. 2013).

As indicated above, we found a significant circadian rhythm in urinary cadmium excretion over 24h (*Paper IV*). Such circadian rhythm has also been seen by others (Kanabrocki et al. 2008). In mixed-effects models, the time point of sampling was found to be a significant determinant of urinary cadmium in spot urine samples. When the different sampling times were compared to each other, the urinary cadmium excretion was generally significantly higher in the second morning sample (09:30) and significantly lower in the afternoon samples (14:30, 17:30 and 22:00) compared to overnight samples.

Factors such as urinary protein excretion or urinary flow rate could partly but not fully explain the variation in urinary cadmium excretion over 24h when included in multivariate models. Another possible

factor that might affect urinary cadmium excretion (as well as protein excretion and urinary flow) is circadian variations in the kidney function. Such circadian rhythm was seen in glomerular filtration rate and renal plasma flow in a study of 11 healthy individuals during bed rest and standardized protein intake (Koopman et al. 1989). The glomerular filtration rate was found to be higher during the daytime compared with overnight, and the urinary flow rate was found to have the same circadian rhythm as the glomerular filtration rate (Koopman et al. 1989). A study of occupationally exposed workers revealed a decreased urinary cadmium excretion overnight, which the authors attributed to the glomerular filtration rate (Yokoyama et al. 2000). A significant effect of glomerular filtration rate on urinary cadmium excretion was also found among 19 occupationally exposed individuals using water loading and water restrictions (Araki and Aono 1989), and among lead workers (Weaver et al. 2011).

Consequently, even though cadmium has a long biological half-time in humans, resulting in a stable long-term cadmium level, the short-term variations in urinary cadmium excretions caused by natural physiological changes will still have an effect on the cadmium measured in urine, causing an attenuation of the epidemiological dose-response relationship in a study of health effects.

## 5.3 Aspects of the choice of biomarkers for cadmium body burden and sampling strategy

Studies on low-exposure populations, as well as epidemiological studies on rare or subtle effects, place increased demands on the study design and choice of biomarker. If the exposure levels in an epidemiological study are not accurately characterized, the estimated relationship between exposure and health effects in humans (the estimated regression coefficient) tends to be underestimated (Rappaport and Kupper 2008). The performance of the biomarker also

affects the number of samples needed to achieve valid results in terms of a high power in the study (Li et al. 2010; Lin et al. 2005; Rappaport and Kupper 2008).

## Measurement strategy and analytical method

Theoretically, urinary 24h cadmium excretion should represent the cadmium body burden most reliably, since this is the average excretion over a longer time (Nawrot et al. 2010; Nordberg et al. 2007). However, 24h urine samples are difficult to collect, and the high risk of incomplete and contaminated samples makes 24h sampling inappropriate for most epidemiological studies. Spot urine samples or blood samples are more feasible to collect in these kinds of studies. Therefore, overnight spot urine samples (first morning samples) and blood samples were compared to separate 24h urine samples collected under controlled circumstances (hospitalization) in *Papers I & III*. There was a strong association ($r_p$>0.74, p<0.001) between the measures in overnight urine samples and the 24h urinary cadmium excretion (*Paper I*); this indicates that the overnight urine samples represent the 24h urinary excretion relatively well, as described previously by others (Berlin et al. 1985). Blood cadmium also represented the 24h urinary cadmium excretion relatively well ($r_p$=0.48, p<0.001), but when the study population was stratified for smoking habits, the association was only present for smokers. Overnight urine samples and blood samples were also significantly associated with kidney cadmium, as discussed above.

When the cadmium levels in 24h and overnight urine samples were compared within individuals in *Paper III*, significant differences were found between the two samples, indicating that the measurement strategy will affect the results, and thus comparisons within and between studies require similar measurement strategies.

Further investigation in study population 2 (*Paper IV*) showed a significant circadian rhythm even during the daytime for most

cadmium measures in spot urine samples. It is therefore clear that a specific time point of sampling must be used if results are to be compared within and between studies. The best time point would be overnight samples (first morning samples), since the conditions tend to be more stable overnight. Additional evidence for this need for consistency in time of sampling is provided by the investigation of variability in *Paper IV*, with its comparison between spot urine samples and time-specific samples (overnight urine samples). When cadmium measures in overnight urine samples were used instead of spot urine samples collected throughout the day, the ICC increased and the total variability decreased for most urinary cadmium measures.

In *Paper III*, we showed the importance of correcting urinary cadmium concentrations for molybdenum oxide-based interference if certain ICP-MS techniques are used, since this interference greatly affects the interpretation of the results.

## Adjusting urinary concentrations in spot urine samples for dilution

Spot urine samples need to be adjusted for dilution. In the present thesis, this was achieved by calculating the cadmium excretion rate or by adjusting the cadmium concentration for urinary creatinine concentration or specific gravity. Numerous studies have investigated the optimal adjustment method for cadmium concentrations in spot urine, with inconsistent results (Araki and Aono 1989; Berlin et al. 1985; Carrieri et al. 2001; Elkins et al. 1974; Greenberg and Levine 1989; Mason et al. 1998; Pearson et al. 2009; Sata et al. 1995; Suwazono et al. 2005). Our results showed only moderate differences in the performance of the different adjustment methods used in this thesis. However, adjusting the cadmium concentration using the urinary creatinine concentration (i.e. the cadmium to creatinine concentration ratio) would be the best choice when adjusting cadmium concentrations in spot urine samples for dilution, see below.

When adjusting for creatinine concentration, a constant excretion of urinary creatinine is assumed. However, there is evidence showing that adjusting for cadmium concentration using creatinine concentration is not a perfect method. In study population 1, the urinary creatinine excretion was affected by the urinary flow rate (Akerstrom et al. 2012), a phenomenon also demonstrated by others (Greenberg and Levine 1989; Trachtenberg et al. 2010). In *Paper II*, urinary flow rate was a significant determinant for urinary cadmium within individuals even after adjusting for creatinine, and significant correlations were found between the urinary cadmium concentration adjusted for creatinine and the urinary creatinine concentration. Nevertheless, urinary cadmium concentration adjusted for creatinine showed a somewhat stronger association with kidney cadmium compared to the other measures of cadmium in urine (*Paper I*, Table 2 & Figure 2). Urinary cadmium concentration adjusted for creatinine was also less affected by normal physiological variations compared to cadmium concentrations adjusted for specific gravity or cadmium excretion rates (*Paper II*).

Adjustment for dilution reduced the variance when the cadmium concentration was adjusted for creatinine, but not when specific gravity was used. Urinary cadmium concentrations adjusted for creatinine also had somewhat higher reproducibility (i.e. higher ICC) compared to cadmium concentrations adjusted for specific gravity (ICC=0.89 vs. ICC=0.75, *Paper IV*). In addition, using urinary cadmium concentrations adjusted for creatinine concentration reproduced the gender difference (*Papers I, III, & IV*) seen in kidney cadmium (Barregard et al. 2010).

The cadmium excretion rate performed generally well and required somewhat smaller study sizes compared to urinary cadmium concentrations adjusted for creatinine (*Paper IV*, discussed more below) but these small differences might not justify the extra work involved in collecting timed urine samples in a study.

## Appropriate sample size when using urinary biomarkers for cadmium exposure

When planning a study, the appropriate sample size required to obtain valid results is of great importance. The optimal sample size in a study is reached when a high accuracy can be achieved (preferably a bias of 20% or lower) with a reasonable number of samples to collected. The optimal sample size might vary depending on the choice of measure of cadmium exposure and the choice of sampling strategy. Our results indicate that different study types place different demands on the measures of cadmium in urine and the sampling strategy. Thus, the choice of biomarker for cadmium exposure and the choice of sampling strategy in a study will affect the optimal sample size.

In epidemiological individually-based studies where dose-response relationships are studied, the relation between the within- and between-individual variability is important for achieving a high accuracy in the estimated regression slope. According to Equation 3, the accuracy can also be increased by increasing the number of samples per individual in a study. *Paper IV* showed that choosing a specific time point of sampling, such as overnight urine samples, increases the ICC for all cadmium measures and thus decreases the required number of repeated measurements per individual to one. Consequently, if only spot urine samples taken throughout the day are accessible in a study, a single measurement of urinary cadmium per individual does not seem to be sufficient to achieve a bias of 20% or less. Among a group of women who each provided two 24h urine samples (3-9 months apart), four 24h urine samples per participant were required to achieve the same power (Gunier et al. 2013).

In studies comparing the cadmium exposure level between two groups, the number of samples per individual did not have a large effect on the group sizes, indicating that a high study power would be better achieved by increasing group sizes than by increasing the number of repeated samples per individual (*Paper IV*). This has also been shown

for urinary proteins (Stengel et al. 1999). In this kind of study, the most important factor affecting the required sample size is the expected difference between the two groups compared. It is therefore crucial to make a prior estimate of the expected difference, since going from a 50% difference between the two groups to a 25% difference results in a threefold increase in the required sample size. It is also important to remember that a strong gender difference was seen in *Paper IV* (Supplementary Information), with a larger variability among women, meaning that much larger group sizes are required if studies are carried out among women only. The larger variance may be caused by differences in iron status (Berglund et al. 1994; Flanagan et al. 1978; Julin et al. 2011; Vahter et al. 1996) and/or other factors affecting women more than men.

## 5.4 Validity and generalizability

Neither of the two study populations was randomly selected from the environmentally exposed population, due to ethical and/or practical reasons. Nevertheless, we believe that they are representative for the healthy part of the environmentally low-level exposed population without exposure from any industrial sources, in terms of the aspects studied in this thesis. Study population 1 included never- and ever-smoking men and women aged 24-70. Personal information was collected for all participants, and only two participants were found to have had a low occupational cadmium exposure in the past. Although all kidney donors were recruited at the transplantation unit, we believe that they represent the environmentally exposed population with regard to the relationship between cadmium in kidney, urine, and blood.

Study population 2 was recruited from our department and among students at the university, and consisted of never-smoking men and women aged 23-59. Compliance with the extensive urine sample collection schedule was of great importance for this study, and so the study participants were not randomly selected. Background

information was collected for each study participant, and no one was found to have a present or past occupational exposure to cadmium. The study population might have had a more sedentary work compared to the entire environmentally exposed population, but we do not think that either urinary excretion itself or variability in cadmium and protein excretion will be affected by physical activity. A very small pilot study performed at our department gave no indication of an effect of physical activity on cadmium excretion (data not shown). However, in a study comparing 21 professional athletes to 26 sedentary individuals, an increased urinary cadmium excretion was found among the group of athletes (Llerena et al. 2012).

Although neither study population was randomly selected from the general population, their level of cadmium exposure corresponds well to the low-level environmentally exposed general population (Barregard et al. 2010; EFSA 2009; Järup et al. 1998; Nordberg et al. 2007; Sand and Becker 2012; WHO 2007).

A higher number of study participants generally improves the ability to achieve valid results. Study population 1 consisted of 152 participants, 109 of whom had a kidney biopsy available for cadmium analysis. This makes this study considerably larger than other studies of the relationship between cadmium in kidney, urine, and blood among the environmentally exposed population (Nilsson et al. 2000; Nilsson et al. 1995; Orlowski et al. 1998; Satarug et al. 2002). Study population 2, consisting of 30 participants (all never-smokers), was also larger than in other studies of mechanisms such as circadian rhythms (Araki et al. 1986; Koopman et al. 1989; Yokoyama et al. 2000).

Since two different study populations were used in this thesis, some of the aspects of interest, including determinants for urinary cadmium excretion and the circadian rhythm of urinary cadmium excretion, could be studied in both study populations. In these cases, similar

results were found for both study populations, which strengthens the findings.

When studying small effects at low levels of the biomarkers, the accuracy of the chemical analyses is of great importance. All cadmium analyses were performed together with external quality control samples, with satisfactory results. In addition, the urinary cadmium concentrations in study population 1 were reanalysed in *Paper III*, and the samples that had been corrected for molybdenum oxide-based interference both times agreed strongly with the initial analysis.

Another potential problem when analysing low-level exposures is values below the limit of detection. In study population 1 (*Papers I & III*), only a few values were below the limit of detection, and these were estimated according to the distribution of the data (Hornung and Reed 1990). For study population 2 (*Papers II & IV*), a higher percentage of the urinary cadmium concentrations were below the limit of detection. The values were estimated in the same way as for study population 1, but when studying variability (*Paper IV*), individuals with 50% or more of their urinary cadmium concentrations below the limit of detection were excluded to minimize the potential risk of attenuating the total variability. The kidney cadmium concentration was determined from a small biopsy, and hence an uneven distribution of cadmium in the kidney could potentially introduce some bias. However, all biopsies were classified as representative of the kidney cortex by an experienced pathologist, and the geometric standard deviation of the kidney cortex concentration was similar to results in autopsy studies with larger biopsies (Barregard et al. 2010). The 24h urine samples from study population 1 were collected at the hospital, which we believe improved the quality of these otherwise difficult-to-collect samples.

# 6  CONCLUSIONS

- There was a strong but nonlinear association between urinary cadmium and kidney cadmium, with slower elimination of cadmium at high kidney cadmium. The association between cadmium in blood and kidney cadmium was weaker. A kidney cadmium of 25 µg/g corresponded to a urinary cadmium of 0.42 µg/g creatinine (a urinary cadmium to kidney cadmium ratio of 1:60). Previous estimates of the urinary cadmium to kidney cadmium ratio may underestimate kidney cadmium at low urinary cadmium.

- Aside from kidney cadmium, cadmium in blood and urinary albumin were significant determinants for urinary cadmium excretion in multivariate models. Kidney cadmium explained about 70% of the urinary cadmium excretion for an average participant.

- Between 0.007% and 0.011% of the total kidney cadmium burden was excreted in urine over 24h. The biological half-time of cadmium in kidney was estimated to be about 30 years.

- Significant associations between urinary cadmium and urinary proteins were found within participants at low urinary cadmium levels, indicating that this association is caused by normal physiology rather than renal toxicity. Consequently, the risk of kidney damage may be overestimated at low urinary cadmium levels.

- Molybdenum oxide-based interference can result in an overestimation of cadmium concentrations when urinary cadmium is analysed using certain ICP-MS techniques. Thus,

correction for molybdenum oxide-based interference may be needed when urinary cadmium is analysed in this way.

- Between-individual variability dominated the total variability for most measures of urinary cadmium excretion. Urinary cadmium excretion showed a circadian rhythm during the day, and time point of sampling was a significant factor even after more determinants had been included in the models.

- In terms of achieving a high power with a limited number of samples, the choice of biomarker for urinary cadmium excretion was found to be more important in individually-based studies of exposure-response relationships than in studies comparing cadmium levels of different groups.

- Samples from first morning urine represent both kidney cadmium and 24h urinary excretion of cadmium relatively well and are feasible to use in larger studies. Cadmium concentrations adjusted for dilution using creatinine concentration or specific gravity generally perform well, but our results indicate that cadmium concentrations adjusted for creatinine are less influenced by urinary protein excretion and result in a slightly higher accuracy in individually-based studies of exposure-response relationships.

# 7  FUTURE RESEARCH

This thesis contributes new knowledge about cadmium biomonitoring at low-level cadmium exposure, using studies carried out on two different Swedish study populations with comparatively very low environmental cadmium exposure. The results still need to be verified for a wider range of cadmium exposures, to establish the range of data on which these results can be applied. This is particularly important for the results that differ from existing estimates derived from populations with higher exposure levels; that is, the relationship between urinary cadmium and kidney cadmium and the association between urinary proteins and urinary cadmium.

By using this new data, it should also be possible to update the metabolic model of cadmium in humans created by Kjellström and Nordberg (1978).

In recent years, cadmium exposure has been assessed by the use of food questionnaires. There is a need for more data on the relationship between these dietary assessments of cadmium intake and biomarkers of cadmium in blood and urine.

The studies described in this thesis, especially *Paper IV*, show that the short-term variability of urinary cadmium excretion should affect the study design. Our results are based on healthy non-smoking individuals with mainly sedentary work, and more studies need to be done to provide additional data on other populations, including smokers.

The determinants available in these studies could not fully explain either the circadian rhythm in urinary cadmium excretion (*Papers III & IV*) or the association between urinary cadmium and urinary proteins in the absence of an effect of cadmium toxicity (*Papers I, II & IV*). Different kinds of normal physiological variation have been presented as possible explanations, but there is a need for more studies identifying such natural variations and other factors which may be relevant for cadmium biomonitoring.

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# 9 REFERENCES

Adams SV, Newcomb PA, Shafer MM, Atkinson C, Bowles EJ, Newton KM, Lampe JW (2011) Sources of cadmium exposure among healthy premenopausal women. Sci Total Environ 409(9):1632-7

Akerstrom M, Lundh T, Barregard L, Sallsten G (2012) Sampling of urinary cadmium: differences between 24-h urine and overnight spot urine sampling, and impact of adjustment for dilution. Int Arch Occup Environ Health 85(2):189-96

Andersson L, Haraldsson B, Johansson C, Barregard L (2008) Methodological issues on the use of urinary alpha-1-microglobuline in epidemiological studies. Nephrol Dial Transplant 23(4):1252-6

Araki S, Aono H (1989) Effects of water restriction and water loading on daily urinary excretion of heavy metals and organic substances in metal workers. Br J Ind Med 46(6):389-92

Araki S, Aono H, Yokoyama K, Murata K (1986) Filterable plasma concentration, glomerular filtration, tubular balance, and renal clearance of heavy metals and organic substances in metal workers. Arch Environ Health 41(4):216-21

Arisawa K, Nakano A, Honda S, Saito H (1997) Reproducibility of urinary beta 2-microglobulin and cadmium excretion among residents in a cadmium-polluted area during a 3-year period. Toxicol Lett 91(2):147-52

Bakoush O, Grubb A, Rippe B, Tencer J (2001) Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. Kidney Int 60(5):1904-9

Barany E, Bergdahl I, Schütz A, Skerfving S, Oskarsson A (1997) Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. J Anal At Spectrom 12(9):1005

Barregard L, Fabricius-Lagging E, Lundh T, Mölne J, Wallin M, Olausson M, Modigh C, Sallsten G (2010) Cadmium, mercury, and lead in kidney cortex of living kidney donors: Impact of different exposure sources. Environ Res 110(1):47-54

Barregård L, Svalander C, Schütz A, Westberg G, Sällsten G, Blohmé I, Mölne J, Attman PO, Haglind P (1999) Cadmium, mercury,

and lead in kidney cortex of the general Swedish population: a study of biopsies from living kidney donors. Environ Health Perspect 107(11):867-71

Berglund M, Åkesson A, Nermell B, Vahter M (1994) Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environ Health Perspect 102(12):1058-66

Berlin A, Alessio L, Sesana G, Dell'Orto A, Ghezzi I (1985) Problems concerning the usefulness of adjustment of urinary cadmium for creatinine and specific gravity. Int Arch Occup Environ Health 55(2):107-11

Bernard A (2008) Biomarkers of metal toxicity in population studies: research potential and interpretation issues. J Toxicol Environ Health A 71(18):1259-65

Börjesson J, Bellander T, Järup L, Elinder CG, Mattsson S (1997) In vivo analysis of cadmium in battery workers versus measurements of blood, urine, and workplace air. Occup Environ Med 54(6):424-31

Börjesson J, Gerhardsson L, Schütz A, Perfekt R, Mattsson S, Skerfving S (2001) Kidney cadmium as compared to other markers of cadmium exposure in workers at a secondary metal smelter. Am J Ind Med 39(1):19-28

Brunekeef B, Noy D, Clausing P (1987) Variability of exposure measurements in environmental epidemiology. Am J Epidemiol 125(5):892-898

Carrieri M, Trevisan A, Bartolucci GB (2001) Adjustment to concentration-dilution of spot urine samples: correlation between specific gravity and creatinine. Int Arch Occup Environ Health 74(1):63-7

Chaumont A, De Winter F, Dumont X, Haufroid V, Bernard A (2010) The threshold level of urinary cadmium associated with increased urinary excretion of retinol-binding protein and beta 2-microglobulin: a re-assessment in a large cohort of nickel-cadmium battery workers. Occup Environ Med 68(4):257-64

Chaumont A, Nickmilder M, Dumont X, Lundh T, Skerfving S, Bernard A (2012) Associations between proteins and heavy metals in urine at low environmental exposures: evidence of reverse causality. Toxicol Lett 210(3):345-52

Chaumont A, Voisin C, Deumer G, Haufroid V, Annesi-Maesano I, Roels H, Thijs L, Staessen J, Bernard A (2013) Associations of urinary cadmium with age and urinary proteins: further evidence of physiological variations unrelated to metal accumulation and toxicity. Environ Health Perspect 121(9):1047-53

Chen L, Lei L, Jin T, Nordberg M, Nordberg GF (2006) Plasma metallothionein antibody, urinary cadmium, and renal dysfunction in a Chinese type 2 diabetic population. Diabetes Care 29(12):2682-7

Christensen EI, Verroust PJ, Nielsen R (2009) Receptor-mediated endocytosis in renal proximal tubule. Pflugers Arch 458(6):1039-48

Christoffersson JO, Welinder H, Spang G, Mattsson S, Skerfving S (1987) Cadmium concentration in the kidney cortex of occupationally exposed workers measured in vivo using X-ray fluorescence analysis. Environ Res 42(2):489-99

de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, Smerhovsky Z, Cikrt M, Trzcinka-Ochocka M, Razniewska G, Jakubowski M, Bernard A (2006) Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ Health Perspect 114(4):584-90

EFSA (2009) Cadmium in food - Scientific opinion of the Panel on Contaminants in the Food Chain. EFSA J 980:1-139

Elinder CG, Nordberg M, Palm B (1990) Is cadmium released from metallothionein in rejected human kidneys? Biol Met 2(4):219-22

Elkins HB, Pagnotto LD, Smith HL (1974) Concentration adjustments in urinalysis. Am Ind Hyg Assoc J 35(9):559-65

Ellis KJ, Vartsky D, Zanzi I, Cohn SH, Yasumura S (1979) Cadmium: in vivo measurement in smokers and nonsmokers. Science 205(4403):323-5

Engström A, Michaelsson K, Suwazono Y, Wolk A, Vahter M, Åkesson A (2011) Long-term cadmium exposure and the association with bone mineral density and fractures in a population-based study among women. J Bone Miner Res 26(3):486-95

Engström A, Michaelsson K, Vahter M, Julin B, Wolk A, Åkesson A (2012) Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. Bone 50(6):1372-8

Engström B, Nordberg GF (1979) Factors influencing absorption and retention of oral 109Cd in mice: age, pretreatment and subsequent treatment with non-radioactive cadmium. Acta Pharmacol Toxicol (Copenh) 45(4):315-24

Ferraro PM, Costanzi S, Naticchia A, Sturniolo A, Gambaro G (2010) Low level exposure to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-2006. BMC Public Health 10:304

Flanagan PR, McLellan JS, Haist J, Cherian G, Chamberlain MJ, Valberg LS (1978) Increased dietary cadmium absorption in mice and human subjects with iron deficiency. Gastroenterology 74(5 Pt 1):841-6

Friberg L (1950) Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning; a clinical and experimental study. Acta Med Scand Suppl 240:1-124

Greenberg GN, Levine RJ (1989) Urinary creatinine excretion is not stable: a new method for assessing urinary toxic substance concentrations. J Occup Med 31(10):832-8

Gunier RB, Horn-Ross PL, Canchola AJ, Duffy CN, Reynolds P, Hertz A, Garcia E, Rull RP (2013) Determinants and within-person variability of urinary cadmium concentrations among women in northern California. Environ Health Perspect 121(6):643-9

Haddam N, Samira S, Dumont X, Taleb A, Lison D, Haufroid V, Bernard A (2011) Confounders in the assessment of the renal effects associated with low-level urinary cadmium: an analysis in industrial workers. Environ Health 10:37

Hagino N, Kono M (1955) A study on the cause of Itai-itai-disease. In: Proc 17th Mtg Japanese Soc of Clinical Surgeons 1955

Heederik D, Boleij JSM, Kromhout H, Smid T (1991) Use and Analysis of Exposure Monitoring Data in Occupational Epidemiology: An Example of an Epidemiological Study in the Dutch Animal Food Industry. Appl Occup Environ Hyg 6(6):458-465

Hong F, Jin T, Zhang A (2004) Risk assessment on renal dysfunction caused by co-exposure to arsenic and cadmium using benchmark dose calculation in a Chinese population. Biometals 17(5):573-80

Hornung RW, Reed LD (1990) Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 5:46-51

Ikeda M, Moriguchi J, Ezaki T, Fukui Y, Ukai H, Okamoto S, Shimbo S, Sakurai H (2005) Smoking-induced increase in urinary cadmium levels among Japanese women. Int Arch Occup Environ Health 78(7):533-40

Jarrett JM, Xiao G, Caldwell KL, Henahan D, Shakirova G, Jones RL (2008) Eliminating molybdenum oxide interference in urine cadmium biomonitoring using ICP-DRC-MS. J Analy At Spectrom 23(7):962-967

Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure - a review of the literature and a risk estimate. Scand J Work Environ Health 24 Suppl 1:1-51

Järup L, Hellström L, Alfvén T, Carlsson MD, Grubb A, Persson B, Pettersson C, Spång G, Schütz A, Elinder CG (2000) Low level exposure to cadmium and early kidney damage: the OSCAR study. Occup Environ Med 57(10):668-72

Julin B, Vahter M, Amzal B, Wolk A, Berglund M, Åkesson A (2011) Relation between dietary cadmium intake and biomarkers of cadmium exposure in premenopausal women accounting for body iron stores. Environ Health 10:105

Järup L, Åkesson A (2009) Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238(3):201-8

Julin B, Wolk A, Bergkvist L, Bottai M, Åkesson A (2012) Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. Cancer Res 72(6):1459-66

Kanabrocki EL, Sothern RB, Ryan MD, Kahn S, Augustine G, Johnson C, Foley S, Gathing A, Eastman G, Friedman N, Nemchausky BA, Kaplan E (2008) Circadian characteristics of serum calcium, magnesium and eight trace elements and of

their metallo-moieties in urine of healthy middle-aged men. Clin Ter 159(5):329-46 [In Italian]

Kasiske BL, Umen AJ (1986) The influence of age, sex, race, and body habitus on kidney weight in humans. Arch Pathol Lab Med 110(1):55-60

Kjellström T, Nordberg GF (1978) A kinetic model of cadmium metabolism in the human being. Environ Res 16(1-3):248-69

Koopman MG, Koomen GC, Krediet RT, de Moor EA, Hoek FJ, Arisz L (1989) Circadian rhythm of glomerular filtration rate in normal individuals. Clin Sci (Lond) 77(1):105-11

Li Z, Romanoff LC, Lewin MD, Porter EN, Trinidad DA, Needham LL, Patterson DG Jr, Sjödin A (2010) Variability of urinary concentrations of polycyclic aromatic hydrocarbon metabolite in general population and comparison of spot, first-morning, and 24-h void sampling. J Expo Sci Environ Epidemiol 20(6):526-35

Lin YS, Kupper LL, Rappaport SM (2005) Air samples versus biomarkers for epidemiology. Occup Environ Med 62(11):750-60

Llerena F, Maynar M, Barrientos G, Palomo R, Robles MC, Caballero MJ (2012) Comparison of urine toxic metals concentrations in athletes and in sedentary subjects living in the same area of Extremadura (Spain). Eur J Appl Physiol 112(8):3027-31

Mason HJ, Williams N, Armitage S, Morgan M, Green S, Perrin B, Morgan WD (1999) Follow up of workers previously exposed to silver solder containing cadmium. Occup Environ Med 56(8):553-8

Mason HJ, Williams NR, Morgan MG, Stevenson AJ, Armitage S (1998) Influence of biological and analytical variation on urine measurements for monitoring exposure to cadmium. Occup Environ Med 55(2):132-7

Nawrot TS, Staessen JA, Roels HA, Munters E, Cuypers A, Richart T, Ruttens A, Smeets K, Clijsters H, Vangronsveld J (2010) Cadmium exposure in the population: from health risks to strategies of prevention. Biometals 23(5):769-82

Nilsson U, Schütz A, Bensryd I, Nilsson A, Skerfving S, Mattsson S (2000) Cadmium levels in kidney cortex in Swedish farmers. Environ Res 82(1):53-9

Nilsson U, Schütz A, Skerfving S, Mattsson S (1995) Cadmium in kidneys in Swedes measured in vivo using X-ray fluorescence analysis. Int Arch Occup Environ Health 67(6):405-11

Nordberg G, Fowler B, Nordberg M, Friberg L (eds)(2007) Handbook on the Toxicology of Metals. Elsevier, London

Nordberg G, Jin T, Wu X, Lu J, Chen L, Liang Y, Lei L, Hong F, Bergdahl IA, Nordberg M (2012) Kidney dysfunction and cadmium exposure--factors influencing dose-response relationships. J Trace Elem Med Biol 26(2-3):197-200

Nordberg GF, Jin T, Wu X, Lu J, Chen L, Lei L, Hong F, Nordberg M (2009) Prevalence of kidney dysfunction in humans - relationship to cadmium dose, metallothionein, immunological and metabolic factors. Biochimie 91(10):1282-5

Olsson IM, Bensryd I, Lundh T, Ottosson H, Skerfving S, Oskarsson A (2002) Cadmium in blood and urine - impact of sex, age, dietary intake, iron status, and former smoking - association of renal effects. Environ Health Perspect 110(12):1185-90

Orlowski C, Piotrowski JK, Subdys JK, Gross A (1998) Urinary cadmium as indicator of renal cadmium in humans: an autopsy study. Hum Exp Toxicol 17(6):302-6

Pearson MA, Lu C, Schmotzer BJ, Waller LA, Riederer AM (2009) Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children. J Expo Sci Environ Epidemiol 19(3):336-42

Penders J, Delanghe JR (2004) Alpha 1-microglobulin: clinical laboratory aspects and applications. Clin Chim Acta 346(2):107-18

Prozialeck WC, Edwards JR (2010) Early biomarkers of cadmium exposure and nephrotoxicity. Biometals 23(5):793-809

Rappaport SM, Kupper LL (2008) Quantitative Exposure Assessment. Stephen Rappaport, El Cerrito, California, U.S.A

Roels HA, Lauwerys RR, Buchet JP, Bernard A, Chettle DR, Harvey TC, Al-Haddad IK (1981) In vivo measurement of liver and kidney cadmium in workers exposed to this metal: its significance with respect to cadmium in blood and urine. Environ Res 26(1):217-40

Sand S, Becker W (2012) Assessment of dietary cadmium exposure in Sweden and population health concern including scenario analysis. Food Chem Toxicol 50(3-4):536-44

Sata F, Araki S, Yokoyama K, Murata K (1995) Adjustment of creatinine-adjusted values in urine to urinary flow rate: a study of eleven heavy metals and organic substances. Int Arch Occup Environ Health 68(1):64-8

Satarug S, Baker JR, Reilly PE, Moore MR, Williams DJ (2002) Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. Arch Environ Health 57(1):69-77

Satarug S, Garrett SH, Sens MA, Sens DA (2010) Cadmium, environmental exposure, and health outcomes. Environ Health Perspect 118(2):182-90

Seiffert (1897) Diseases in zinc smelter workers and hygienic precautions taken. Dtsch Vierteljahrschr Offentl Gesundhpfl 29:419-454 [In German]

Stengel B, Watier L, Chouquet C, Cenee S, Philippon C, Hemon D (1999) Influence of renal biomarker variability on the design and interpretation of occupational or environmental studies. Toxicol Lett 106(1):69-77

Suwazono Y, Åkesson A, Alfven T, Järup L, Vahter M (2005) Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10(2-3):117-26

Suzuki Y, Endo Y, Ogawa M, Matsuda M, Nakajima Y, Onda N, Iwasaki M, Tsugane S (2008) Determination of sub-ppb cadmium in urine by solid-phase extraction and inductively coupled plasma-mass spectrometry. Anal Sci 24(8):1049-52

Svartengren M, Elinder CG, Friberg L, Lind B (1986) Distribution and concentration of cadmium in human kidney. Environ Res 39(1):1-7

Symanski E, Greeson NM (2002) Assessment of variability in biomonitoring data using a large database of biological measures of exposure. AIHA J (Fairfax, Va) 63(4):390-401

Thomas LD, Michaelsson K, Julin B, Wolk A, Åkesson A (2011) Dietary cadmium exposure and fracture incidence among men: a population-based prospective cohort study. J Bone Miner Res 26(7):1601-8

Trachtenberg F, Barregard L, McKinlay S (2010) The influence of urinary flow rate on mercury excretion in children. J Trace Elem Med Biol 24(1):31-5

Trevisan A, Nicoletto G, Maso S, Grandesso G, Odynets A, Secondin L (1994) Biological monitoring of cadmium exposure: reliability of spot urine samples. Int Arch Occup Environ Health 65(6):373-5

Vahter M (1982). Assessment of human exposure to lead and cadmium through biological monitoring. Report prepared for UNEP/WHO. Stockholm: National Swedish Institute of Environmental Medicine and Karolinska Institute.

Vahter M, Berglund M, Nermell B, Åkesson A (1996) Bioavailability of cadmium from shellfish and mixed diet in women. Toxicol Appl Pharmacol 136(2):332-41

Weaver VM, Kim NS, Jaar BG, Schwartz BS, Parsons PJ, Steuerwald AJ, Todd AC, Simon D, Lee BK (2011) Associations of low-level urine cadmium with kidney function in lead workers. Occup Environ Med 68(4):250-6

WHO (2011) Evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives Geneva

WHO (2007) Health risks of heavy metals from long-range transpoundary air pollution. Copenhagen

Yamagami T, Suna T, Fukui Y, Ohashi F, Takada S, Sakurai H, Aoshima K, Ikeda M (2008) Biological variations in cadmium, alpha 1-microglobulin, beta 2-microglobulin and N-acetyl-beta-D-glucosaminidase in adult women in a non-polluted area. Int Arch Occup Environ Health 81(3):263-71

Yokoyama K, Araki S, Sato H, Aono H (2000) Circadian rhythms of seven heavy metals in plasma, erythrocytes and urine in men: observation in metal workers. Ind Health 38(2):205-12

Åkesson A, Barregard L, Bergdahl I, Nordberg G, Nordberg M, Skerfving S (2014) Non-Renal Effects and the Risk Assessment of Environmental Cadmium Exposure. Environ Health Perspect DOI:10.1289/ehp.1370110 [epub ahead of print]

Åkesson A, Julin B, Wolk A (2008) Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a

population-based prospective cohort study. Cancer Res 68(15):6435-41

Åkesson A, Lundh T, Vahter M, Bjellerup P, Lidfeldt J, Nerbrand C, Samsioe G, Strömberg U, Skerfving S (2005) Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. Environ Health Perspect 113(11):1627-31