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CADMIUM EXPOSURE AND RISK OF KIDNEY EFFECTS AND BONE FRACTURES: POPULATION-BASED STUDIES IN ENGLAND AND SWEDEN.

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To Lars Järup

ABSTRACT

Cadmium (Cd) is a toxic metal with no beneficial biological function. The dissemination of cadmium to the surface environment, by industrial and agricultural practices, has led to increased human exposure. Food is the main source of exposure in the general non-smoking population however in areas close to industrial sources, contact with contaminated environmental media may also be important. Previous studies have shown toxic effects of cadmium on the kidneys and bone, however, considerable uncertainty remains over the exposure levels at which these toxic effects may start to occur and the clinical relevance of the early effects observed following long-term, low-level cadmium exposure.

The aims of this thesis were: 1) To assess cadmium body burden and early signs of kidney dysfunction in a population exposed to industrial cadmium emissions and to develop and validate an air dispersion model of these emissions. 2) To identify urinary metabolites, associated with cadmium exposure, using metabolic profiling techniques. 3) To prospectively assess the association between validated estimates of dietary cadmium exposure and chronic kidney disease (CKD) incidence, kidney stone incidence, and fracture incidence, in two large population-based cohorts of men and women.

In a population-based sample of 180 subjects, living close to a zinc smelter in Avonmouth, Southwest England, urinary cadmium concentrations (median = 0.22 nmol Cd/mmol creatinine) were in the same range as those where associations with kidney and/or bone effects have been observed previously. Three percent had concentrations above 1nmol Cd/mmol creatinine (~1µg/g) – the point of departure for tubular proteinuria set by the European Food Safety Authority in 2009. Modelled air cadmium concentrations from the smelter were strongly correlated with those from air monitoring sites (R^2=0.84) and were a significant predictor of urinary cadmium (p=0.04). In a cross-sectional analysis, a significant dose-response relationship between urinary cadmium and one of the biomarkers of early tubular dysfunction (N-acetyl-β-d-glucosaminidase) was observed. Metabolic profiling identified six urinary metabolites, either related to mitochondrial metabolism or one carbon metabolism, associated with urinary cadmium.

Two large population-based cohorts of men and women from Central Sweden were used to investigate the association between dietary cadmium exposure and incidence of CKD, kidney stones and fractures. Median dietary cadmium exposure levels in our study populations were 19µg/day in men and 13µg/day women. During an average of 12 years of follow-up, we ascertained 599 incident cases of CKD among men (481,591 person-years) and 253 among women (415,432 person-years). We did not observe an association between dietary Cd and rate of CKD in men, hazard ratio (HR) 0.97 (95% CI 0.77-1.21) or women HR 0.74 (95% CI 0.53-1.04), either before or after adjustment for potential confounders. During an average of 13 years of follow-up, we ascertained

707 incident cases of kidney stone among men (421,611 person-years) and 290 among women (403,575 person-years). Likewise, we did not observe an association between dietary Cd and rate of kidney stones in men HR 0.97 (95% CI 0.77-1.23) or women HR 0.99 (95%CI 0.89-1.43), either before or after adjustment for potential confounders.

We ascertained 2,183 cases of any fracture and 374 cases of hip fracture, during a 10-year follow-up of 20,173 Swedish men. In the multivariable adjusted model, dietary cadmium was associated with a statistically significant 19% (HR: 1.19, 95%CI: 1.06-1.34) higher rate of any fracture, comparing highest tertile with lowest. Hip fracture rates were also higher in the highest tertile of cadmium exposure but only statistically significant among never smokers, with a 70% (HR: 1.70, 95%CI: 1.04-2.77) higher rate. This study provides the first data on hip fracture rates in relation to cadmium exposure and is the first to report an excess risk of any fracture associated with long-term low-level cadmium exposure in men.

The results of this thesis suggest that the adverse effects of cadmium exposure around the Avonmouth smelter may be detected in urinary biomarkers. In addition, the results of the prospective studies do not support a role of dietary cadmium exposure, at the level seen in the general population, in the development of CKD or kidney stones. However, the results do provide further evidence of increase fracture risk in relation to cadmium exposure. In conjunction with recent findings, the results of this thesis suggest that bone may be a more sensitive target of cadmium toxicity than the kidney, in terms of clinically relevant outcomes.

LIST OF PUBLICATIONS

I. **Thomas LDK**, Hodgson S, Nieuwenhuijsen M, and Jarup L. Early Kidney Damage in a Population Exposed to Cadmium and Other Heavy Metals. *Environ Health Perspect*. (2009) 117:181–184

II. Ellis JK, Athersuch TJ, **Thomas LDK**, Teichert F, Perez-Trujillo M, Svendsen C, Spurgeon DJ, Singh R, Jarup L, Bundy JG, Keun HC. Metabolic profiling detects early effects of environmental and lifestyle exposure to cadmium in a human population. *BMC Med*. (2012);10(1):61.

III. **Thomas LDK**, Elinder C-G, Prütz KG, Wolk A, Åkesson A. Dietary cadmium exposure and chronic kidney disease: A population-based prospective cohort study of men & women. SUBMITTED FOR PUBLICATION

IV. **Thomas LDK,** Elinder CG, Tiselius HG, Wolk A, Akesson A. Dietary cadmium exposure and kidney stone incidence: A population-based prospective cohort study of men & women. *Environ Int*. Jul 1 2013;59C:148-151.

V. **Thomas LDK**, Michaëlsson K, Julin B, Wolk A, Åkesson A. Dietary cadmium exposure and fracture incidence among men: A population-based prospective cohort study. *J Bone Min Res*. (2011) 26, 1601-8.

RELATED PUBLICATIONS

Julin B, Wolk A, **Thomas LDK**, Akesson A. Exposure to cadmium from food and risk of cardiovascular disease in men: a population-based prospective cohort study. *Eur J Epidemiol.* Aug 24 2013.

Thomas LDK, Elinder CG, Tiselius HG, Wolk A, Akesson A. Ascorbic Acid supplements and kidney stone incidence among men: a prospective study. *JAMA Intern Med*. Mar 11 2013;173(5):386-388.

Tiselius HG, Akesson A, **Thomas LDK**. Ascorbic Acid supplements and kidney stone risk-reply. *JAMA Intern Med*. Jul 22 2013;173(14):1384a-1384.

Spurgeon DJ, Lawlor A, Hooper HL, Wadsworth R, Svendsen C, **Thomas LDK**, Ellis JK, Bundy JG, Keun HC, Jarup L. Outdoor and indoor cadmium distributions near an abandoned smelting works and their relations to human exposure. *Environ Pollut* 2011;159(12):3425-32.

Hodgson S, **Thomas LDK**, Fattore E, Lind PM, Alfven T, Hellström L, Håkansson H, Carubelli G, Fanelli R, Jarup L. Bone mineral density changes in relation to environmental PCB exposure. *Environ Health Perspect*. 2008 Sep;116(9):1162-6.

CONTENTS

LIST OF ABBREVIATIONS

ADMS	Atmospheric Dispersion Modelling System
BMD	Bone mineral density
BMI	Body mass index
Cd	Cadmium
CI	Confidence interval
CKD	Chronic kidney disease
COSM	Cohort of Swedish Men
CVD	Cardiovascular disease
D-Cd	Dietary cadmium exposure
DEXA	Dual-energy x-ray absorptiometry
DTM1	Divalent Metal Transporter 1
FFQ	Food frequency questionnaire
GFR	Glomerular filtration rate
GIS	Geographical Information Systems
HR	Hazard ratio
ICP-MS	Inductively coupled plasma mass spectrometry
LMWP	Low-molecular weight protein
MT	Metallothionein
NMR	Nuclear magnetic resonance
OR	Odds ratio
ROS	Reactive oxygen species
QALYs	Quality adjusted life years
SMC	Swedish Mammography Cohort
U-A1M	Urinary α-1-microglobulin
U-Cd	Urinary cadmium
U-NAG	Urinary N-acetyl-β-$_D$-glucosaminidase
U-RBP	Urinary retinol-binding protein

1 INTRODUCTION

This thesis focuses on kidney effects and bone fractures in relation to long-term low-level exposure to cadmium. The studies covered in this thesis use a range of study designs, assess exposure using both dietary and urinary cadmium and investigate both biomarker and clinical outcomes.

The diet represents the main source of cadmium exposure in the general non-smoking population however in the vicinity of polluting industries, contact with contaminated environmental media, such as air and house dust, may be an additional source of exposure. There is a need in such areas to assess and characterize exposure and health effects in the local population. Considerable information regarding exposure and toxic effects within the body can be gleaned from urinary composition. While urinary biomarkers are already in use, their full potential remains to be explored. New metabolic profiling techniques offer a useful tool for identifying novel biomarkers but so far their use has been largely confined to experimental animal studies.

Exposure to cadmium in the diet takes place throughout life and, therefore, even in areas with no particular cadmium contamination and where concentrations in food are generally low, exposure levels may be sufficient to be of public health concern. The kidneys and bones have long been recognized as targets of cadmium toxicity however the impact of long-term low-level cadmium exposure on these structures remains to be clarified.

2 BACKGROUND

2.1 CADMIUM

Cadmium (Cd) is a toxic metal with no beneficial function in the human body. While humans have always been exposed to cadmium in the environment, human activity has brought us into greater contact with the metal. Since its discovery in 1817, cadmium has been used in a wide range of applications. These uses include in metal alloys and corrosion resistant plaiting, red and yellow pigments, stabilizers for plastics and in nickel-cadmium batteries.[1]

2.1.1 Cadmium in the environment

Cadmium is ubiquitous, occurring in the environment as a result of both natural and anthropogenic processes. Natural soils typically contain between 0.1-0.4 mg/kg, however, background concentrations may be elevated in areas underlain by sedimentary rocks such as black shales and phosphate deposits, or bodies of zinc and lead ore.[1] It is estimated that the median cadmium concentration in European topsoils is 0.14 mg/kg.[2]

Industrial emissions, mining wastes, and the application of phosphate fertilizers and sewage sludge to agricultural land, have resulted in increased cadmium concentrations in soils.[1] In the UK, the main sources of industrial emissions are metal production, electricity generation, manufacturing and other forms of industrial combustion.[3] Since 1970, emissions of cadmium to air in the UK have declined by 92%.[3] This fall can in part be accounted for by the closure of a large metal processing facility in Avonmouth, Southwest England.[3] Mine tailings and effluent may cause significant contamination of surface soils in areas where metal extraction has occurred.[1] Indeed, some of the highest soil cadmium concentrations ever recorded were found in the village of Shipham, Somerset where zinc mining took place between the 17th and 19th century.[1]

Non-ferrous metal processing, such as lead and zinc smelting, is a major source of metal emissions. A review of European inventory data for 2003 identified the sector as the third largest in terms of cadmium releases.[4] Restricted to a few large-scale operations, emissions from this sector are often responsible for some of the highest concentrations of metals in the environment. Previous studies have shown that these elevated concentrations may result in additional human exposure to metals.[5] However, little is known about the public health implications for populations living in the vicinity of such operations.

Over the last century, phosphate fertilisers and sewage sludge have been applied to agricultural land to improve crop yield. This practice has inadvertently contributed to elevated cadmium concentrations in agricultural soils. Cadmium is present as a trace element in the phosphate deposits used in mineral fertilizer production. The cadmium

concentrations in raw phosphate show considerable variation, and this difference is reflected in the final product. Apatite from the Kola Peninsula, for example, has a very low cadmium concentration (0.3 mg Cd/kg P_2O_5), while the concentrations in crude phosphate from Taiba in Senegal are over a thousand times higher (200 mg Cd / kg P_2O_5).[6] Methods for reducing the cadmium content of fertilizers increase the cost of production and, therefore, the use of these products tends to be restricted to areas where regulatory controls require the use of low cadmium fertilizers. Levels of cadmium in Swedish arable soil are estimated to have increased by around 33% in the past 100 years[7] but are now thought to have stabilised.

The rate of uptake of cadmium from soil is dependent on the soil pH, organic content, salinity and concentration of other elements as well as the plant species and cultivar. The bioavailability of cadmium is strongly influenced by soil pH, with plant uptake of cadmium decreasing as soil pH increases. Cadmium concentrations in Swedish arable soils are on the whole lower than in other European countries. This is not, however, reflected in the cadmium levels in Swedish-grown crops, which tend to have similar concentrations to those from elsewhere in Europe. This is due to the fact that soil pH in Sweden is approximately one unit lower than in Central Europe with approximately 30% of soils having a pH of 6.0 or less.[8] In contrast, Shipham in Southwest England has some of the highest soil cadmium concentrations ever recorded yet the bioavailability of cadmium in the soil has been found to be relatively low as a consequence of the high soil pH.[1]

2.1.2 Exposure sources

Exposure to cadmium from food

Food is the primary source of cadmium exposure in the general non-smoking population.[9] All foods contain cadmium irrespective of whether they are produced by organic or conventional methods. Cadmium concentrations in food are routinely monitored by organisations such as the National Food Agency in Sweden (Livsmedelsverket) and the Food Standards Agency in the UK. Data from these surveys indicate that the highest concentrations are found in foods such as offal, shellfish and certain seeds. These food categories tend to form only a small proportion of the diet and, as a consequence, foods such as grains and vegetables represent the bulk of dietary cadmium exposure.[9] Vegetarians have higher dietary exposure due to their high consumption of cereals, nuts, oilseeds and pulses.[9] Exposure levels are also elevated in those regularly consuming bivalves and wild mushrooms.[9] The rate of cadmium uptake by food crops is dependant both on plant type and cadmium bioavailability. Cadmium is present in food in its inorganic form either as salts or attached to proteins such as metallothionein (MT). Exposure to cadmium in the diet takes place over a lifetime and therefore even in areas with no particular cadmium contamination and where levels in food are generally low, exposure levels may be sufficient to be of public health concern.

Exposure to cadmium from tobacco smoke

Tobacco is an important source of cadmium exposure among smokers. The cadmium content of tobacco leaves is relatively high with each cigarette containing around 1-2 μg of cadmium, depending on the origin of the tobacco.[1] It is estimated that approximately 10% of this cadmium is inhaled and between 10-50% of inhaled cadmium is absorbed.[10,11] As a consequence cadmium concentrations in the blood, urine and kidney cortex[10,12] are substantially higher is smokers compared to never smokers.

Other sources of cadmium exposure

The cadmium concentrations in air, drinking water and dust are generally low and do not represent important sources of exposure. However, around industrial sites and in the occupationally exposed, exposure from these sources may be elevated. There is some evidence that in areas with contaminated soils, ingestion of house dust is potentially an important route of exposure to cadmium.[5] Among the occupationally exposed, the inhalation of contaminated air and the ingestion of contaminated dust may be an important source of exposure.[1]

2.1.3 Uptake and distribution

Absorption of cadmium from the gastrointestinal tract

Gastrointestinal absorption of cadmium in adults is around 5%.[13] Absorption takes place mainly in the small intestine through active uptake by the divalent-metal transporter (DTM1).[14] Cadmium is absorbed mainly in its ionic form, however, some absorption of cadmium bound to organic ligands such as glutathione and metallothionein may also take place.[15] MT is a high-affinity metal-binding protein involved in both essential element homeostasis and the detoxification of toxic metals. DMT1 is primarily responsible for the uptake of iron but has a high affinity for Cd^{2+}.[14,16] In those with low iron status, such as is commonly seen in women of fertile age, DMT1 is up-regulated leading to increased cadmium absorption from the digestive tract.[16] Epidemiological studies have shown increasing blood cadmium with decreasing serum ferritin in women of fertile age.[17,18] It is because of this effect on DMT-1, that women generally have a higher cadmium body-burden than men.[19] The presence of other divalent or trivalent cations in the diet may compete for absorption with cadmium, and as a consequence, dietary levels of trace elements and minerals, including calcium, manganese and zinc may affect the rate of cadmium absorption.[17,18,20,21] Dietary factors, such as fibre content, may also influence the rate of cadmium absorption from the gastrointestinal tract.[22-24]

Absorption of cadmium from the lungs

Absorption of cadmium from the lungs is an important source of exposure among smokers and the occupationally exposed. Uptake of cadmium from the lungs is estimated at between 10-50%.[10,11] A number of factors determine the absorption of inhaled cadmium these include, particle size, deposition, mucociliary and alveolar

clearance, chemical species and solubility. Data on the respiratory absorption of cadmium in humans comes mainly from studies comparing smokers and non-smokers. Based on organ cadmium burden, Elinder et al. calculated that approximately 50% of cadmium inhaled via cigarette smoke is absorbed.[10]

Distribution and excretion of cadmium

Once absorbed, cadmium is transported in the bloodstream to the liver. Initially cadmium in blood is mainly found bound to albumin in the plasma, however, over time concentrations in red blood cells increase. On reaching the liver the cadmium-albumin complex is taken-up and degraded, and the free cadmium then induces and binds to MT. This low-molecular weight, cysteine-rich protein plays an important role in detoxification. Cadmium concentrations in the liver then decline as cadmium is transported to other tissues most notably the kidney. The Cd-MT-complex is small in size and, along with similar low-molecular weight proteins, can be filtered across the glomerular membrane and into the primary urine. It is then reabsorbed by kidney tubular cells, is released from the MT-complex and accumulates in the kidney cortex. The half-life of cadmium in the human kidney is estimated at 10-30 years.[25,26] Cadmium is excreted primarily in urine.

2.1.4 Biomarkers of exposure

Blood

Blood cadmium is regarded primarily as a biomarker of recent exposure; however, long-term exposure may also contribute to blood cadmium concentrations. Based on concentrations in an occupationally exposed population, Järup et al proposed a two compartment model, the first compartment reflecting recent exposure with a half-life of approximately 100 days and the second reflecting long-term exposure with a half-life of 7-16 years.[25] Cadmium in blood is mainly bound to erythrocytes and is typically measured by inductively coupled plasma mass spectrometry (ICP-MS) either in whole blood or in erythrocytes only.

Urine

Urinary cadmium is regarded as a good marker of long-term exposure to cadmium and has been shown to reflect concentrations in the kidneys.[27,28] Urinary dilution varies greatly both between and within individuals and it is essential that this dilution is taken into account when assessing the amount of cadmium being excreted in urine. "If uncorrected for degree of dilution, concentrations of any two otherwise unrelated solutes of urine will inevitably be positively correlated".[29] Factors affecting urine dilution include fluid intake, physical activity and temperature. There are several methods available to allow for the effect of dilution.

Urine collected over a 24-hour period provides the best method for assessing urinary cadmium excretion, as the total amount can be calculated based on the concentration and urinary volume. This method also avoids any effect of diurnal variation in urinary

cadmium excretion. However, the collection of all urine passed over a 24-hour period requires considerable commitment from participants and samples may therefore be incomplete.[30] As a consequence, it is more common to collect spot-urine samples and adjust for dilution based on one of two methods: urinary creatinine, specific gravity.[31]

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body with around 1-2% of muscle creatine being converted to creatinine each day. This fairly constant rate of creatinine excretion in human urine is the basis for creatinine adjustment of spot urine measurements however this method does have several limitations. Creatinine excretion reflects skeletal muscle mass[32] and this is on average higher in men than in women and typically declines with age.[33] It also reflects dietary intake of meat rich in creatine and creatinine. As a consequence creatinine adjustment tends to overestimate urinary cadmium concentrations in women compared to men and in the young compared to the old.[31] Specific gravity is simply a density measurement. Like creatinine adjustment it has several limitations for example it may be elevated by glucosuria or proteinuria.[31]

In non-smoking individuals with no significant occupational or environmental exposure, urinary cadmium concentrations are usually in the range 0.02-0.7μg Cd/g creatinine.[34] Levels typically increase with age up to 50-60 years before decreasing. Urinary cadmium levels in smokers are typically 1.5-2 times higher than those in non-smokers.[9]

2.1.5 Health effects

The health effects of cadmium exposure were first reported in the 1850s among occupationally exposed populations. Only with the emergence of Itai-Itai disease were the risks to environmentally exposed populations recognised. This most serious outbreak of cadmium poisoning occurred in Toyama Prefecture, Japan, at the start of the 20th century. Those with the condition experienced severe pain as a result of the multiple fractures that occurred within their bodies, and because of this, the disease was dubbed ‘Itai-Itai’, meaning ‘ouch-ouch’ in Japanese. Those with Itai-Itai disease were found to have a combination of osteoporosis and osteomalacia and showed multiple fractures of the long bone as well as compression fractures of the spine. Milkman’s pseudo fractures, which are characteristic of osteomalacia, were identified by x-ray and biochemical findings indicative of osteomalacia were reported, including increased serum levels of alkaline phosphatase and decreases in calcium and phosphate. Reduced bone mineral density characteristic of osteoporosis, and impaired renal function was also found in those with the condition.

The cause of Itai-Itai disease was not recognised until the 1950s[35] when river water contaminated with mining effluent was identified as the source. Water from the Jinzu River was used for washing, cooking, drinking and the irrigation of rice paddies, leading to high cadmium exposure in the local population. Post-menopausal women

appear to have been particularly vulnerable to the effects of cadmium exposure and it is thought that low vitamin D status may also have played a role in disease development. More recently, studies have shown toxic effects at much lower levels of exposure. The health effects of long-term, low-level cadmium exposure are thought to include adverse effects on the kidneys (see section 2.4) and bones (see section 2.6) as well as cancer.[36] Data from animal studies have indicated that cadmium may play a part in the aetiology of diabetes and cardiovascular disease, however, the association in humans has so far been inconclusive.[37-40]

Cadmium and cancer

The International Agency for Research on Cancer (IARC) classified cadmium as a known human carcinogen (group I) on the basis of sufficient evidence for carcinogenicity in both humans and experimental animals.[41] More recently, epidemiological studies have reported statistically significant increased risk of cancers of the bladder,[42] lung,[43,44] prostate,[45] endometrium[46] and breast[47,48] in relation to long-term low-level exposure to cadmium. Further evidence, at low-levels of exposure, is needed in order to confirm these findings.

2.2 HEALTH RISK ASSESSMENT

The European Food Safety Authority (EFSA) carried out a risk assessment for cadmium in 2009 in which the detrimental effects of cadmium on the kidney were considered the critical effect.[9] Based on a meta-analysis of urinary cadmium (µg/g creatinine) in relation to beta-2-microglobulin (B2M) excretion (see section 2.4.2), the report recommended that the cadmium burden on the kidney should not exceed cadmium levels in urine of 1µg/g creatinine (point of departure). A tolerable weekly intake (TWI) was then derived by translating urinary cadmium to long-term dietary cadmium exposure using population-based toxicokinetic modelling;[26] a sub-cohort of the SMC was used to establish this link.[49] Based on this, the TWI was lowered from 7µg Cd/kg body weight to 2.5µg Cd/kg body weight.

In contrast, the risk assessment carried out by the WHO/FAO Joint Expert Committee on Food Additives and Contaminants (JECFA) in 2011, concluded that urinary cadmium excretion of less than 5.24µg Cd/g creatinine (5th–95th percentiles 4.94–5.57) was not associated with an increased excretion of B2M.[50] Based on this, they derived a provisional tolerable monthly intake (PTMI) using a one-compartment toxicokinetic model to determine a corresponding dietary exposure. Using the lower bound of the 5th population percentile of dietary cadmium exposure, this gave a PTMI of 25µg/kg bodyweight; this is equivalent to a TWI of 6.25µg/kg bodyweight. The main difference between the two assessments was that EFSA took inter-individual variation of urinary cadmium, within each dose-group, into account whereas JECFA did not.

2.3 KIDNEY

The kidneys serve several essential functions within the human body. These include the filtration and excretion of toxic substances, regulation of acid-base balance, maintenance of electrolyte (primarily sodium and potassium) concentrations, extracellular fluid volume and the production of hormones such as calcitriol and erythropoietin. Blood is filtered through the kidneys continuously in order to remove toxins, including both the endogenous waste products of metabolism such as urea and creatinine, and exogenous substances such as cadmium. The kidneys' role in the removal of waste from the body, make these organs particularly vulnerable to the effects of toxic substances.

Healthy kidneys contain roughly one million functioning units called nephrons, each of which is made up of a glomerulus and a renal tubule (See **figure 1**). The glomerulus is composed of a fine network of capillaries that are clustered together in a cup-like structure known as the Bowman's capsule. The structure of the glomerulus allows waste products and some water and salt to pass from the blood into the Bowman's capsule. The glomerular filtrate or primary urine then passes down a series of specialized tubules where the reabsorption and secretion of various solutes takes place. The liquid remaining in the tubules at the end of this process is excreted as urine. Damage to either the glomerulus or renal tubule may result in kidney dysfunction.

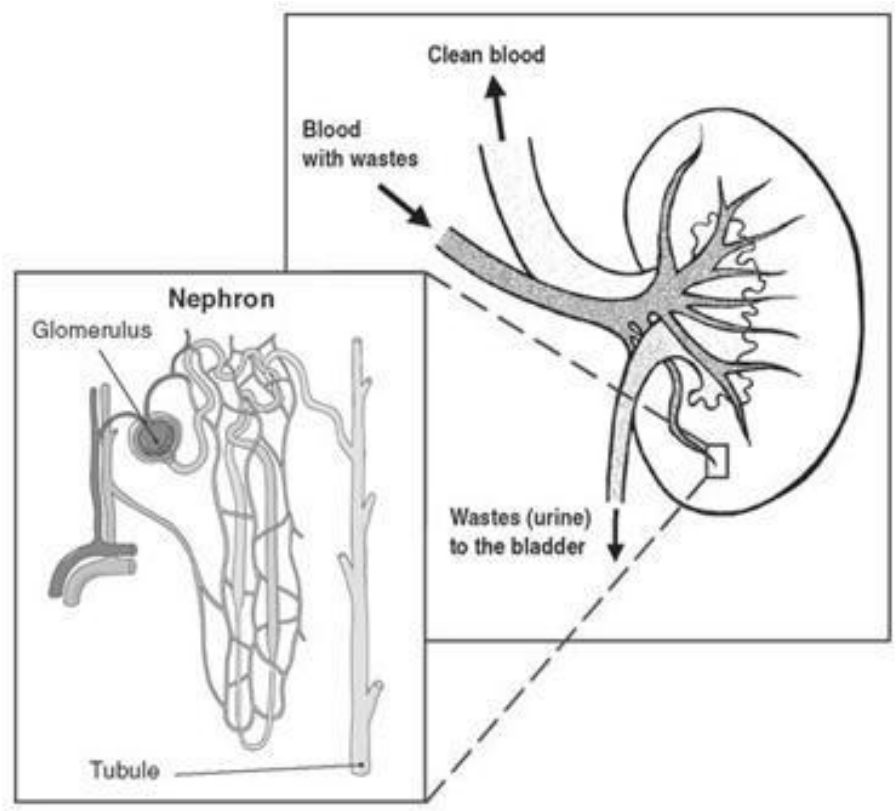

Figure 1. Physiology of the human kidney.

2.3.1 The glomerulus

The kidneys are supplied with blood by the renal arteries, left and right, and receive approximately 20% of cardiac output. On entering the kidney the arteries branch into successively smaller blood vessels before forming the capillaries of the glomerulus. High pressure within the capillaries facilitates ultrafiltration of the blood with fluids and solutes being forced out of the capillaries and into the Bowman's capsule. The filtrate must pass through the glomerular capillary endothelial cells, glomerular basement membrane, and podocytes in order to enter the lumen of the Bowman's capsule. Pores within the endothelium are between 70 -100nm in diameter and allow the free filtration of fluids, solutes and proteins. Podocytes line the Bowman's capsule and control the filtration of larger proteins such as serum albumin. The molecular size of albumin is above the threshold size of the pores in the basement barrier and elevated albuminuria, therefore, indicates damage of the integrity of the glomerular filtration barrier.

The glomerular filtration rate (GFR) is the rate at which blood is filtered through the glomeruli and is a measure of overall renal function. Prospective studies have shown that a decrease in the GFR translates into increased risk of renal failure but also cardiovascular disease and overall mortality.[51] The glomerular filtration can be directly measured using the infusion of water-soluble small molecules, such as inulin, that are freely filtered through the glomerulus. GFR is usually standardized for body surface area and expressed in mL/min/1.73 m^2. In healthy, young people the mean GFR is usually in the range 105-120 mL/min/1.73 m^2. After peaking at around 20-30 years of age, GFR then declines by 0.5-1 mL per year, especially after the age of 50.[52-54] Calculation of the actual glomerular filtration rate by measurement of an external filtration marker is cumbersome and impractical. As a consequence, GFR is often estimated using endogenous substances such as creatinine. Creatinine is freely filtered through the glomerulus and elevated concentrations of creatinine in the plasma therefore reflect impaired GFR. Measurement of plasma creatinine provides a crude assessment of renal function and is the most common method for estimating GFR. There are, however, methodological problems associated with the measurement of plasma creatinine, the most important of these being that creatinine is not only filtered but also actively secreted into the urine by the proximal tubuli. As a consequence, the use of endogenous creatinine clearance will overestimate the GFR, particularly at relatively low GFR. Also, creatinine production is higher in those with higher muscle mass. The effect of muscle mass can, to some extent, be taken into account using formulas which include variables such as age, gender, ethnicity and weight. Of these the MDRD and CKD-EPI[55] formulas are considered the most accurate. Cystatin C is another endogenous marker that can be used to estimate GFR and this marker has the advantage that it is not influenced by muscle mass to the same extent.[56]

2.3.2 Chronic kidney disease

CKD is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or a combination of the two, and is an increasing public health issue. It is characterized by a gradual decline in renal function and is divided into 5 stages according to the severity of the disease. The diagnosis of CKD is based primarily on GFR or eGFR with other diagnostic factors include blood or protein in the urine. In mild to moderate cases (stages 1-3), sufferers are unlikely to experience any symptoms, however, in more advanced cases (stages 4-5) symptoms may include fatigue, weight loss, fluid retention, anaemia and nausea. Complications include increased all cause and cardiovascular mortality, cognitive decline, mineral and bone disorders, and fractures. In mild to moderate cases treatment focuses on slowing the progression of disease, treating underlying causes and reducing the risk of developing cardiovascular disease. However, those with end-stage kidney failure (stage 5 CKD) will require dialysis or kidney transplant. Screening and intervention can prevent the progression of the disease, and where management strategies have been implemented the incidence of end-stage kidney disease has been reduced.[57]

Chronic kidney disease prevalence and trends

The worldwide prevalence of CKD is estimated at between 8–16% and is set to increase.[57] The treatment costs associated with CKD are very high particularly in the later stages where renal replacement therapy is required. It is estimated that between 2–3% of the health-care expenditure in developed countries is used to provide treatment for patients with end-stage kidney disease even though they account for only 0.1–0.2% of the total population.[57] The UK National Health Service estimated that, for the year 2009-10, the cost of treating CKD amounted to £1.44–1.45 billion.[58]

Chronic kidney disease risk factors

Globally, the three main causes of CKD are diabetes, hypertension and ageing. Less common causes include: infection; renal artery stenosis; blockages to the flow of urine, such as may be caused by kidney stones; polycystic kidney disease; haemolytic uremic syndrome; drug- and toxin-induced kidney damage.

Toxins present in the diet or environment may play a role in the development of CKD, for example, Balkan endemic nephropathy is associated with chronic dietary exposure to aristolochic acid.[59] This nephrotoxin, present in certain plants, can enter the food chain through contaminated crops; it is also present in some of the plants used in herbal medicines. High rates of renal failure have also been identified in Mesoamerica and Sri Lanka, however, the cause of this renal damage has not yet been confirmed. Recent findings from effected areas of Mesoamerica suggest that dehydration and the use of non-steroidal anti-inflammatory drugs (NSAIDS) is a likely cause.[60] While diabetes and hypertension are the major causes, nephrotoxicants may also contribute to disease progression and there is therefore a clear need for these potentially modifiable risk factors to be identified.

2.3.3 The renal tubule

The kidney tubule can be divided into three main sections: the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule. Each section has a highly specialized role. The proximal convoluted tubule reabsorbs approximately two thirds of the water and salt and all organic solutes such as glucose, amino acids and low-molecular weight proteins such a B2M. The filtrate then passes to the Loop of Henle where the salt content of the filtrate is further reduced. The final section is the distal convoluted tubule. Here the active transport of ions takes place, regulated by the endocrine system. For example, parathyroid hormone signals the distal convoluted tubule to absorb more calcium and excrete more phosphate. The filtrate remaining at the end of this process is collected in a series of ducts before passing out of the kidney as urine.

2.3.4 Tubular dysfunction

In its most severe form, the tubular dysfunction associated with cadmium exposure displays major features of acquired Fanconi syndrome, the causes of which include exposure to medication and ingested toxins.[61] Tubular dysfunction, such as that associated with cadmium exposure, rarely gives rise to symptoms or clinical disease making its prevalence within the population difficult to determine.

2.3.5 Kidney stones

Kidney stones (renal calculi or nephrolithiasis) are hard crystalline concretions that form in the kidneys. Composed of calcium oxalate, calcium phosphate, uric acid or cystine the stones may vary in size from a few crystals to several centimetres across. Small stones may pass out of the kidney via the ureter without causing any problems, however, stones of sufficient size can become lodged in the ureter resulting in the severe pain of renal colic. The pain is almost always caused by a dilation of the system above the stone - renal pelvis, calyces and ureter – as a consequence of the obstruction to urinary flow. The symptoms of renal colic are highly characteristic and seldom pose a diagnostic dilemma.[62] The diagnosis is usually confirmed using imaging techniques such a CT (computed tomography) or ultrasound scans. In Sweden, the vast majority of stones are diagnosed in hospital (Personal communication H-G Tiselius). Most stones do not require treatment and will pass on their own within a few days, although patients are likely to require analgesics. Less commonly, stones will be broken up using techniques such extracorporeal shockwave lithotripsy or removed by surgery.

Stones composed of calcium salts are by far the most common and are formed when urine becomes supersaturated. Supersaturation is expressed as the ratio of urinary calcium oxalate or calcium phosphate concentration to its solubility.[63] At levels above 1, stones can nucleate and grow, promoting stone formation. Calcium oxalate grown takes place over epithelial deposits of calcium phosphate or Randall's plaques.[64] Calcareous stones are therefore often composed of a combination of calcium oxalate and calcium phosphate. The analysis of kidney stone material collected from 3,176 men, treated with extracorporeal shockwave lithotripsy in Stockholm County, found

calcium oxalate to be the dominant component in 92.6% (H.-G. Tiselius: unpublished data).

Kidney stone risk factors

The causes of kidney stones are not fully understood, however, it is clear that urinary composition is the main driving factor. Such abnormal urinary composition may be either metabolic or environmental in origin.[65] Elevated urinary levels of calcium, oxalate and uric acid and low levels of citrate are known to be important in stone formation. Hypercalciuria is the most important pathophysiological factor[62] and contributes to calcium-stone formation both by increasing urinary saturation of calcium salts and by inactivating negatively charged urinary inhibitors.[65] Hypercalciuria may be caused by increased intestinal calcium absorption or less commonly by hyperparathyroidism.

The composition of urine is influenced by the diet and several dietary factors have been proposed to modify the risk of kidney stones. Nutrients such as sodium, sucrose, animal protein and oxalate are thought to increase stone risk while magnesium and potassium are thought to be protective. Fluid intake is one of the most important modifiable factors governing stone formation since urinary concentration, rather than the overall amount of crystallizing solutes, is what ultimately governs stone formation. Other factors associated with urinary stones include high body mass index (BMI), vitamin C supplement use[66] and diabetes.

Kidney stone incidence and trends

Kidney stones are a very common and highly recurrent disorder. The lifetime risk of stone formation in the US exceeds 12% in men[67] and 6% in women[68] with a yearly incidence in North America and Europe of around 0.1-0.4%.[65] The occurrence of kidney stones shows age, sex and race differences as well as geographic variation.[69] The reason for the sex difference is not clear but may in part be related to oestrogen levels. Incidence is highest among white males and lowest among black females. Among men, the incidence peaks between 40 and 60 years of age, while among women the incidence is highest in the late 20's.[69]

2.4 CADMIUM AND KIDNEY DYSFUNCTION

The kidney is the main site of cadmium accumulation within the human body, and has been recognized as a critical target organ of cadmium toxicity for over 60 years. It is estimated that around 50% of the body-burden is found in the kidneys.[50] Autopsy data suggests that long-term oral exposure to cadmium may lead to a variety of progressive histopathological changes, including glomerular basal cell damage, interstitial fibrosis and epithelial cell damage of proximal tubules with limited tubular cell regeneration. Tubular dysfunction, characterized by an increased excretion of low-molecular weight proteins and intracellular tubular enzymes, is widely regarded as the first sign of an

adverse effect.[36,70] At high levels of exposure there is evidence of irreversible renal toxicity[71-76] and increased mortality.[73-75] The renal effects of cadmium at lower exposures are more uncertain; in particular it is unclear whether long-term, low-level exposure to cadmium contributes to the development of clinically relevant outcomes such as CKD.

2.4.1 Cadmium and glomerular dysfunction

As well as effects on the kidney tubule, cadmium may also reduce the glomerular filtration rate. In the Itai-Itai endemic areas of Japan, where cadmium exposure levels are some of the highest in the World, an association between urinary cadmium and increased mortality from renal disease has been reported.[73-75] Other studies conducted in this area have suggested that the proteinuria observed in these populations may progress to decreased glomerular filtration rate (GFR).[71,72,76] A previous ecological study carried out in a cadmium polluted area of Sweden, reported an increased risk of end-stage renal disease (start of renal replacement therapy) in relation to proximity to cadmium emitting industries (Mantel-Haenszel rate ratio 1.8, 95%CI: 1.3 - 2.3), however ecological bias cannot be ruled out as a potential explanation for this apparent association.[77]

Potential glomerular effects of long-term low-level cadmium exposure have also been investigated.[78-82] Estimated glomerular filtration rate (eGFR) in individuals with normal kidney function are, however, not sufficiently accurate to reveal early, or relatively small effects in the GFR.[83] In addition, biomarkers of dose and effect are likely to be similarly affected by GFR and confounding by a non-cadmium dependent effect of smoking cannot be ruled out. As a result, the findings of these studies are difficult to interpret. A population-based, nested case-referent study, prospectively explored the association between erythrocyte cadmium and end-stage renal disease and observed no statistically significant association based on 118 cases.[84]

2.4.2 Cadmium and tubular dysfunction

In order to exert its toxic effect upon the proximal tubule, cadmium must enter the cells and be present in the cytoplasm as a free inorganic ion (Cd^{2+}). Cadmium in blood plasma is bound to MT. Along with other low-molecular weight proteins, MT can be efficiently cleared from blood plasma by glomerular filtration before being reabsorbed from the primary urine by cells of the proximal tubule. In vivo, the primary pathway for cadmium uptake, by proximal tubular cells, is apical endocytosis of cadmium bound to metallothionein. This transport of cadmium to cells of the kidney tubule has been demonstrated in animal experiments. Only once the MT-Cd-complex has been degraded in endo-lysosomes is Cd^{2+} free to move into the cytosolic compartment, this translocation may occur via the divalent metal transporter (DMT1). Cd^{2+} ions within the cytosol interfere with various cellular processes by generating reactive oxygen species (ROS). These ROS deplete endogenous radical scavengers and damage a variety of transport proteins including the Na(+)/K(+)-ATPase. Cd^{2+} ions also cause mitochondrial swelling and release of cytochrome C. These changes may cause

affected cells to undergo apoptosis. The S1 segment of the kidney proximal tubule is a major target of chronic Cd^{2+} toxicity. In its most severe form, the tubular dysfunction associated with cadmium exposure displays major features of acquired Fanconi syndrome including the impaired reabsorption of proteins, amino acids, glucose, bicarbonate and phosphate.[61]

Biomarkers of tubular dysfunction

Urinary biomarkers provide useful information on the toxic effects taking place within the kidney. Damage to kidney tubular cells leads to decreased tubular reabsorption of low-molecular weight proteins and increased cell death, these changes can be detected in urine as an increased excretion of proteins and enzymes. A brief description of the four main biomarkers, used to assess cadmium induced tubular damage, is given below. It is important to note that these biomarkers are not specific to any particular exposure and indicate potential health impairment rather than any overt illness.

- **Urinary beta-2-microglobulin (U-B2M)**
  This low-molecular weight protein has been widely used. As the protein is unstable in acidic urine, it is necessary to add a buffer to the urine samples to prevent its degradation.
- **Urinary retinol binding protein (U-RBP)**
  This LMWP is more stable than B2M at the physiological pH of urine, however, it is also slightly less sensitive to tubular dysfunction.
- **Urinary alpha-1-microglobulin (U-A1M)**
  Also known as protein HC, this LMWP is less sensitive and less specific to tubular dysfunction than B2M.
- **Urinary N-acetyl-β-$_D$-glucosaminidase (U-NAG)**
  NAG is a lysosomal enzyme present in high concentrations in the proximal tubule. Increased NAG activity may occur as a consequence of effects other than renal damage. While total NAG is sometimes used, only the activity of the membrane-bound isozyme NAG-b is an indicator of cell shedding. The use of total NAG relies on the assumption that there is a correlation between NAG-b and total NAG. Alternative explanations for elevated NAG excretion may be exocytosis and interference with enzyme activity by inhibitors.

Epidemiology

Tubular proteinuria was first reported in the 1950s in populations occupationally exposed to cadmium.[85,86] Subsequent studies identified the same type of tubular dysfunction in populations exposed to high levels of environmental cadmium, including the Itai-Itai endemic areas of Japan[76] and industrial contaminated areas of China.[87,88] Within Europe, studies have focused on populations living in the vicinity of metal smelters in Belgium[89] and a nickel-cadmium battery factory in Sweden.[90]

More recently, studies have shown a positive association between the urinary excretion of cadmium and tubular proteinuria, at low levels of exposure.[90,91] There has, however, been much debate over whether this excretion of low-molecular weight proteins (below the threshold of 0.3 B2M mg/g creatinine) reflects early changes that may progress to clinically significant kidney damage, or simply a transient change in tubular reabsorption.[92,93]

Co-excretion of low molecular weight proteins

It has recently been suggested that the apparent association between proteinuria and low-level urinary cadmium may reflect co-excretion rather than any toxic effect.[94-96] Most of the cadmium circulating in plasma is bound to proteins, and to MT in particular. MT shares the same affinity for tubular binding sites as other LMW proteins. Normal inter-individual variation in the absorption of LMW proteins is therefore likely to be reflected in the urinary excretion of both tubular biomarkers (B2M, RBP, A1M) and cadmium. The results of three studies carried out in populations with low-level cadmium exposure suggest that factors such as diuresis, current smoking and co-excretion may account for the apparent associations. Use of these biomarkers in low exposed populations may, therefore, result in an overestimation of the adverse effects of cadmium on kidney function.

NAG is a biomarker of increased cell turnover rather than reduced reabsorption. No studies have so far investigated whether the apparent association between U-NAG and U-Cd at low exposures may similarly be accounted for by normal inter-individual variation. We may, however, reasonable expect that factors such as smoking as diuresis are important.

2.4.3 Cadmium and kidney stones

Cadmium has been proposed to increase kidney stone formation through increased urinary calcium excretion.[97] Calcium is a major component of the majority of kidney stones and increased urinary calcium excretion is a recognized risk factor for stone formation. A correlation between urinary cadmium and urinary calcium has been observed in a number of studies.

A link between cadmium exposure and kidney stones was first suggested by Friberg in 1950 based on observations gathered from an occupationally exposed population working in the manufacture of alkaline accumulators.[86] Subsequent studies provided further case reports from occupationally exposed populations both in England and Sweden.[98,99] These were then followed by several studies in which kidney stone prevalence among the exposed was compared to that among a control or less exposed group. The first of these reported higher urinary cadmium ($p=0.025$) among those workers with a history of kidney stones ($n=8$) compared to those without. However, the prevalence (14%) among exposed workers was not found to be statistically significantly different to that among a control group (9%).[71] An American study also reported a higher prevalence of kidney stones ($n=9$) among cadmium-exposed workers

compared to controls (prevalence: unexposed 3%; exposed 18%; p=0.07).[100] Similarly, a study among Swedish solderers, observed a higher prevalence of kidney stones (n=10) in relation to blood cadmium levels with a prevalence of 9% in the low exposure group compared to 35% in the high exposure group (p=0.04).[101] Each of these studies, however, was based on a small number of cases (n≤10) and did not adjust for age or other potential confounders. These early studies provided useful descriptive data however, due to the methodological limitations highlighted, no firm conclusions can be drawn as to whether the prevalence of kidney stones was indeed higher among those occupationally exposed. Järup & Elinder (1993) reported a significantly increased incidence rate ratios and dose-response relationships in relation to both estimated cumulative exposure based on work history and cumulative blood cadmium with analyses adjusted for age.[102]

Only recently has the kidney stone risk associated with long-term low-level cadmium exposure in the general population started to be explored. One previous cross-sectional study among a representative sample of the non-institutionalized US population (National Health and Nutrition Examination Survey (NHANES) III) showed a higher prevalence (OR 1.40 95% CI: 1.06-1.86) of self-reported kidney stones among women with urinary cadmium levels >1 μg/g compared to those with ≤1μg/g, however, no significantly association was observed among men (OR 0.89 95%CI: 0.67-1.19).[103] Data from both occupationally exposed populations and the general population therefore remains inconclusive.

2.5 BONE

The skeleton performs a number of functions, these include supporting the body, allowing movement, protects internal organs, producing blood cells and acting as a calcium and phosphate store. It is comprised of 206 bones each of which is being continuously remodelled in response to various stimuli.

Mineralized bone or osseous tissue is classified into two main types – cortical (compact) and trabecular bone (cancellous) – that differ considerably in their porosity and microstructure. The ratio of trabecular bone to cortical bone varies depending on the properties required at a given skeletal site. Cortical bone tissue has a low porosity (5-10%) and forms a hard outer shell in long bones. Trabecular bone, by contrast, has a high porosity (50-90%) and is primarily found in the end sections of long bones (such as the neck of the femur), vertebrae, scull, pelvis etc. This open structure of mineralised plates and rods is both strong and lightweight and provides room for bone marrow. Approximately 80% of the bone mass is composed of cortical bone while the remaining 20% is trabecular bone. The metabolic rate of trabecular bone is much greater than that of cortical bone with an annual turnover rate of 30% and 3%, respectively.

Osseous tissue is a dense type of connective tissue and is composed primarily of calcium hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$] and collagen. Living and dead cells are embedded in the mineralized organic matrix. The two main cell types are osteoblasts (bone forming) and osteoclasts (bone resorption). Osteoblasts are mononucleate cells that are located on the bone surface. They excrete a protein mixture known as osteoid, which is primarily composed of type I collagen, and enzymes such as alkaline phosphatase, which is involved in the mineralization of osteoid. As osteoblasts mature they become entrapped in this newly produced bone matrix and develop into osteocytes. The primary functions of osteoblasts are bone formation, matrix maintenance and calcium homeostasis, they are also involved in regulating the bones response to stress and mechanical load. Osteoclasts are specialized cells of the haemopoetic cell line and are responsible for breaking down bone. These large multinucleated cells are located in resorption pits on the bone surface and secrete enzymes that dissolve the mineral substrate. The process of resorption and formation are coupled and in healthy bone resorption is balanced by bone formation.

2.5.1 Bone mineral density

Bone mineral density is a measure of the mineral content of bone. Peak bone mineral density is achieved in the second decade of life and is dependent on a number of factors including sex steroid production and weight-bearing exercise in the peri-pubertal years. By middle age the absorption of bone starts to exceed formation, leading to a loss of bone mass. Data from prospective population studies indicate that BMD provides very similar information on fracture risk in both sexes.[104]

The most widely validated technique for assessing BMD is dual energy X-ray absorptiometry (DXA). This technique allows the bone mineral density to be measured at any given skeletal site. The results are given as a T-score or Z-score, both are expressed as the number of standard deviations a patient's BMD differs from the reference. The T-score is the bone mineral density (BMD) at a given site, compared to the young normal reference mean. The prevalence of osteoporosis defined by the T-score depends upon the reference range used. For the proximal femur it is recommended to use the US reference data, generated from the NHANES III study in women, as the standard.[105,106] DXA bone density measurements taken at the hip have the highest predictive value for hip fracture[107] and this site is therefore preferred for diagnostic purposes.[106] The Z-Score is the BMD compared to an age, sex and ethnicity matched reference. In premenopausal women and men below the age of 50, z-scores are a preferred index for assessing bone mineral density.

2.5.2 Osteoporosis and fractures

Osteoporosis is "a systemic skeletal disease characterized by low bone mineral mass and micro-architectural deterioration of bone tissue with consequent increase in bone fragility and increased fracture risk".[108] It is defined by the World Health Organization (WHO), as a femoral neck BMD lying 2.5 standard deviations or more below the young adult mean value (a T-score of <-2.5 SD).[108,109] Osteoporosis reflects an

imbalance between bone formation and bone resorption, in which bone resorption dominates. Genetic and environmental factors contribute to the pathogenesis of osteoporosis.

A healthy skeleton should be able to withstand low energy impact such as a fall from standing height and fractures sustained in this way are, therefore, indicative of osteoporosis. The only symptoms of osteoporosis occur in relation to fractures and many of those with osteoporosis will be unaware of their condition until they sustain their first fragility fracture. The most common sites of osteoporotic fractures are the distal forearm, proximal humerus, vertebra, femoral neck and pelvis.[110] Compression fractures of the vertebra lead to the height loss and curvature of the spine (kyphosis) that is common among those with osteoporosis.

Osteoporosis and its related fractures are increasingly recognized as a major health care problem and are associated with reduced quality of life, reduced life expectancy and high treatment cost for public health services. The consequences of an osteoporotic fracture vary according to the fracture site, with vertebral and hip fractures being of particular concern with regards to morbidity and mortality. Excess mortality associated with vertebral and hip fractures is well described.[111] Overall, hip fractures are associated with the greatest morbidity and mortality, and incur the highest treatment costs for health services. Mortality in the first year after a hip fracture, is 10-15%.[112]

Known risk factors for low bone density include; advanced age, female sex, postmenopausal status, physical inactivity, low bodyweight, smoking, high alcohol consumption, low sun exposure, glucocorticoid use, genetic factors, previous fall injuries, and other medical conditions. While bone mineral density is an important determinant of future fracture risk, a number of other risk factors have also been identified which are partially or wholly independent of BMD. These factors include smoking, neurological factors, impaired vision and body mass index.

Smoking as a risk factor for osteoporosis

Smoking is known to cause reduced BMD and may act on bone through a variety of both direct and indirect effects, including inhibition of collagen synthesis, increased cell turnover, down-regulation of cell proliferation, induction of premature osteoblast cell death, anti-estrogenic effects, impaired calcium absorption, reduced body weight, increased oxidative stress, vascular effects, and hypercortisolism.[113] Non-skeletal effects of smoking, such as impaired balance and lowered muscular strength, also may increase the risk of fractures.[114] Exposure to cadmium from cigarette smoke is high, and cadmium may contribute to the negative effects of smoking on the maintenance of bone mass.

Osteoporosis and the role of sex hormones

Sex hormones play and important role in maintaining bone mass. In both men and women, cortical and trabecular bone density increase significantly during puberty in

response to the actions of sex steroid hormones.[115] The subsequent decline in hormone levels is in part responsible for the age related reduction in bone mineral density seen in both sexes.

The main physiological effect of oestrogen on bone is to inhibit bone resorption. In women, most oestrogen is produced in the ovaries up until the onset of menopause. The conversion of androgens to oestrogens, by aromatization, takes place in adipose tissue, and among postmenopausal women, adipose tissue represents the main source of endogenous oestrogens. Higher body weight, therefore, provides some protection against a fracture both by cushioning bones in the event of a fall and by boosting oestrogen levels. In men, testosterone exerts an indirect effect on bone, through the skeletal aromatization of testosterone to oestrogen.[116] Compared with women, men attain greater peak bone mass, and have larger and stronger bones in young adulthood.[117]

Incidence and trends in osteoporosis

Globally, the number of osteoporotic fractures is set to increase markedly over the next few decades, largely due to increased life expectancy in developing countries. In Europe, hip fractures are expected to reach 4.5 million per year by 2050.[118] The age- and sex-specific incidence of hip fracture shows considerable geographical variation and even within Europe, the risk can vary by 10-fold between countries.[119,120] The reasons for this geographic variation in fracture risk are not known and cannot be fully explained by differences in demographics. Osteoporosis is widely regarded as a disease of middle aged and elderly women and remains an under recognized cause of morbidity and mortality in men. One-third of all hip fractures worldwide occurring in men.[118] The age-adjusted incidence of osteoporotic fractures is particularly high in Norway and Sweden.[121,122] Around 70,000 osteoporotic fractures occur every year in Sweden, including 18,000 hip fractures.[112] Statistically, half of all Swedish women and one in four Swedish men are expected to sustain an osteoporotic fracture during their lifetime.[110,112] In Sweden, the total cost of osteoporotic fracture – including treatment costs (1.4 billion Euros/year) and loss of disability adjusted life years (2.7 billion Euros/year) – is estimated at 4.1 billion Euros/year.[123]

2.6 CADMIUM AND BONE

The effects of cadmium on bone were first identified following the emergence of Itai-Itai disease (see section 2.1.5). Those with the disease suffered multiple fractures and were found to have a combination of osteoporosis and osteomalacia. More recent studies have reported an association between cadmium exposure and bone effects in populations with long-term, low-level environmental exposure. Little is known about the public health impact of low-level cadmium exposure in terms of its effects on fracture risk.

Several studies have shown an association between environmental exposure to cadmium and lower BMD and increased osteoporosis risk.[88,124-127] However, only four previous studies have assessed fracture risk in relation to urinary cadmium.[124,128,129] The PheeCad study reported a higher risk of any fractures in women (RR=1.73, 95% CI 1.16–2.57) and a non-significantly increased risk of height loss in men (RR=1.60 95% CI 0.94–2.72) associated with a doubling of urinary cadmium.[124] This is consistent with the finding of a Swedish study, which reported an 18% (95% CI 1.01–1.37) increased risk of forearm fracture per unit increment in urinary cadmium (nmol Cd/ mmol creatinine) in those over 50 years of age.[128] A study in China found a higher prevalence of fracture in those living in a cadmium-polluted area compared to those in the control area (n=790).[126] For those living in the highly polluted area (mean U-Cd 9.2-13 µg/g cr) compared to the control area (mean U-Cd 1.6-1.8 µg/g cr), the age standardized relative risk (SRR) was 4.1(95% CI; 1.55–6.61) for men and 2.5 (95% CI; 1.42–3.54) for women. All three studies were carried out in areas with known industrial contamination, this may have contributed to exposure and levels are therefore likely to be higher than in our study. However, increased fracture risk in relation to cadmium exposure has also been reported among women living in an area of Sweden with no significant industrial sources.[129] This study found that never-smoking women with urinary cadmium levels of 0.5 mg/g of creatinine or higher had an odds ratio (OR) of 2.03 (95% CI 1.33–3.09) for any fracture, an OR of 2.06 (95% CI 1.28–3.32) for osteoporotic fractures, and an OR of 2.18 (95% CI 1.20–3.94) for forearm fractures compared with those with lower urinary cadmium levels.[129]

Mechanism of cadmium osteotoxicity

Experimental data also show effects of cadmium on bone and support a causal relationship. The mechanism by which cadmium exerts its negative effect on bone remains to be clarified. It has been proposed that the effects may be mediated by initial kidney damage and/or result from a direct effect on the skeleton.[130] Experimental data supports a direct effect of cadmium on bone with decreased bone formation and increased bone resorption at cadmium concentrations relevant to human exposures.[130-132]

Epidemiological studies have shown cadmium exposure to be associated with increased bone resorption and lower levels of parathyroid hormone. These results suggest that cadmium-associated calciuria is most likely a result of increased bone resorption rather than the result of cadmium induced decreased tubular reabsorption.[132,133] The changes in bone-resorption markers and BMD were observed even in the absence of cadmium-induced renal tubular dysfunction,[131,133] this may be seen as further support for a direct effect on bone. It is important to note however, that there is also support for a kidney-mediated effect, with reported associations between cadmium exposure and kidney tubular damage and osteoporosis.[88,126,127] The two proposed mechanisms do not conflict and may co-exist.

2.7 METABOLIC PROFILING (METABONOMICS)

Metabonomics is defined as 'the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification'.[134] Toxic substances induce pathophysiological perturbations resulting in disturbance in the ratios and concentrations, binding or fluxes of endogenous biochemicals. The metabolites in body fluids, are in dynamic equilibrium with those inside cells and tissues and, consequently, abnormal cellular processes following a toxic or metabolic insult will be reflected in altered body fluid compositions.[134] Such changes to the metabolic profile are characteristic of the disease or toxic process. High-resolution nuclear magnetic resonance (^{1}H-NMR) spectroscopy is one analytical method that can be used to obtain a metabolic profile from biological sample. The metabolic fingerprint obtained by NMR, is very complex and may contain upwards of several thousand resolved lines. Specialist statistical methods have therefore been developed in order to interpret this data and identify the relevant metabolites.

2.8 CADMIUM IN AVONMOUTH

Avonmouth is a town in Southwest England. Situated just to the northwest of Bristol, the area is dominated by the docks and has a long history of heavy industry. For over 70 years Avonmouth was the site of the largest lead and zinc smelter of its kind in the World, however, there is evidence of lead and zinc smelting operations in the area dating back as far as the 18th century.[135] Avonmouth is close to the village of Shipham, famous for the highest soil concentrations of cadmium ever recorded.

During it operation the smelter emitted large amounts of toxic metals, including cadmium, lead, mercury and arsenic to both air and water. When it closed in 2003, the smelter was the largest point source of atmospheric cadmium emissions in the United Kingdom accounting for nearly 30% of U.K. point source emissions.[136] Soil sampling carried out in the vicinity of the smelter has shown a significant build-up of metal contamination in the soil up to 15 km from the smelter (See **figure 2**).[137] Similarly, moss surveys, which aim to monitor atmospheric deposition of metals, also identify higher concentrations around Avonmouth prior to the closure of the site.[138]

Close to 50,000 people live within 5 km of the smelter and there has, therefore, been concern that emissions from the site may have led to increased cadmium exposure in the local population. It is thought that human exposure in this area may have occurred both directly through inhalation of contaminated air and indirectly through the ingestion of home-grown vegetables and house dust.

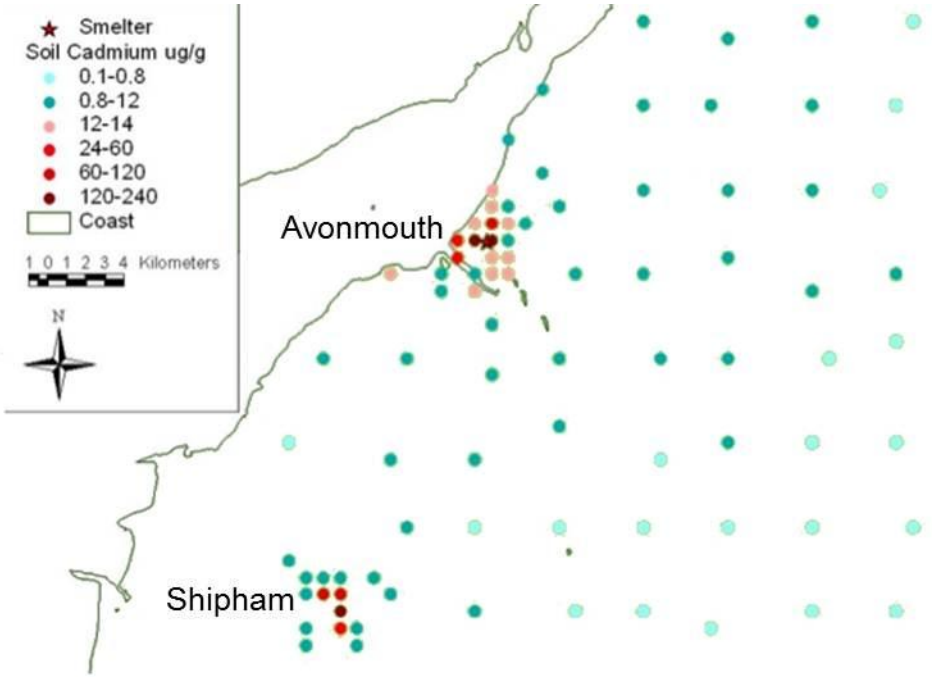

Figure 2 Map showing concentrations of cadmium in soil around Avonmouth. Data used by kind permission of the Centre for Ecology and Hydrology, UK.

3 AIMS

The overall aim of this thesis was to assess the association between cadmium exposure and bone and kidney effects.

The specific objectives were:

- To assess cadmium body burden and early signs of kidney damage in a population exposed to industrial cadmium emissions (Avonmouth) (**Paper I**).
- To develop and validate an air dispersion model of cadmium emissions to be used in large-scale studies on health effects (**Paper I**).
- To identify urinary biomarkers associated with cadmium exposure using metabolic profiling techniques (metabonomics/metabolomics) (**Paper II**).
- To prospectively assess the association between dietary cadmium exposure and chronic kidney disease incidence (**Paper III**), in two large population-based cohorts of men and women (COSM & SMC).
- To prospectively assess the association between dietary cadmium exposure and kidney stone incidence (**Paper IV**) in COSM & SMC.
- To prospectively assess the association between dietary cadmium exposure and all fracture and hip fracture incidence (**Paper V**) in COSM & SMC.

Figure 3. Aerial view of the Britannia Zinc Smelter in Avonmouth. From "A History of the Zinc Smelting Industry in Britain." E.J.Cocks & B. Walters. Published by George G. Harrap & Co. Ltd. 1968

4 MATERIALS AND METHODS

4.1 STUDY POPULATIONS

This thesis is based on data from populations in both England and Sweden. The English data was gathered from a sample of the general population living in Avonmouth, Southwest England, and recruited specifically for this study. The Swedish data is from two large, population-based prospective cohorts of men and women, living in three counties of central Sweden. The Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM) have been used extensively to investigate the role of nutritional and lifestyle factors in the development of disease.

4.1.1 Avonmouth (Paper I & II)

Avonmouth was the site of a large lead and zinc smelter, which is known to have emitted large quantities of cadmium to air (see section 2.8). There was concern that these emissions may have led to increase cadmium exposure in the local population. In order to assess cadmium exposure in this population, we aimed to recruit a representative sample of those living in the areas most exposed to atmospheric emissions from the site.

Air dispersion modelling (see section 4.2.1) was used to estimate exposure around the smelter. Using the validated model we identified the 'exposed area' and divided this into high, medium and low exposure bands. Postcodes falling within these bands were then identified using GIS (Geographical Information Systems). This data was then cross-referenced with National Health Service (NHS) patient registration data to identify potential participants. In order to address differences in anticipated response rates between population groups, we stratified potential participants into age, sex and exposure groups. A random sample was then taken from each group. We over sampled from groups such as young males as this group tends to have the lowest response rate.

Those invited were sent written information about the study and were required to return a signed consent form in order to be enrolled. The consent form also asked for permission to inform their general practitioner (GP) of their biomarker results. Participants were then asked to complete a self-administered questionnaire, including questions on lifestyle (e.g. smoking, consumption of home-grown vegetables etc.), medical history and residential history. Participants were also sent a sample bottle and were asked to provide first-voided, morning urine. Questionnaires and samples were then either taken to one of two GP surgeries where freezers had been provided or were collected from participants' homes. Urine samples were frozen on the day of collection.

Following the analysis of urinary samples, the participants were provided with a copy of their individual biomarker results and information on whether their biomarker levels were over the reference level. Participants were advised to consult their GP if their biomarker results were outside the reference range and therefore potentially of concern.

We also provided GPs with their patients' biomarker results. As the biomarkers measured are not used in clinical practice, we also provided advice to GPs on how the results should be interpreted. The study findings (summary results containing no personal identifiable data) were provided both to government agencies such as the Health Protection Agency and the Environment Agency as well as to the local press.

Of the 865 people invited to take part in the study, 180 participated (response rate 21%). This response rate is typical of epidemiological studies of this sort in the UK. Bristol South and Central Ethics Committee granted ethical approval for the collection and use of the data, and Bristol North Primary Care Trust provided Research and Development approval.

4.1.2 Swedish Mammography Cohort (Papers III & IV)

The SMC was established between 1987 and 1990. All women living in the counties of Uppsala and Västmanland, in central Sweden and born between 1914 and 1948 were invited to mammography screening and asked to complete a self-administered questionnaire (response rate 74%). In 1997, a second questionnaire was sent to those women who were still alive and living in the study area, in order to update and expand the exposure information (response rate 70%). This questionnaire included over 350 questions on diet and lifestyle, including a 96-item food frequency questionnaire (FFQ). In the papers included in this thesis we have used only the data from the 1997 questionnaire. The 1997 data was used as it provides data on smoking habits (not included in 1987) and allows more direct comparison with the COSM data. Written information was sent along with the study questionnaire; return of a completed questionnaire was considered to imply informed consent. The Regional Ethical Review Board in Stockholm granted ethical approval, for the collection and use of this data.

For details of the exclusions relevant to each study please refer to **Figure 4**. In brief, from the baseline population (n=39,227) we excluded those with incorrect or incomplete national registration numbers, those who reported an implausible energy intake (±3 SD of mean log transformed energy) and those with pre-baseline diagnosis of cancer (not including non-melanoma skin cancer). We also excluded those with a history of diabetes based on self-report and Diabetes Register data (n= 1,920). For **Paper III** we additionally excluded those who were diagnosed with kidney disease (*International Classification of Disease system, 10th revision* code (ICD-10) N00-19 and N25-29) prior to baseline, based on data from the National Hospital Discharge Register and the National Outpatient Register, and those who started renal replacement therapy based on data from the Renal Replacement Register (n= 839). For **Paper IV** we additionally excluded those diagnosed with kidney stones (National Hospital Discharge Register and the National Outpatient Register) prior to baseline (n= 1,718) as the disease is known to be highly recurrent and the advice given to stone formers may influence their exposure to dietary cadmium.

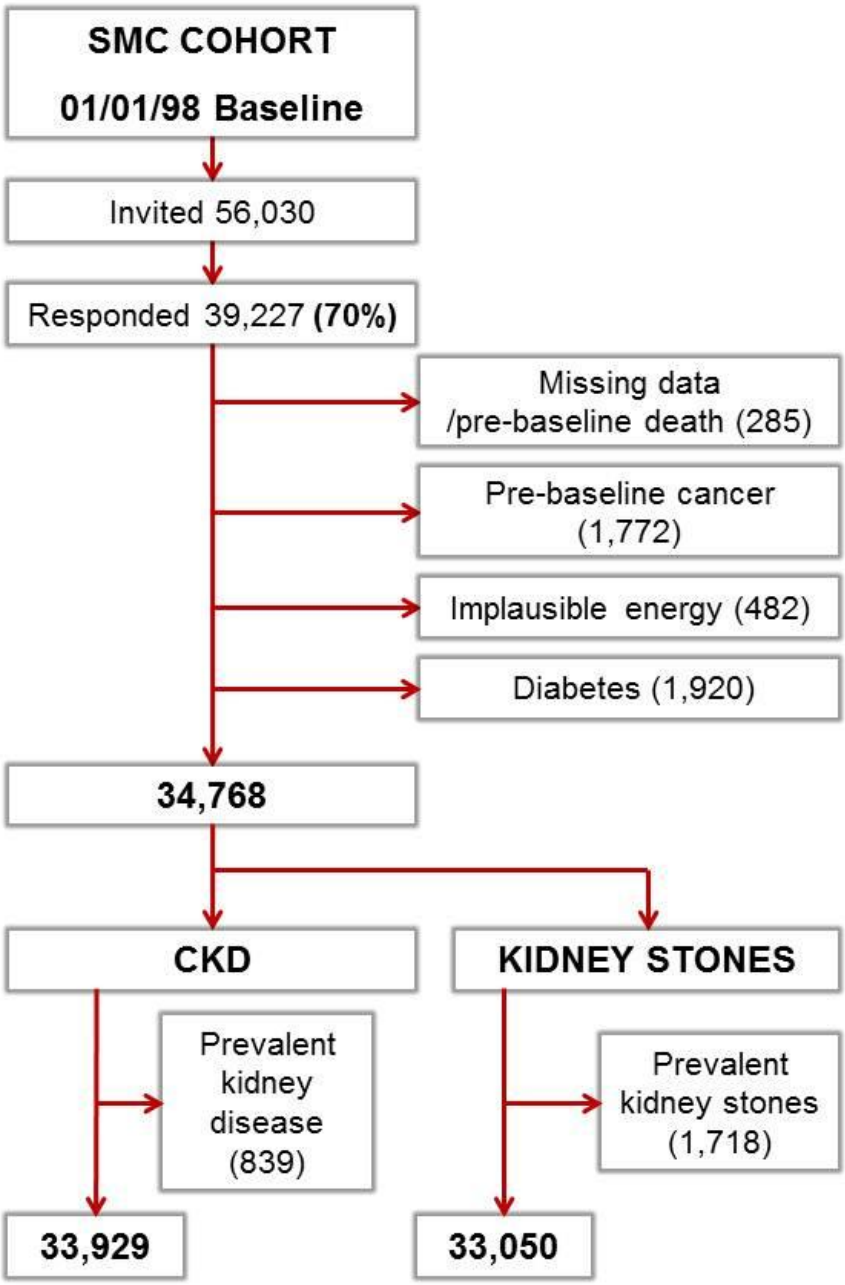

Figure 4. The Swedish Mammography Cohort (SMC): Source and study population for **Papers III & IV**.

4.1.3 Cohort of Swedish Men (Paper III, IV & V)

The COSM was established in 1997 when all men born between 1918 and 1952, and residing in Västmanland and Örebro counties, were invited to participate (n= 100,303). The self-administered questionnaire included over 350 questions including a 96-item food frequency questionnaire; 48,860 men returned the baseline questionnaire (response rate 49%). The questionnaires completed in 1997 by the SMC and COSM cohorts are very similar with the exception of some sex specific questions. The COSM cohort is representative of the Swedish male population with regards to age distribution, weight and education level.[139] Written information was sent along with the study questionnaire; return of a completed questionnaire was considered to imply informed consent. The Regional Ethical Review Board in Stockholm granted ethical approval, for use of this data.

For details of the exclusions relevant to each study please refer to **Figure 5**. In brief, from the baseline population (n=48,850) we excluded those with incorrect or incomplete national registration numbers, those who reported an implausible energy intake (±3 SD of mean log transformed energy) and those with pre-baseline diagnosis of cancer (not including non-melanoma skin cancer). We also excluded those with a history of diabetes based on self-report and register data (n= 4,256). For **Paper III** we additionally excluded those who were diagnosed with kidney disease (ICD-10: N00-19 and N25-29) prior to baseline, based on data from the National Hospital Discharge Register and the National Outpatient Register, and those who started renal replacement therapy based on data from the Renal Replacement Register (n=705). For **Paper IV** we additionally excluded those diagnosed with kidney stones (National Hospital Discharge Register and the National Outpatient Register) prior to baseline (n=5,538). For **Paper V** we included only those men living in Västmanland County, as outpatient fracture data was not available for Örebro County at the time of analysis.

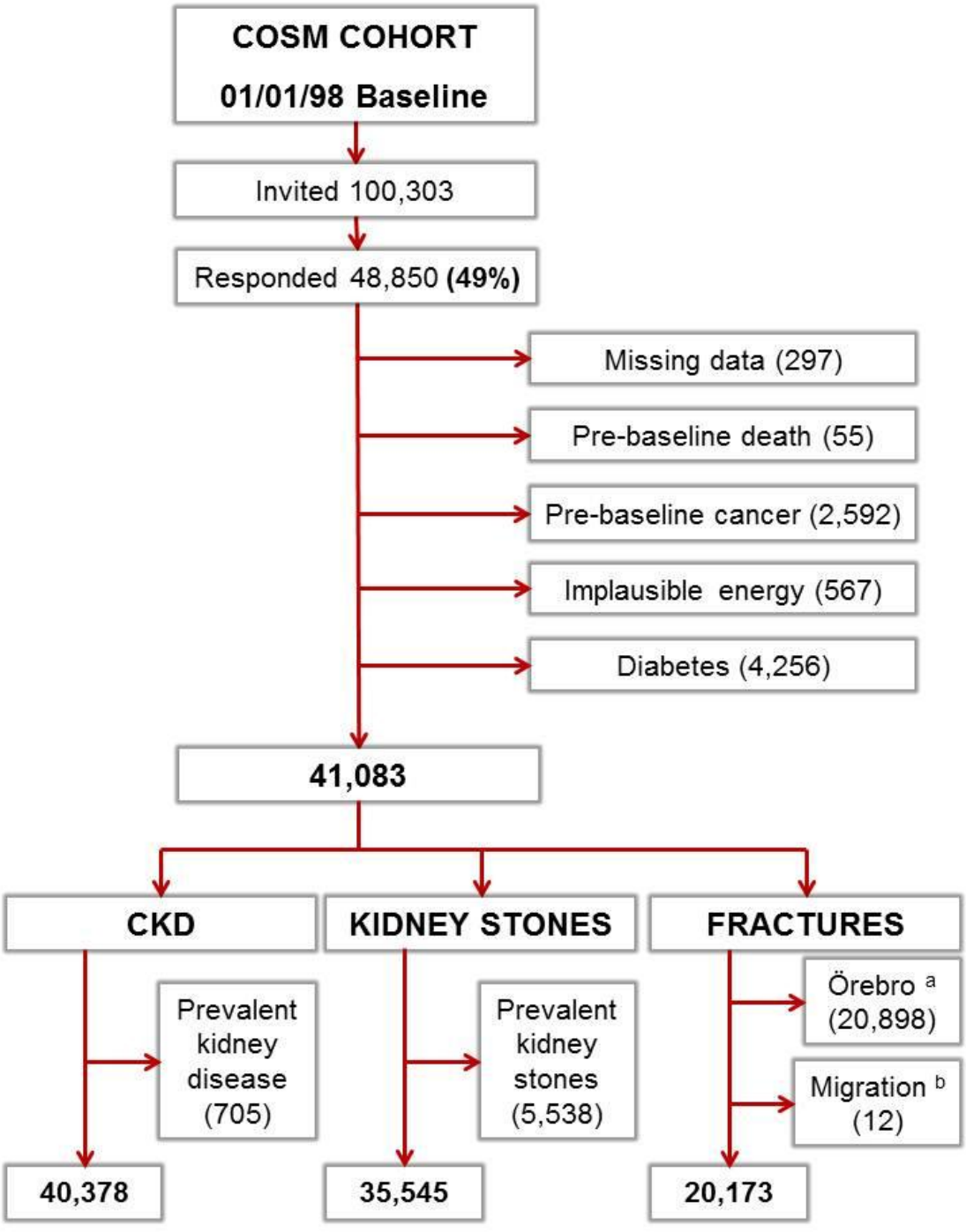

Figure 5: The Cohort of Swedish Men (COSM): source and study populations for **Papers III, IV & V.** [a] Exclusion of those living in Örebro County. [b] Exclusion of those who migrated out of Västmanland prior to baseline.

4.2 EXPOSURE ASSESSMENT METHODS

4.2.1 Air dispersion modelling (Paper I)

We used modelling to provide spatial information on the aerial dispersion of cadmium emissions from the Avonmouth smelter. ADMS-Urban (Atmospheric Dispersion Modelling System software (ADMS-Urban version 2.0; Cambridge Environmental Research Consultants, Cambridge, UK) is an advanced semi-Gaussian model, widely used for environmental impact assessments within the UK. Using this software we were able to model ground level cadmium concentrations around the smelter. The model was then used to identify the potentially exposed population and to stratify this population into high-, medium-, and low-exposure groups prior to the recruitment of study participants.

Specification of the air dispersion model

In order to model point source emissions, such as those from the Britannia Zinc smelter, ADMS requires detailed data on emissions, site characteristics, meteorology and background concentrations, to be imputed. Using this data we were able to model cadmium emissions from the Britannia Zinc smelter between 1996 and 2002 (excluding 1997).

Under Integrated Pollution Control legislation, detailed emissions data from the smelter were reported to regulatory authorities from 1996 to early 2003 when the site closed. This data included cadmium concentration, temperature and flow rate at each of the stacks. During this time, up to 13 stacks were emitting cadmium to air. This data is in the public domain and we were therefore able to access the data through Bridgewater Public Registry.

Data relating to the characteristics of the site was obtained both from Bristol City Council and satellite images. Bristol City Council provided data such as stack location, diameter and height. Satellite images were used to identify significant buildings. These are building which, because of their size and proximity to the emissions point, may impact on the way in which the emissions plume disperses.

We used hourly sequential meteorological data from the Bristol Weather Centre and Filton Meteorological station; the British Atmospheric Data Centre provided the data. This data includes temperature, wind speed and direction. Unfortunately the data from 1997 was incomplete and could not therefore be used. **Figure 6** shows rose diagrams of the wind speed and wind direction data used in the model. These figures clearly show the UK's prevailing south-westerly wind direction.

Background ambient air cadmium concentrations in the United Kingdom[140] are typically between 0.05 and 1.0ng/m^3, we therefore added a background concentration of 0.5ng/m^3 to the model. We also checked emissions records for any other significant sources of cadmium in the study area.

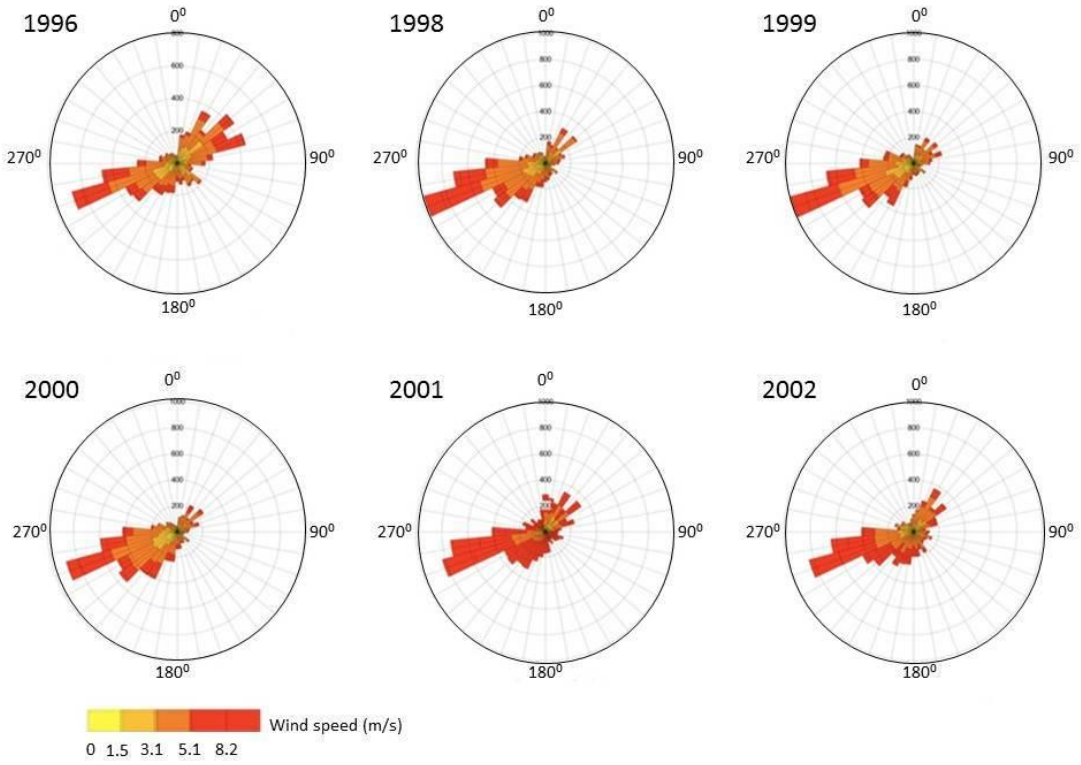

Figure 6. Windrose diagrams, showing the wind speed and direction data used in the air dispersion model.

Validation of the air dispersion model against monitoring data

We validated the model output from the year 2000 using the annual averages from six air monitoring sites. Bristol City Council and Stanger/National Physical Laboratory (Middlesex, England) provided the air monitoring data including the geographic coordinates of each monitoring site. The coordinates were entered into the ADMS model so that point estimates at these locations could be modelled. Output from the year 2000 was used for the validation, since this was the year that provided the most complete dataset. In order to reduce occupational exposure at the site, many of the buildings in which the smelting process took place were to some degree open-sided. This helped to increase ventilation and reduce the temperature however it also meant that there were significant emissions not just through the chimneys but also through gaps in the sides of the buildings. These fugitive emissions are thought to have been significant but could not been included in the model since they were never quantified. The final model used was an average of the output for 1996–2002 (excluding 1997).

4.2.2 Urinary cadmium assessment (Papers I & II)

First voided morning urine samples were collected in cadmium-free tubes, frozen on the day of collection and kept frozen until analysis. Samples were analysed for cadmium using inductively coupled plasma mass spectrometry (ICP-MS). The Health and Safety Laboratory (HSL) in Buxton, England carried out the analysis according to standard operating procedures. HSL is a major government laboratory specializing in occupational health and safety, the laboratory participates in inter-laboratory quality control procedures. We adjusted urinary cadmium values to urinary creatinine to account for differences in urinary dilution.

4.2.3 Assessment of diet and lifestyle factors (Papers I-V)

Papers I & II

Data on medical history, residential history and lifestyle factors such as smoking, were collected via a self-administered questionnaire. The questionnaire was completed by the study participants and returned with the urine sample.

Papers III, IV & V

Diet was assessed in 1997 using a 96-item, self-administered, semi-quantitative, food frequency questionnaire. Participants were asked to report their typical consumption of various food items over the previous year. Frequency of consumption for each food type was reported using eight predefined categories ranging from never/seldom to four times a day. Open-ended questions were used to report consumption of bread and dairy products. The validity of the baseline FFQ has been assessed among a random sample of 248 men aged 40-74 years living in the study area. Each man completed the FFQ along with 14 repeated 24-hour dietary recall interviews over the period of one year. For macronutrients the Spearman's rank correlation coefficient between the FFQ and an average of the 14 repeated 24-hour recalls was 0.65.[141] The self-administered questionnaire completed at baseline (1997) by both cohorts also included questions on physical characteristics such as weight and height, lifestyle factors such as physical activity and smoking habits and health questions including use of certain medicines and supplements. The physical activity questionnaire data has also been validated against 7-day activity records and accelerometers.[142]

4.2.4 Dietary cadmium assessment (Papers III, IV & V)

Dietary cadmium (D-Cd) exposure was calculated using data from the FFQ and a database of the cadmium content of all foods available on the Swedish market, as previously described.[46,49] This comprehensive database, covering 422 food items, has been constructed using data obtained from the Swedish National Food Agency (NFA, Uppsala, Sweden). For a small number of food items for which Swedish data was not available, Finnish or Danish data has been used. Dietary cadmium exposure was calculated by multiplying the frequency of consumption of each food type by its cadmium content using age-specific (<53, 53-65, >65 years) portion sizes. Intake of energy and specific nutrients was also calculated in this way.

Dietary cadmium exposure was positively correlated with energy intake (r=0.76 among women and r =0.79 among men) and we therefore adjusted dietary cadmium estimates to the mean energy intake in the cohorts (men 2,600 kcal/day; women 1,700 kcal/day) using the residual method.[143] Energy adjustment is standard practice in nutritional epidemiology and is based on the concept that the composition of the diet, independent of total caloric intake, is of primary interest. This adjustment also reduces the artificial between person variation introduced by under- and over-reporting of food intake in the FFQ.

This method for estimating dietary cadmium exposure has been validated in a sample of 680 non-smoking women from the SMC.[26] Cross-classification of FFQ-estimated dietary cadmium exposure, and urinary cadmium concentrations, gave a sensitivity of 51% and a specificity of 58%. The Pearson's correlation coefficient between measured and modelled urinary cadmium (based on dietary cadmium) was 0.2 when accounting for within person variability.[144]

4.3 BIOMARKER BIOMARKERS OF EFFECT

4.3.1 Biomarkers of tubular dysfunction (Paper I)

Urine samples were analysed for three biomarkers of tubular damage [NAG (U-NAG), RBP (U-RBP), and A1M (U-A1M)]. The reader is referred to section 2.3.3 for a full description of these biomarkers. The HSL (see 4.2.2) determined U-NAG by fixed time incubation, U-RBP by enzyme-linked immunosorbent assay, and U-A1M by a non-competitive immunoassay, and adjusted biomarker concentrations to that of urinary creatinine to account for differences in urine dilution. As there are no standard reference values for any of the effect biomarkers we used the 97.5th percentiles from a UK cohort of 320 working subjects with no history of exposure to nephrotoxins for U-NAG and U-RBP (Mason H, personal communication). For the U-A1M reference level we used the 95th percentile of a non-occupationally exposed, healthy Swedish population of working age.[145] These reference values were used as a cut-off, both in the reporting of results to participants and their GPs, and in logistic regression models.

4.3.2 Metabolic profiling (Paper II)

High-resolution ^{1}H NMR spectroscopy (metabonomics/metabolomics) was used to acquire urinary metabolic profiles for 178 of the 180 adults recruited from the Avonmouth area (see **Paper I**). For a full description of the ^{1}H NMR process used please see 'NMR data acquisition' on page 2 of **Paper II**. Urinary levels of 8-oxo-deoxyguanosine were also measured, using mass spectrometry, as a marker of systemic oxidative stress. Assignment of peaks to specific metabolites was based on the addition of known standards to the biological samples, together with published literature,[146] on-line metabolomics databases and statistical total correlation spectroscopy (STOCSY).[147] Metabolic profiling is a relatively new technique and

much of the knowledge used to assign spectral peaks has been gathered from experimental animal studies.

4.4 CASE ASCERTAINMENT (PAPERS III, IV & V)

First incident cases of CKD (**Paper III**) and kidney stones (**Paper IV**) were ascertained from the 1st of January 1998 to the 31st of December 2010 by computerized linkage of the study population to the National Hospital Discharge Register and the Outpatient Register. Incident fracture (**Paper V**) cases were ascertained from the 1st of January 1998 to the 31st of December 2008 by linkage to the National Hospital Discharge Register and the Regional Hospital Diagnosis Register. Regional data was only available for Västmanland county, therefore, for **Paper V** we excluded those men who moved out of the county, using data from the Population Register. Information on date of death was obtained from the Swedish Death Register.

The Hospital Discharge Registry (also called the National Inpatient Registry (IPR)) has covered 100% of the country since 1987, while coverage of the National Outpatient Register was assessed at approximately 80% in 2007. As of 2011, 99% of all somatic and psychiatric hospital discharges were registered in the IPR and a primary diagnosis was listed for 99% of all discharges. A review of the validity of the IPR found positive predictive values (PPVs) of 85-95% for most diagnoses given in the IPR, with a PPV for hip fracture of 95-98%.[148]

4.5 STATISTICAL METHODS

4.5.1 Correlation (Paper I)

We assessed the correlation between urinary biomarkers of both dose (U-Cd) and effect (A1M, NAG, RBP) on ln-transformed data using Pearson's two-tailed correlation coefficient. Pearson's was chosen since the biomarkers were log-normally distributed and did not show a nonlinear relationship when plotted.

4.5.2 Linear Regression (Paper I)

We used linear regression to assess the relationship between natural log (ln) U-Cd and a set of independent variables—sex, age, ADMS category (modelled cadmium concentrations), and smoking status. The median value was taken as the representative concentration for each ADMS category.

4.5.3 Logistic Regression (Paper I)

In order to allow more direct comparison with previous studies, we calculated odds ratios for the prevalence of U-NAG above the reference value using a logistic regression model. The U-Cd categories were chosen *a priori* based on the OSCAR (Osteoporosis - Cadmium as a Risk Factor) study.[90] The lowest U-Cd category (U-

Cd <0.3 nmol/mmol creatinine) was used as the reference group and the analysis was adjusted for sex. We used Egret software (version 3.2; Cytel Software Corp., Cambridge, MA, USA) to test for trend across the odds ratios. SPSS software was used (SPSS Inc., Chicago, IL, USA) in all other analysis included in **Paper I**. We assessed statistical significance at the 95% level and all tests were 2-tailed.

4.5.4 Metabolic profiling (Paper II)

The analysis of metabolic profile data requires complex specialist statistical methods. For a full description of the analysis please see page 4, **Paper II**. In brief, we used multivariate and univariate analysis to identify metabolites that were correlated with lifestyle or biological factors. Data were imported and manipulated in Matlab (Math-works, Natick, MA, USA) using in-house code for automatic phasing, baseline correction, and referencing chemical shifts to the TSP resonance at δ 0. In order to account for differences in urinary dilution, each spectrum was normalized by the median-fold change to a reference spectrum generated by calculation of the median of all spectra at each spectral point.[149] In metabolic profiling, it is crucial to normalize spectra to compensate for differences in dilution. Specific changes in metabolite levels are then visible as changes in the concentrations of a few metabolites relative to the concentrations of all other metabolites. This method makes use of all the data available and avoids some of the problems can be associated with the use of any one metabolite, such as creatinine.

The spectra were analysed at two resolutions: (1) reduced resolution for partial least squares (PLS) regression analysis; and (2) high resolution for covariance/correlation analysis. The analysis identified 6 specific metabolites (3-hydroxyisovalerate (3-HV), dimethylglycine (DMG), citrate, 4-deoxy-erythronic acid (4-DEA), creatinine and creatine) that were correlated with lifestyle of biological factors. For each metabolite, we measured the area under the relevant spectral peaks. These data were then analysed by multiple linear regression (MLR) using SPSS (v19, IBM, Armonk, NY, USA) controlling for major confounding factors (age, sex and smoking status). For citrate, further analysis was carried out to investigate its association with smoking. Three separate MLR models were developed. The first excluded individuals with missing smoking data (n = 3); the second additionally excluded all individuals classed as current smokers (n = 32); and the third excluded current (n = 32) and past smokers (n = 35) as well as those with missing data. Additionally, we used non-parametric tests to test for a significant ($P< 0.05$) difference or trend, respectively, in the concentration of citrate between the three classes of smoker (current-, past-, and never smoked).

Urinary-8-oxodG was quantified in samples from the upper and lower 15th percentile of U-Cd concentration. The analysis was not carried out for the full sample due to financial constraints. The U-8-oxodG concentrations were normalized to the creatinine concentration and a non-parametric Mann-Whitney test was used to test for any significant ($P<0.05$) difference between the groups.

4.5.5 Cox proportional hazards model (Papers III, IV & V)

Each participant contributed to follow-up time from the start of follow-up until the date of diagnosis, death, migration (**Paper V**) or end of follow-up (31st of December 2010 **Papers III & IV**, 31st December 2008 **Paper V**) whichever occurred first. The 1st of January 1998 was taken as the start of follow-up for both cohorts. Hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated using the Cox proportional hazards regression model with age (in years) as the underlying time-scale. The Schoenfeld's residuals test was used to check for violation of the proportional hazards assumption; we did not find any violation. We tested the linear trends across categories using the median dietary cadmium values within each category as a continuous variable; median values were used so as to limit the impact of outliers. All reported p-values are from two-sided statistical tests and significance was taken as <0.05. Multivariable models were adjusted for established risk factors (see **figures 8 & 9** for variables included) with missing values treated as a separate missing category. All models (**Papers III, IV & V**) were stratified by smoking status (ever/never) in order to investigate possible effect modification. Smoking is major source of cadmium exposure and is also associated with CKD and fractures. In additional analyses, we adjusted for fruit and vegetable intake (**Paper V**) as these foods both contain cadmium and are a significant source of vitamins and minerals that are potentially beneficial to bone health. These foods were weakly to moderately correlated with dietary cadmium.

4.5.6 Spline (Paper V)

In order to flexibly model the association between dietary cadmium exposure and fracture risk, we used restricted cubic-spline analysis. Three knot-positions were specified based on percentiles. The median dietary cadmium exposure was used as the reference as the uncertainty in the model is lowest at this point.

4.5.7 Combined analysis (Paper V)

We investigated the association between dietary cadmium exposure and fracture incidence in relation to smoking status (never, past, or current) using ''never smoker'' with ''lowest tertile of dietary cadmium exposure'' as the reference. We also examined the association between dietary cadmium exposure and fracture incidence in relation to fruit and vegetable consumption (tertiles, g/d) using ''high fruit and vegetable intake'' with ''lowest tertile of dietary cadmium exposure'' as the reference category. For both analyses an interaction term was added to the multivariable adjusted Cox model in order to test for a statistical significance interaction using the likelihood-ratio test. Stata software (Stata versions 10 & 12; Stata Corp., College Station, TX, USA) was used for all of the analyses in **Papers III-V**.

4.5.8 Aetiological fraction and economic cost of fractures

In additional analyses, not included in the original paper (**Paper V**), we calculated the aetiological fraction to allow the economic costs of fractures (all fractures sites) attributable to dietary cadmium exposure to be calculated. This work was an assignment from the Swedish Chemicals Agency.[150] The aetiological fraction is the proportion of morbidity that can be explained by a particular exposure (assuming that it is causal), in this case dietary cadmium. The formula is as follows:

$$Aetiological\ fraction = p\ (RR - 1)/RR$$

Where p is the prevalence of exposure among the cases, i.e. the number of cases in the exposed group divided by the total number of cases, and RR is the relative risk. We used the Cox proportional hazards model to calculate the HR for those with dietary cadmium exposure above the median, using those below the median as the reference. The fully adjusted model, as given in **Paper V**, was used in the analysis. The HR is equivalent to the RR and can be used in place of the RR in the equation.

Once the proportion of fractures attributable to dietary cadmium exposure had been calculated, this information was combined with data on the total cost of fractures in Sweden.[123] The report by Strom et al. takes account of fractures that occur in the over-50 age group – this is comparable to the age distribution in our cohort (45-79years at baseline). The report distinguishes between health care costs of treatment and social care that arise during the first year, and health care costs arising after the first year. In addition, the costs of deaths, chronically reduced quality of life and functional impairment in the form of loss of quality adjusted life years (QALYs), are presented as well as administrative costs.

5 RESULTS

5.1 CADMIUM AND TUBULAR DYSFUNCTION (PAPER I)

We recruited 180 (74 men, 106 women) participants from the local Avonmouth population, to take part in our study. The response rate was 21% and is fairly typical for epidemiological studies of this kind in the UK. As expected, the response rate increased with age (in years: 18–29, 10%; 30–49, 15%; ≥50, 32%) and was higher in women (24%) than in men (17%). Using data from the census we compared responders with non-responders in terms of socioeconomic status and found no significant differences. Of the study participants, 61% were never-smokers, 20% had smoked in the past and 18% were current smokers; smoking data were not available for three participants, these entries were left as missing values. Seventeen participants (15 men, 2 women) reported that they had been employed at the smelter; employment information was not available for three participants.

We modelled ground-level air cadmium concentrations using air dispersion modelling software (See section 4.2.1). **Figure 7** shows the modelled cadmium levels and the monitoring station locations. Modelled ground-level air cadmium concentrations were validated against data from six monitoring sites using annual averages for the year 2000. We found that the model underestimated cadmium concentrations but showed a good correlation (R^2 = 0.84) with measured values (Figure 2, **Paper I**). Ambient air cadmium concentrations at each of the six monitoring sites exceeded the World Health Organization (WHO) Air Quality Guidelines for Europe of 5 ng/m3.[151]

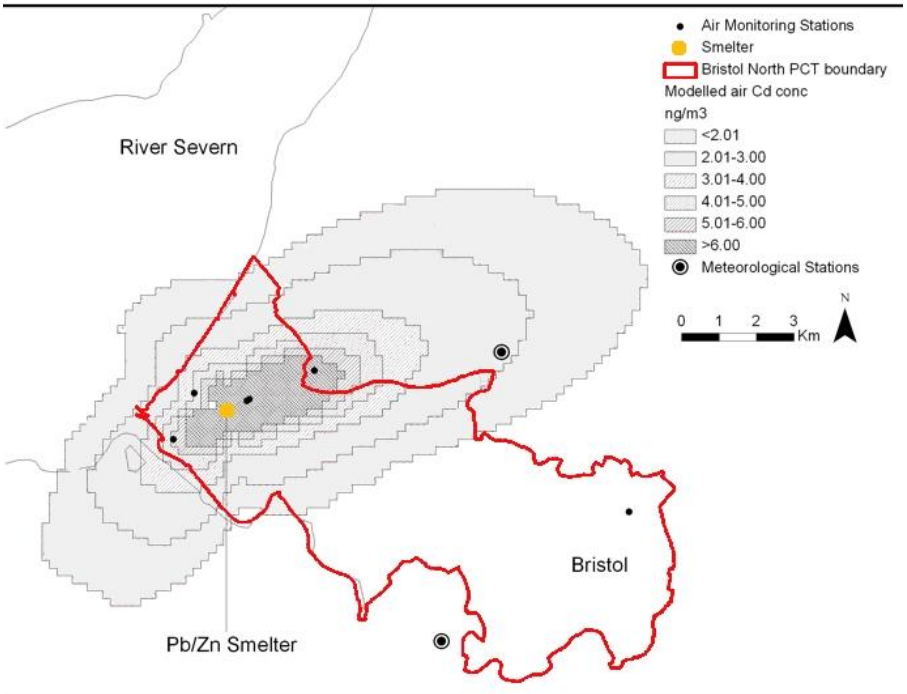

Figure 7. Map showing modelled air cadmium concentrations (conc), the six air monitoring sites, and the site of the lead/zinc smelter. Two of the air-monitoring sites are within 100m of each other.

In our population sample, we found median U-Cd concentrations of 0.22 nmol/mmol creatinine in men (non-smokers 0.18/smokers 0.40) and 0.34 nmol/mmol creatinine in women (non-smokers 0.31/smokers 0.46), after excluding the occupationally exposed. These levels are in the same range as those found in other studies that detected early kidney damage and low bone mineral density (Table 1, **Paper I**). U-Cd was log normally distributed, we therefore used log-transformed data in showing U-cadmium as a function of sex (female vs. male), age, ADMS-modelled cadmium concentration levels, and smoking status (never, past, current smokers). Modelled atmospheric cadmium concentrations were found to be a significant predictor ($p=0.04$) of ln(U-Cd) (Table 2, **Paper I**).

We observed significant correlations between ln(U-Cd) and ln(U-NAG) for men (Pearson's $r= 0.328$, $p= 0.004$), women (Pearson's $r= 0.399$, $p<0.001$) and men and women combined ($n=180$) $r= 0.380$ ($p\leq0.001$) (Figure 3, **Paper I**). We also observed a significant correlation between U-Cd and U-A1M in women (Pearson's $r= 0.220$, $p= 0.03$). We did not observe a significant correlation between urinary cadmium and U-RBP. In order to allow more direct comparison with other studies, we calculated odds ratios using urinary cadmium categories chosen *a priori* based on those used in the OSCAR study.[90] We found a significant dose-response relationship between U-Cd and the prevalence of U-NAG above the reference level ($n=17$), with odds ratios of 2.64 [95% confidence interval (95% CI), 0.70–9.97] and 3.64 (95% CI, 0.98–13.5) for U-Cd levels of 0.3 to <0.5 and levels ≥0.5 nmol/mmol creatinine, respectively (p for trend = 0.045). The dose–response trend did not change when we excluded current and past smokers, although the total number of cases was reduced (Table 3, **Paper I**).

5.2 CADMIUM AND METABOLIC PROFILING (PAPER II)

Metabolic profiling identified six urinary metabolites that were associated with urinary cadmium. All six metabolites are related either to mitochondrial metabolism (citrate, 3-hydroxyisovalerate, 4-deoxy-erythronic acid) or one-carbon metabolism (dimethylglycine, creatinine, creatine). In particular, citrate levels retained a significant correlation to urinary cadmium and smoking status after controlling for age and sex. Urinary 8-oxo-deoxyguanosine levels – a marker oxidative stress – were found to be elevated in individuals with high cadmium exposure. This finding supports the hypothesis that cadmium may cause mitochondrial dysfunction.

5.3 DIETARY CADMIUM EXPOSURE (PAPERS III, IV & V)

Median dietary cadmium exposure levels in our study populations (**Papers III, IV & V**) were 19µg/day in men and 13µg/day women. Those in the highest tertile of dietary cadmium were more likely to: be never smokers; have a lower dietary intake of calcium; have a higher intake of iron, magnesium, potassium, vitamin B6 and vitamin C; drink less alcohol; be more physically active; to consume more fruit and vegetables.

5.4 CADMIUM AND CHRONIC KIDNEY DISEASE (PAPER III)

We ascertained 599 incident cases of CKD among men (in 481,591 person-years) and 253 cases among women (in 415,432 person-years) during 12 years of follow-up. We observed no association between dietary cadmium exposure and CKD incidence in either men (HR 0.97; 95% CI: 0.77-1.21) or women (HR 0.74; 95% CI: 0.53-1.04) in the multivariable adjusted model. Additional adjustment for intake of protein, vegetables and wholegrain, did not change the results (see **figure 8**). We then stratified by smoking status as smoking is a significant source of cadmium exposure and may also be independently associated with CKD. Among never smokers we observed multivariable-adjusted HR 1.17 (95%CI: 0.80-1.69) for men and HR 0.63 (95%CI: 0.40-0.99) for women. The corresponding results for ever-smokers were HR 0.87 (95%CI: 0.65-1.16) for men and HR 0.83 (95%CI: 0.49-1.40) for women (table 2, **Paper III**).

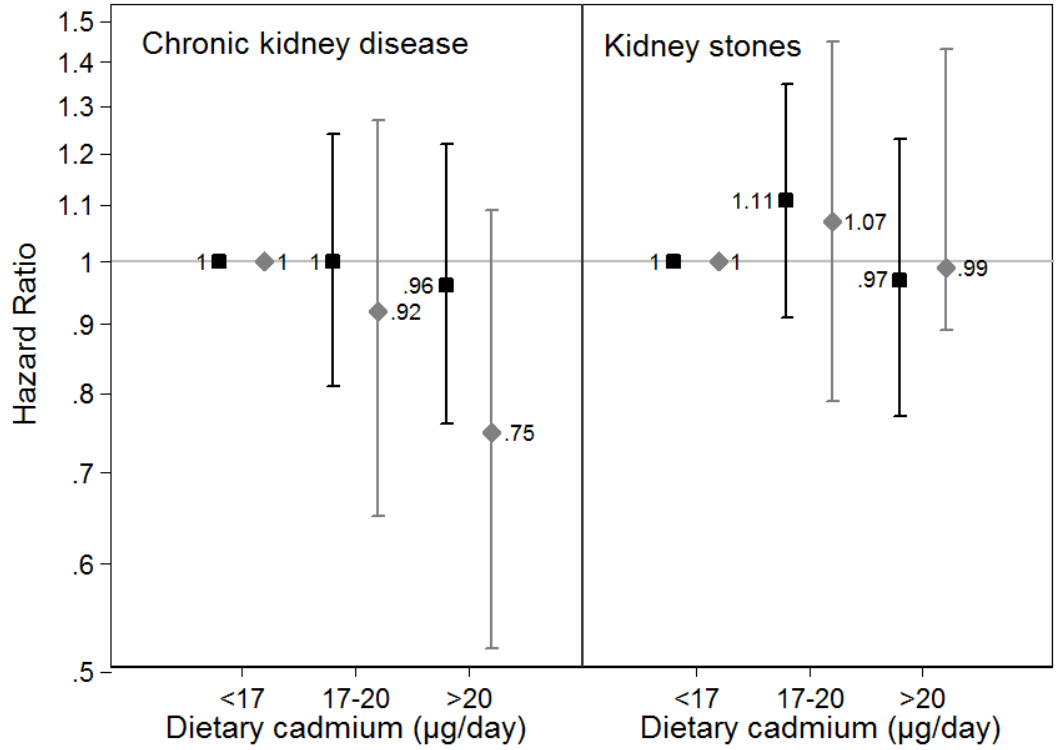

Figure 8. Multivariable adjusted HR and 95%CI for dietary cadmium exposure and chronic kidney disease incidence (**Paper III**) and dietary cadmium and kidney stone incidence (**Paper IV**). Black squares (Men); Grey diamonds (Women). For covariates, see below.
Chronic kidney disease: adjusted for education, BMI (underweight/normal (<25 kg/m^2), overweight (25-29 kg/m^2), obese (≥30 kg/m^2)), smoking (tertiles of pack-years plus non-smoking category), alcohol (quintiles) hypertension (yes/no), aspirin use (yes/no) and dietary intake (quintiles) of iron, protein intake (quintiles) and vegetable and wholegrain (tertiles). Missing values were treated as a separate missing category.
Kidney stones: adjusted for BMI (underweight/normal (<25 kg/m^2), overweight (25–29 kg/m^2), obese (≥30 kg/m^2), alcohol (quintiles plus no alcohol category), cigarette use (tertiles of pack-years plus non-smoking category) and dietary intake (quintiles) of calcium, iron, magnesium, potassium, vitamin B6 and vitamin C.

5.5 CADMIUM AND KIDNEY STONES (PAPER IV)

We ascertained 707 incident cases of kidney stones among men (in 421,611 person-years) and 290 cases among women (in 403,575 person-years) during an average of 13 years of follow-up. We observed no association between dietary cadmium exposure and kidney stone incidence in either men (HR 0.97; 95%CI: 0.77-1.23) or women (HR 0.99; 95%CI: 0.89-1.43) (See **figure 8**). Additional adjustment for hypertension, tea and coffee consumption and use of vitamin C supplements did not change the results (data not shown). We then stratified by smoking status as smoking is a significant source of cadmium exposure and may also be independently associated with kidney stones. Among never smokers we observed multivariable-adjusted HR 0.89 (95%CI: 0.60-1.32) for men and HR 0.72 (95%CI: 0.41-1.24) for women. The corresponding results for ever-smokers were HR 1.06 (95%CI: 0.79-1.43) for men and HR 1.30 (95%CI: 0.79-2.15) for women (table 2, **Paper IV**).

5.6 CADMIUM AND BONE FRACTURES (PAPER V)

We ascertained 2,183 incident cases of any fracture (in 194,617 person-years) and 374 hip fracture cases (in 203,038 person-years) during an average of 10 years of follow-up. In the full multivariable-adjusted model for any fracture (Table 2, **Paper V**), dietary cadmium exposure was associated with a 19% higher rate (HR=1.19, 95% CI 1.06–1.34) of fracture, comparing highest with lowest tertile, and showed a statistically significant dose-dependent trend ($p< 0.01$ for trend). In order to remove any residual effects of smoking not accounted for by the adjustment for pack-years, we stratified the analysis by smoking history (never or ever). Among never smokers only, dietary cadmium exposure was associated with a 21% higher rate (HR=1.21, 95%CI 0.98–1.50) of any fracture ($p= 0.06$ for trend) comparing highest tertile with lowest (see **figure 9)**. Among ever smokers, dietary cadmium exposure was associated with an 18% higher rate (HR=1.18, 95% CI 1.02–1.37) of any fracture ($p=0.03$ for trend) comparing highest tertile with lowest (Table 2 **Paper V**).

For hip fractures (Table 3 **Paper V**), the highest tertile of dietary cadmium exposure was associated with a non-significant 28% higher rate (HR = 1.28, 95% CI 0.97–1.69) of fractures in the multivariable-adjusted model. However, among never smokers only the highest tertile of dietary cadmium was associated with a statistically significant 70% (HR= 1.70, 95% CI 1.04–2.77) dose-dependent ($p=0.03$ for trend) higher rate of hip fracture (See **figure 9**). Spline analysis displayed a linear dose-response relationship between dietary cadmium exposure and increased rate of both any fracture (see **figure 10**) and hip fracture (see **figure 11**).

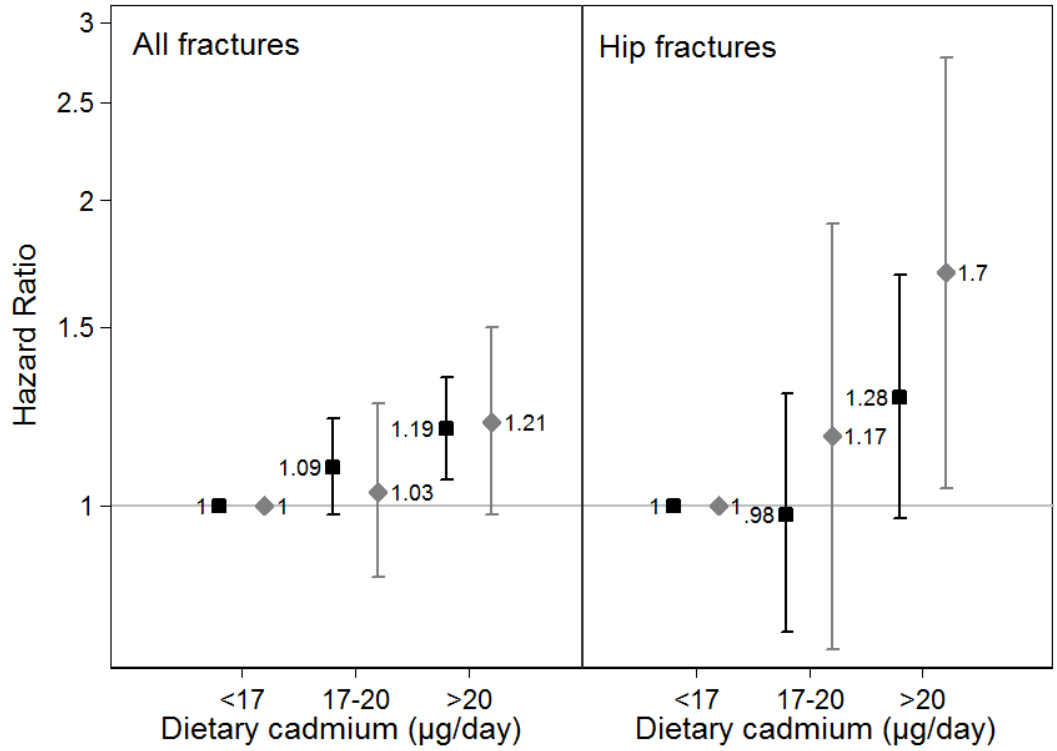

Figure 9. Multivariable adjusted HR and 95%CI for dietary cadmium exposure and incidence of any fracture and dietary cadmium and incidence of hip fracture (**Paper V**). Black squares (All men); Grey diamonds (never-smokers only).

Adjusted for: attained age, height (tertiles), weight (tertiles), education (primary, secondary, tertiary), civil status (single, married/cohabiting, divorced/widowed), employment status (full time, part-time work/student, unemployed, disability pension/retired), alcohol intake (tertiles plus never-drinker category), cortisone use (ever/never), walking/cycling (<40 minutes/day, >40 minutes/day), exercise (<1 hour/week, >1 hour/week), occupational activity (sitting down >50%, sitting down <50%), pack-years (tertiles plus never smoker category), liver disease (yes/no), kidney disease (yes/no), celiac disease (yes/no), inflammatory joint disease (yes/no), and dietary intake of calcium (tertiles) and iron (tertiles), fruit and vegetable intake (tertiles of g/d).

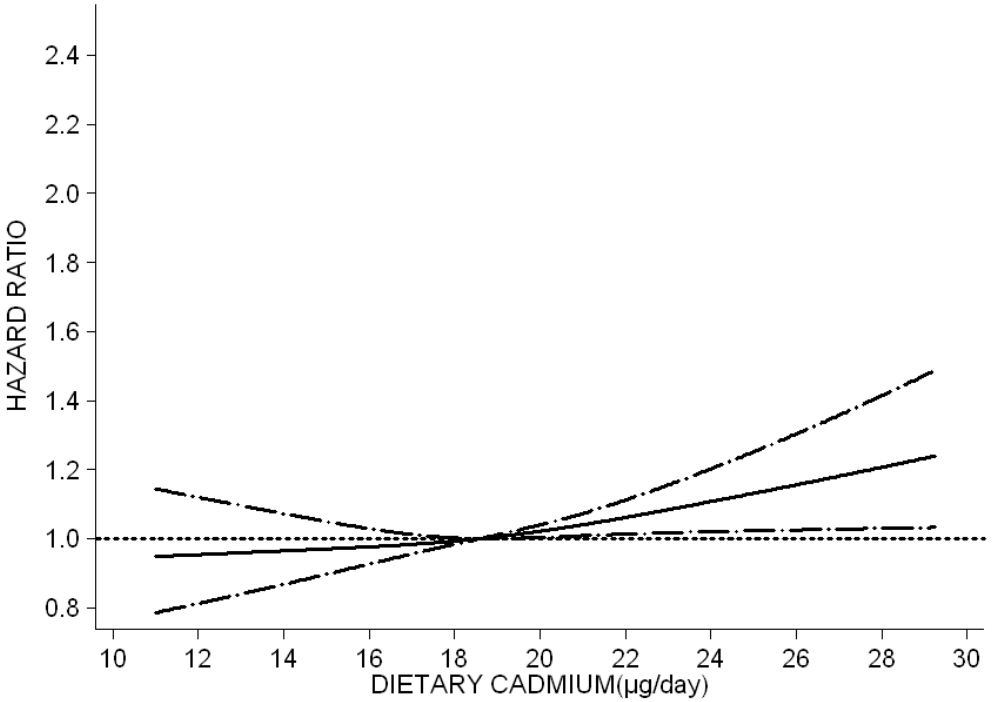

Figure 10. Dietary cadmium exposure and incidence of any fracture. HRs and 95%CIs of dietary cadmium exposure in relation to risk of any fracture using restricted cubic splines. Plot covers the central 98% of the dietary cadmium exposure values. For full details of the variables included in the multivariable adjusted model, please see **Paper V**. Splines (solid line), 95% CIs (long dashed lines), and HR =1.00 reference line (short dashed line).

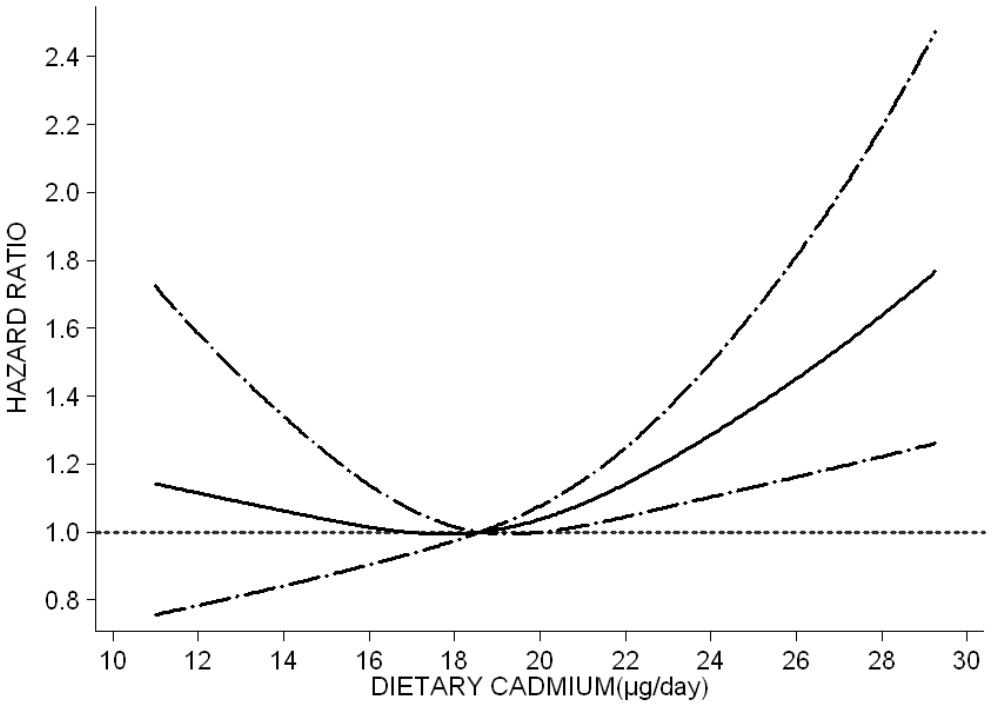

Figure 11. Dietary cadmium exposure and incidence of hip fracture. HRs and 95%CIs of dietary cadmium exposure in relation to risk of hip fracture using restricted cubic splines. For explanation see figure 10.

Smoking is a source of cadmium and is also associated with fracture risk. We therefore wanted to explore the combined effect of dietary cadmium and smoking on the risk of fractures (see **figure 12**). Never smokers in the lowest tertile of dietary cadmium were used as the reference group. Compared with the reference category, current smokers with high dietary cadmium exposure had a statistically significant 36% higher rate of any fracture (HR=1.36, 95% CI 1.15–1.61;).

Fruit and vegetables are thought to be beneficial for bone health and we therefore assessed the rate of fractures by consumption levels of these foods (See **figure 13**). Those in the highest tertile of fruit and vegetable consumption and the highest tertile of dietary cadmium were used as the reference group. We observed 41% higher rates among those with high dietary cadmium exposure and low fruit and vegetable consumption (HR =1.41; 95% CI 1.19–1.68) compared with the reference category.

Since fruit and vegetable consumption was found to be higher on average among never smokers (never: 429g/d; past: 401g/d; current: 347g/d), we assessed the fracture risk in current smokers with a high dietary cadmium exposure and low fruit and vegetable consumption. We observed in this group a 62% higher rate (HR = 1.62, 95% CI 1.32–1.99) of any fracture and a 75% higher rate (HR = 1.75, 95% CI 1.07–2.85) of hip fracture compared with the reference group (never smoker, low dietary cadmium exposure, high fruit and vegetable consumption).

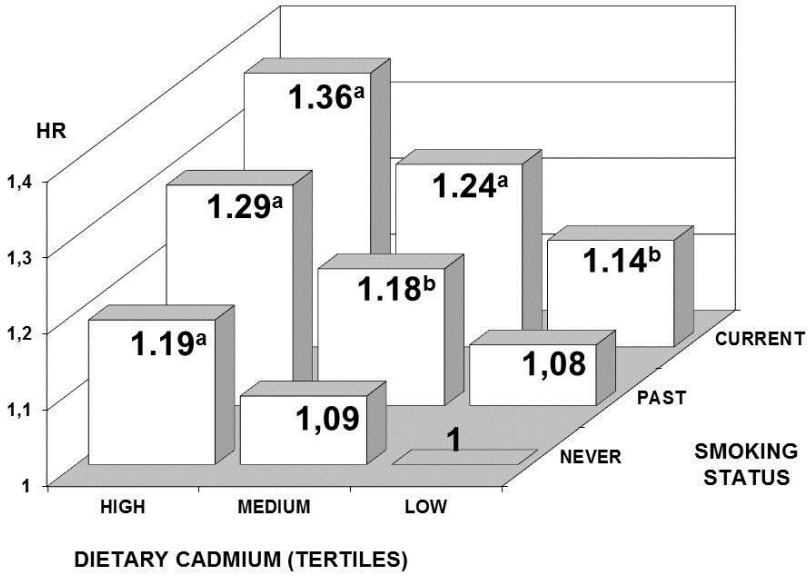

Figure 12. Dietary cadmium exposure and fracture incidence in relation to smoking status. Multivariable adjusted hazard ratios for dietary cadmium exposure and fracture incidence in relation to smoking status (never, past, current) using "never smoker", "low dietary cadmium exposure" as the reference category. For covariates please see **Paper V**. [a] indicates significance at the 0.01 level. [b] indicates significance at the 0.05 level.

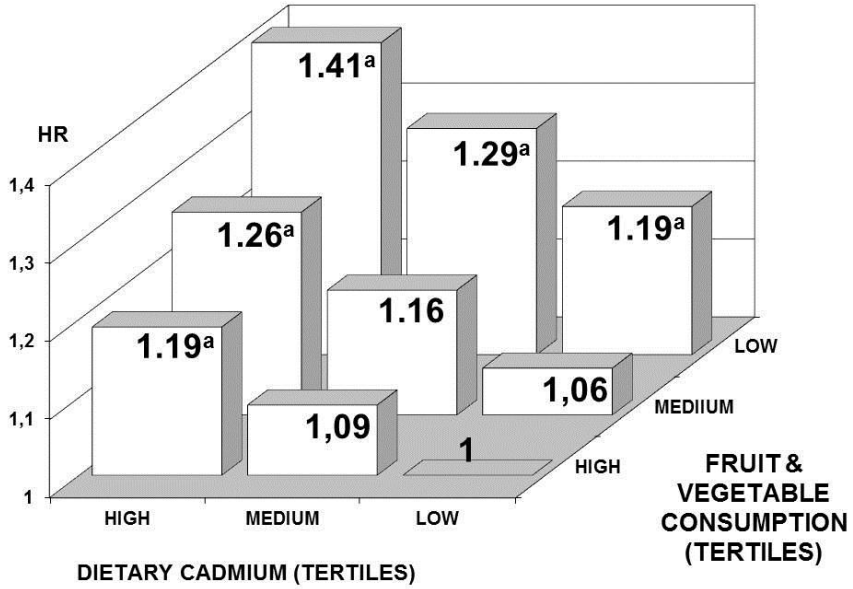

Figure 13. Dietary cadmium exposure and fracture incidence in relation to fruit and vegetable consumption. Multivariable adjusted hazard ratios for dietary cadmium exposure and fracture incidence in relation to fruit and vegetable (tertiles) consumption using "high fruit and vegetable intake" and "low dietary cadmium exposure" as the reference category. Median fruit and vegetable consumption in the highest tertile was 585g compared to 194g in the lowest tertile. [a] indicates significance at the 0.01 level.

Aetiological fraction and economic costs

Based on a reanalysis of the data from Paper V (see section 4.5.8), the aetiological fraction – the proportion of fractures attributable to dietary cadmium exposure – was estimated at 6.8% for men (see **table 1**). From this, the economic costs of fracture in men were calculated (see **table 2**).

Table 1. Data on which calculation of aetiological fraction is based.

Number of fractures in high exposure group	1,115
Total number of fractures	2,183
Prevalence of exposure among cases (p)	0.5108
Hazard ratio (HR)	1.153
Aetiological fraction (95% CI)	6.8 (2.4-10.9)

Table 2. Economic costs (million SEK/year) of all fractures for men in Sweden: total costs and costs attributable to high dietary cadmium exposure.

	Short-term healthcare costs	Long-term healthcare costs	Admin. etc.	Loss of QALYs	Total
Total	2,500	1,600	79	8,900	13,000
High Cd	170	100	5	600	880

A similar analysis was carried out for women and based on data from the SMC.[129,152] The estimated proportions of all fractures that can be explained by dietary cadmium exposure differs between the sexes, as does the relative distribution of the health care costs and quality adjusted life years (QALYs). In women, the estimated the aetiological fraction was 12.7% and the economic cost at 3,300 million SEK/year. Overall, the estimated cost to society of fractures attributable to high dietary cadmium exposure, was estimated at 4.2 billion SEK/year. The costs of deaths and suffering make up about two-thirds of the cost, while health care costs account for a third or SEK 1.46 billion.[150]

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

With any epidemiological study it is important to consider whether the observed results are valid. Results may reflect the true effect of an exposure on the development of disease; however, alternative explanation such as chance, error or confounding may also account for the findings. All of these alternatives explanations must be considered in order to assess the validity of an association. It is not possible to judge whether an association is causal based on the results and validity of any given study however, as this requires wider consideration of other criteria such as those set out by Bradford Hill[153].

6.1.1 Chance

An underlying assumption in most epidemiological studies is that inferences can be drawn for the entire population based on the findings from a sample of that population. One of the major problems in drawing such an inference is that random variation from sample to sample will always affect the results observed. The degree to which chance affects study findings is largely determined by study size, with variability decreasing as sample size increases. It is, therefore, important to quantify the degree to which chance variability may account for the results observed. The studies included in this thesis, use confidence intervals both to indicate statistical significance at the 95% level and to provide a measure of precision.

6.1.2 Error

Error is inherent in epidemiological studies and care must be taken to limit its role. As it is not possible to adjust for error at the data analysis stage, potential sources of error must be addressed in the study design. Error may be introduced as a result of the way in which individuals were selected to participate or the methods used to obtain and report information. Non-differential error, where misclassification is not related to disease or exposure status, typically results in an attenuation of the true association, while systematic error has the potential to bias the results in either direction.

The main sources of error in the studies included in this thesis are most likely non-differential and are therefore unlikely to account for the positive association observed in **Papers I, II and V**. The various types of error affecting epidemiological studies are discussed below.

Exposure misclassification

Three exposure metrics have been used in the papers included in this thesis: modelled air cadmium, urinary cadmium and dietary cadmium. In each case, the methods used to assess exposure were applied in an identical manner across all study participants. Therefore, while each exposure method is subject to error this is most likely non-

differential. Sources of error for each exposure metric are discussed elsewhere: modelled air cadmium (see section 6.2.1); urinary cadmium (see section 2.1.4); dietary cadmium (see section 6.2.3).

In most cases non-differential error leads to an attenuation of the true association, between exposure and disease, however, under certain circumstances this does not hold true. When polychotomous categorization is used, the association in the intermediate category may be biased away from the null[154] and this effect may conceal a dose-response relationship. In nutritional epidemiology it is not common to use dichotomous categorization unless the variable can be defined as exposed and non-exposed (eg. vitamin supplement use). In **Papers III-V** we categorized members of the cohorts into tertiles of dietary cadmium exposure. While we cannot say for certain whether our results for the intermediate category have been biased away from the null, evidence of a dose-response association in the results for **Paper V** suggest that this is not a major concern.

Outcome misclassification

Each outcome has its own challenges with regard to ensuring complete case ascertainment and these are discussed below. The Swedish National Hospital Discharge Register and the National Outpatient Register are considered to be virtually complete,[148] however, it is recognized that some cases may go undiagnosed or may be misdiagnosed. It is important to note, however, that any outcome misclassification in our studies (**Papers III-IV**) is mostly likely non-differential and would, therefore, be expected to attenuate the true association.

Chronic kidney disease

In its early stages, those with CKD may experience only very mild symptoms and be unaware of their condition. Those with risk factors for CKD such as diabetes are more likely to undergo screening and therefore be diagnosed. This difference in case ascertainment is one of the reasons we have chosen to exclude diabetics from our analysis in **Papers III-V**. In addition to screening, variations in methods used to estimate GFR will affect the number of cases of early-stage CKD that are diagnosed.[57] In clinical practice, the renal function (GFR) is often assessed based on simple measurement of plasma or serum creatinine. This is however a crude estimate and a much better estimation of GFR (eGFR) is obtained if appropriate formulas including age and sex are used.[155] While eGFR is unlikely to have been assessed using validated formulas, we believe this is unlikely to be a significant source of error.

Kidney Stones

Data on kidney stones has been gathered from National Hospital Discharge Register and the National Outpatient Register. Kidney stones are typically asymptomatic until they start to cause obstruction in urinary flow resulting in renal colic. The severe pain associated with kidney stones means that most cases are diagnosed in hospital following presentation at acute care or general practice.

Fractures

All hip fractures will require hospital care and therefore will be picked up by the Hospital Discharge Register. For fractures at other sites, outpatient treatment may be sufficient and therefore it is particularly important that complete outpatient data is used. For **Paper V** we restricted our analysis to Västmanland county as, at the time of analysis, fracture data from Regional Hospital Diagnosis Register was not available for Örebro county.

The diagnosis of bone fractures requires the physician to make a judgement both on the degree of fracture and the site, based on X-ray images. This is a potential source of error. In addition, fractures may go undiagnosed; this is particularly true of vertebral compression fractures, which lead to a gradual change in height and posture but which may not be brought to the attention of a physician. A significant proportion of vertebral fractures are asymptomatic and it is thought that only around 30% come to clinical attention in Sweden and the US,[104] with even fewer being entered into hospital registers.[156] In addition, as vertebral compression fractures are progressive, it is difficult to say exactly when the fracture occurred; this may be problematic for survival analysis.

As our study design is prospective and diagnosis is made with no knowledge of the patient's exposure status, misdiagnosis or under-diagnosis of fractures is most likely non-differential and may not, therefore, account for the positive association reported in **Paper V**.

Loss to follow-up

Loss to follow-up represents an important, potential source of bias in prospective cohort studies and can be particularly problematic in cohorts with long follow-ups. Both the SMC and COSM cohorts have been followed for over a decade and are therefore vulnerable. However, linkage of the cohorts to high quality register data has allowed this potential source of bias to be kept to a minimum. Dates of death have been captured by the Swedish Death Register and migration out of the study area (**Paper V**) is recorded in the Swedish Population Register, both registers are considered to be virtually complete. Cases have been ascertained using high quality inpatient and outpatient data (see section 4.4). The Avonmouth study is cross-sectional and loss to follow-up does not therefore apply.

Selection & response bias

Selection bias occurs when there is a systematic difference in the way participants are recruited. In the Avonmouth study, potential participants were selected at random from those eligible. Similarly, in the COSM and SMC cohorts all those who were eligible to participate were invited. Therefore the selection procedure is unlikely to be a source of bias in our studies. The response rate for the Avonmouth study was rather low (21%) and some response bias is therefore likely, however, as participants would be unaware

of their biomarker status we believe that this bias is unlikely to affect the findings (see section 6.1.4).

6.1.3 Confounding and effect modification

A confounder is a factor that is associated with both the exposure and, independently of that exposure, is a risk factor for the disease; that is to say, not on the causal pathway between the exposure and disease. Such factors may account in whole or in part for any observed association if they have not been adjusted for in the statistical analysis. The detailed diet and lifestyle data collected for the COSM and SMC cohorts allowed us to adjust for a number of potential confounders, which were identified from the literature (**Papers III-V**). While we cannot exclude the possibility of uncontrolled or residual confounding we believe this is minimal.

6.1.4 Generalizability

It is also important to consider whether the results are applicable both to the full population from which the participants are drawn and to other populations.

Avonmouth

For the Avonmouth study we aimed to recruit a sample, representative of the local population. We therefore used stratified random sampling to identify potential participants and over sampled from sex and age categories from which we expected a low response rate. Although we were interested in the effect of living close to the smelter, we did not select based on how long people had lived in the area, as this would have affected the generalizability of our findings. Using data from the census we compared responders to non-responders in terms of socioeconomic status and found no significant differences. However, given the low response rate (21%) and the small study size, the study population is unlikely to be fully representative of the Avonmouth population in general. For example, we might reasonably expect that residency time in the area would affect response rate. It is difficult to know, however, whether those who have lived in the area for a long time or those who have only recently moved to the area, would be more concerned and therefore more likely to respond. While response bias may affect the generalizability of our results it is unlikely to affect the validity since participants will be unaware of their biomarker status.

SMC & COSM cohorts

The SMC and COSM cohorts are described as 'population-based cohorts' as participants were recruited from the general population. The results from our studies should, therefore, be generalizable to the population from which they were drawn – that is to say, middle aged and elderly Swedish men and women – provided no significant response bias has taken place. Those who participate may differ from nonparticipants in a number of ways. The response rate was 70% among women in 1997 (SMC) and 49% among men (COSM). The COSM cohort has previously been shown to be

representative of Swedish men in terms of age distribution, education level and prevalence of overweight.[139]

6.2 MAIN FINDINGS AND GENERAL DISCUSSION

6.2.1 Cadmium exposure & tubular dysfunction

Among our population sample of 180 people living in the vicinity of the Avonmouth zinc smelter, we found median urinary cadmium concentrations of 0.18 and 0.31 nmol/mmol creatinine in non-occupationally exposed, non-smoking men and women, respectively. The corresponding data for smokers were 0.40 in men and 0.46 in women. In its 2009 evaluation, EFSA concluded that the cadmium burden on the kidney should not exceed cadmium levels in urine above 1 µg Cd/g creatinine (~1nmol/mmol creatinine) in order to prevent tubular dysfunction. Out of our population sample of 180 people, six participants (3.3%) were found to have U-Cd values greater than 1 nmol/mmol creatinine. As with previous studies,[19] our results identify women as a particularly susceptible subgroup, with significantly higher U-Cd concentrations than men. This difference between the sexes is well established and is thought to result from an up-regulation of the iron-transporters, through which both cadmium and iron are absorbed from the digestive tract, among those with depleted iron stores.[18,19] However a higher creatinine excretion in men than women also contributes to this difference.

Air dispersion modelling and exposure sources

We modelled cadmium emissions from the Avonmouth smelter in order to evaluate whether exposure in the local population was related to emissions from the site. The air dispersion model was constructed using emissions data and validated against air monitoring data. We found a good correlation between ADMS-modelled levels and monitoring data, suggesting that the modelled values were valid estimates of cadmium exposure from the smelter, however, the model underestimated concentrations by around a factor of four. One possible reason for this underestimation is that fugitive emissions, which are thought to have been significant, could not be included in the model because these were never quantified. Fugitive emissions are those that escape through outlets other than the chimneys, for example through windows. Smelting operations produce a great deal of heat and fumes and therefore in order to improve working conditions and reduce occupational exposure it is important to maximise ventilation. At the Avonmouth smelter, some stages in the process took place in buildings that were largely open-sided and therefore fugitive emissions are likely to have been high. The possibility of including fugitive emissions in the air dispersion model, as an area source emission, was discussed. The decision was made not to do this based on the fact that it would require too many assumptions to be made.

We modelled outdoor, ground-level air cadmium concentrations at the residential address of each study participant. It is recognised that this only represents a proxy for

individual air cadmium exposure since indoor and outdoor concentrations can vary considerably and people will not spend all their time at the same address. In addition we only used the current residential address and did not take account of how long they had lived there. These limitations are likely to lead to random misclassification of the air exposure estimate, such that any relationship between modelled air cadmium and urinary cadmium would be biased towards the null. In our analysis modelled air cadmium was found to be a significant predictor of urinary cadmium.

In addition to the anticipated cadmium exposure from the smelter, individuals will have been exposed to cadmium from other sources, in particular, from tobacco smoke, which is known to be a major source of cadmium exposure. In our study, mean U-Cd levels in "ever-smokers" were approximately twice those of "never-smokers"; this is similar to findings in other studies.[34]

Dose-effect relationship and clinical significance

The results of our study show a significant dose–effect relationship between U-Cd and U-NAG in both men and women and a significant dose–effect relationship between U-Cd and U-A1M in women. We also found a significant dose-dependent trend in the prevalence of early renal dysfunction, as assessed by U-NAG, and this trend persisted after exclusion of current and past smokers. This is in line with several recent studies, which have shown effects at low-levels of cadmium exposure.[90,91]

The biomarkers of tubular dysfunction investigated in this study (A1M, RBP and NAG) are not used in clinical practice and indicate potential health impairment rather than any overt disease. Indeed the tubular effects associated with cadmium exposure do not in themselves give rise to symptoms or clinical disease. There is, however, some data to suggest that these changes may progress to glomerular dysfunction if exposure is prolonged. In **Paper I** we have described U-NAG values above the reference as indicating renal 'damage'. Given that the clinical significance of elevated U-NAG concentrations is not clear I now feel that renal 'dysfunction' would be a more appropriate term. In our analysis we have used total NAG (NAG-a & NAG-b), however, only the activity of the membrane-bound isozyme NAG-b is an indicator of cell shedding. The use of total NAG relies on the assumption that there is a correlation between NAG-b and total NAG. This may not always hold true and alternative explanations for elevated NAG excretion include exocytosis and interference with enzyme activity by inhibitors.

The role of co-excretion

It has recently been suggested that the apparent association between proteinuria and low-level urinary cadmium may reflect co-excretion rather than any toxic effect[94-96] (Please see section 2.3.4). The cadmium exposure levels observed in the Avonmouth population are in the range where factors such as diuresis, current smoking and co-excretion may, according to recent research, account for any apparent associations. It is possible, therefore, that our study overestimates the adverse effects of cadmium on

kidney function. However, the main association observed in our study is between U-Cd and U-NAG. NAG is an intracellular enzyme and its presence in urine indicates increased cell turnover (NAG-b) rather than reduced reabsorption. No studies have so far investigated whether the apparent association between U-NAG and U-Cd at low exposures may similarly be accounted for by normal inter-individual variation. We may reasonable expect that, as with low-molecular weight proteins, factors such as smoking or diuresis influence the excretion of U-NAG. In our analysis, we see a stronger association between urinary cadmium and U-NAG after excluding smokers, this suggests that the observed association cannot be accounted for by smoking.

Study design and sample size

The cross-sectional design of this study means that we do not have any information on whether exposure came before effect. This temporal relationship is important when considering causality.[153] This study, which was originally intended as a pilot study, is small in size and as a consequence the power of the study is limited. This may account for the lack of any observed association between urinary cadmium and U-RBP and U-A1M in men. A large population sample would have allowed us to adjust for more variables and would have benefited the study.

Concomitant exposure to other nephrotoxic metals

The biomarkers of tubular dysfunction used in this study are not specific to cadmium. Along with cadmium, the smelter in Avonmouth emitted arsenic, lead, and mercury, all of which are nephrotoxic. It is likely that these metals followed a pattern of dispersion very similar to that of cadmium because they were emitted from the same stacks and share similar chemical properties. The similar patterns of exposure mean that it is not possible to separate the effects of cadmium from those of the other nephrotoxins; our finding could therefore result from exposure to a mix of several nephrotoxic metals. It is important to note however, that while we see an association between modelled emissions from the smelter and urinary cadmium, the main sources of cadmium exposure in this population are likely to be the diet and smoking.

6.2.2 Cadmium and metabolic profiling

NMR-based metabolic profiling (metabonomics/metabolomics) offers a new approach to identifying the biochemical disturbances resulting from exposure to xenobiotics such as cadmium, and in so doing, to aid the discovery of novel biomarkers of toxicity. In addition, the metabolic signature identified by this process could help determine the mechanism of action of a toxicant and the etiology of the associated disease. More broadly, metabolic phenotypes have the potential to help define the complex interaction between lifestyle, environment and genes that ultimately determine disease development.[157]

So far, the use of metabolic profiling techniques has been largely confined to animal models where variability in the population can be closely controlled. Use of the technique in a human population presents new challenges owing to the inherent

variability in factors such as exposure, genotype, age and disease. It has been well documented that metabolic profiling can detect cadmium-induced perturbations of metabolism in experimental animal studies,[158-160] however, no comparable studies have so far been carried out in human populations. Our study using urine samples collected from an uncontrolled human population is therefore the first of its kind.

This study can be viewed as a proof of concept study since metabolic profiling techniques have not previously been applied to an uncontrolled human population in this way. Using an NMR-based approach, we have demonstrated the capacity, in principle, of metabolic profiling to characterize the metabolic consequences of exposure to environmental toxicants, such as Cd and tobacco smoke, in a human population. The results of this study suggest that in identify novel biomarkers and molecular signatures of the effects of exposure, metabolic profiling has the potential to improve risk assessment models and ultimately guide intervention to prevent disease progression.

Associated metabolites

Metabolic profiling identified six urinary metabolites that were associated with urinary cadmium. All six metabolites are related either to mitochondrial metabolism (citrate, 3-hydroxyisovalerate, 4-deoxy-erythronic acid) or one-carbon metabolism (dimethylglycine, creatinine, creatine). After adjustment for age and sex, only citrate levels retained a significant correlation to urinary cadmium, however, it is important to recognise that the power of the study was limited due to the sample size. There is some evidence from previous studies on the biochemical effects of Cd to suggest that the metabolites identified in our study may be related to the underlying mechanisms of Cd toxicity.[161,162]

Creatinine

Creatinine was found to be negatively correlated with age and to be higher in men than in women. These findings highlight some of the known limitation of the creatinine adjustment of urinary biomarkers (see section 2.1.4).

Citrate

Our results identify previously unreported associations between urinary citrate and both urinary cadmium and smoking status. Citrate was positively correlated with U-Cd and negatively correlated smoking status. This difference is perhaps surprising given that smoking is an important source of cadmium exposure, however, it is important to remember that cadmium is just one of over 98 hazardous components identified in tobacco smoke.[163] The increase in urinary citrate levels associated with urinary cadmium may result from cadmium-induced tubule toxicity that affects citrate handling in the kidney.[164] We did not, however, observe a direct association between citrate levels and levels of urinary NAG – a marker of tubular dysfunction.

Smoking may reduce urinary citrate levels due to reduced capacity for oxidative metabolism in mitochondria. Previous studies have shown an association between chronic smoking and decreased activity of complex IV and III in the lymphocyte mitochondrial electron transfer chain, this was found to return to normal after cessation of smoking.[165,166] In our study, however, past smokers were found to have intermediate urinary citrate levels, relative to current smokers and never smoked. This observation suggests that metabolic differences persist after the cessation of smoking.

These findings are also of interest in regard to kidney stones, since citrate is an important inhibitor of calcium phosphate crystal growth.[167] This data provides one possible explanation for why any increased excretion in urinary calcium associated with cadmium exposure may not result in increased stone risk. Based on these finding we might also expect to see an elevated risk of kidney stones associated with smoking however this does not appear to be the case.

Cadmium and U-8-oxodG

Urinary 8-oxo-deoxyguanosine is a marker oxidative stress. Our analysis found 8-oxo-deoxyguanosine to be elevated in individuals with high cadmium exposure. This finding supports the hypothesis that cadmium may cause mitochondrial dysfunction. These findings are consistent with those reported from a population in Bangladesh.[168]

Study limitations

The study has several limitations that should be considered. Firstly, the sample size was rather small and as a consequence the study may have lacked power to detect associations, our findings therefore need to be confirmed in larger studies. A larger sample size and more detailed lifestyle and dietary data would also allow for adjustment for more potential confounders. The study would also have benefited from additional biological samples, such as blood plasma, as analysis of these samples would help to determine whether the observed associations indicated a toxic effect of cadmium or simply reflected renal function through co-excretion.

6.2.3 Dietary cadmium exposure

By estimating dietary cadmium exposure we are able to assess the association between cadmium from the diet independently of that from other sources such as tobacco smoke. This is clearly relevant from a public health perspective since dietary exposure represents the primary source of cadmium exposure at the population level. In addition, epidemiological studies relying on biomarker data are often limit in size due to financial constraints. Dietary cadmium estimates can be performed at relatively low cost, improving the feasible of large-scale epidemiological studies. It is important, however, to consider the validity of the estimates.

Dietary cadmium levels

The estimated dietary cadmium exposure (median intake 19µg/day men; 13µg/day women) levels in our two cohorts were in the same range as those observed both in the United States and elsewhere in Europe: Catalonia men 15.7 µg/day, women 12.0 µg/day[169]; UK 12.0 µg/day[170]; United States men 19.3 µg/day, women 18.5 µg/day[171]; United States 11.4 µg/day (for an adult of 60kg)[172]; and Denmark 16.0 µg/day.[173] An assessment of dietary cadmium levels in Sweden, estimate weekly dietary cadmium intake at 1µg/kg b.w./week[174]; this is lower than in our study populations (see **figure 14)**.

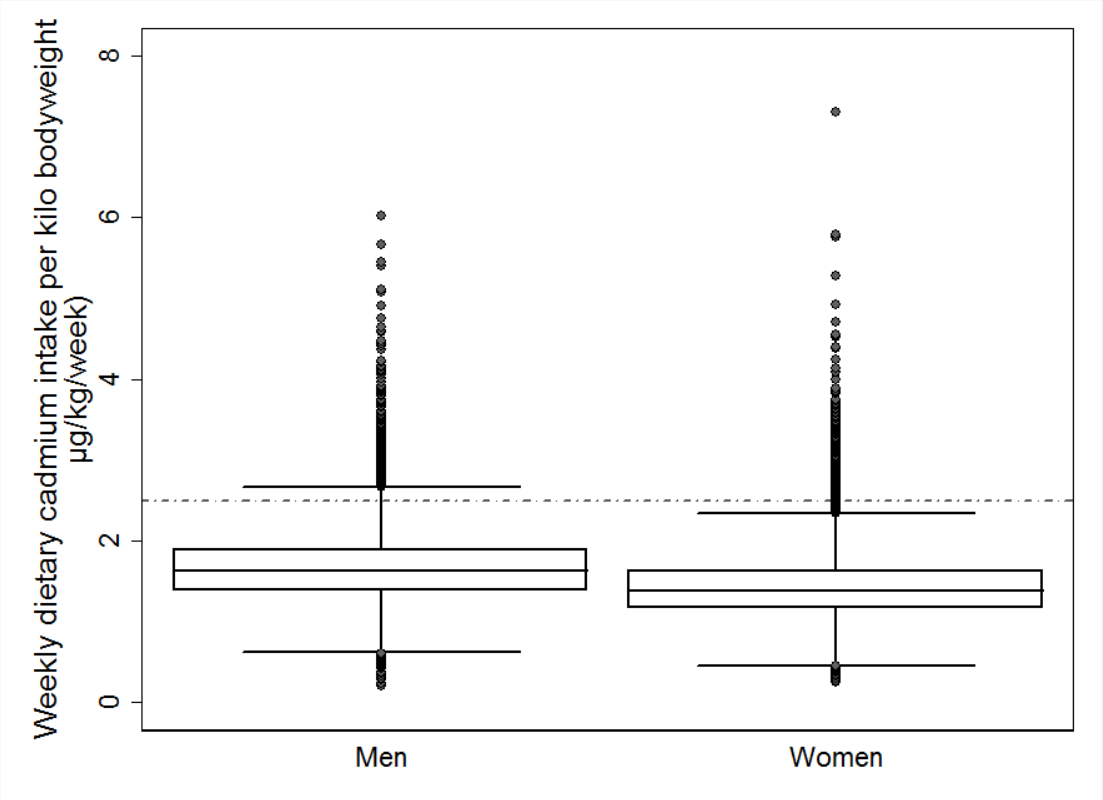

Figure 14. Boxplot showing weekly dietary cadmium intake per kilo bodyweight for men and women. EFSA TWI of 2.5 µg/kg/week (short dashed line).

A risk assessment of cadmium carried out by the European Food Safety Authority (EFSA) set a tolerable weekly intake (TWI) for cadmium of 2.5µg/kg body weight, based on the detrimental effects of cadmium on the kidney.[9] In our study populations the TWI was exceeded in 3.3% of men and 1.6% of women (see **figure 14**.). EFSA also concluded that the cadmium burden on the kidney should not exceed cadmium levels in urine above 1 µg Cd/g creatinine. Among a subsample of 2,688 women from the SMC cohort, the median urinary cadmium concentration was 0.34 µg/g creatinine,[129] with only 1.7% having concentrations >1 µg/g creatinine, indicating that exposure levels in our populations are largely below those at which detrimental effects on the kidney would be expected based on current knowledge.

Sources of dietary cadmium intake

The foods contributing most to dietary cadmium exposure are healthy foods such as wholegrain and vegetables.[9] In our cohorts these foods account for 80% of the total dietary cadmium exposure.[49] It is for this reason that among our study populations we observe a higher intake of several nutrients and a lower prevalence of smoking in those with the highest cadmium exposure. In our analyses we have adjusted for several nutrients and/or food groups, as these are a potential source of confounding.

The validity of dietary exposure estimates

The dietary cadmium exposure estimates used in this thesis have been validated previously. Among a subsample of women from the SMC, the questionnaire-based estimates of dietary cadmium were validated against model-predicted urinary cadmium concentrations. Urinary concentrations were modelled using a one-compartment model with a standard first-order elimination, taking the exponential shape of the elimination rate of Cd, as well as factors such as gastrointestinal absorption etc. into account. This validation gave a Pearson correlation of 0.2 (95% CI: 0.1-0.3), the sensitivity (58%) and specificity (51%) were also modest. Sensitivity was defined as the probability of being classified as high in the FFQ-estimated dietary cadmium if urinary cadmium was also high and specificity as the probability of being classified as low in FFQ-cadmium if also urinary cadmium was low.

There are several possible reasons for the rather modest agreement between dietary cadmium estimates and urinary cadmium. Firstly, some degree of measurement error in the dietary assessment is unavoidable. In completing the FFQ participants are required to recall and report their typical dietary intake, this is a difficult task and is inevitably a source of error. In our analysis, we have adjusted dietary cadmium exposure to the mean energy intake in the cohorts (men 2,600 kcal/day; women 1,700 kcal/day) and excluded those with implausible energy intake in order to reduce the effect of under and over reporting. The use of mean cadmium concentrations for each type of food is also a source of measurement error since cadmium is present in food as a contaminant and concentrations, in a given foodstuff, can vary much more widely for contaminants than for nutrients.

Secondly, the rate of cadmium absorption from the diet is dependent both on the composition of the food with which it is ingested and the nutritional status of the individual. Dietary cadmium intake may not therefore fully capture internal dose. Iron status, in particular, is known to be an important determinant owing to it effect on the regulation of DTM1 (see section 2.1.3). In a previous study from our unit, it was observed that dietary cadmium intake, measured in duplicate portions, was a reasonable predictor of biomarkers of both long-term kidney accumulation (urine) and short-term exposure (blood) and that predictions were substantially improved by taking individual iron status into account.[175] The size of the study cohorts means that it is not feasible to collect data on serum ferritin for all study participants. Moreover, serum ferritin in

postmenopausal women may not reflect their iron status during their pre-menopausal years, when much of the cadmium absorption is likely to have taken place.

Thirdly, urinary cadmium is a measure of exposure from all sources. While the diet is the dominant source of exposure in the general, non-smoking population, it is not the only source. The two exposure metrics are therefore not directly comparable.

Fourthly, while biomarkers represent the best method for assessing internal dose they are not without their limitations. Urinary cadmium is generally considered to be a good biomarker of cadmium concentrations in the kidney.[27,28] It is recognized, however, that factors such as diurnal variation, dilution adjustment and analytical method are sources of variation. Accumulated levels within the kidney are our primary interest when considering kidney related outcomes such as CKD and kidney stones, however, levels in the kidney may not be as relevant when considering cadmium's effects on bone. A recent study among a subsample of the SMC cohort concluded that the use of urinary or dietary cadmium exposure alone, underestimated the adverse effect of cadmium in terms of reduced BMD, risk of osteoporosis and risk of fractures (see **figure 15**) and that by combining the two exposure estimates the level of misclassification was substantially reduced.[152] These results suggested that urinary cadmium may not fully capture the internal dose and provides important support for the use of FFQ-based dietary cadmium estimates in large-scale epidemiological studies.

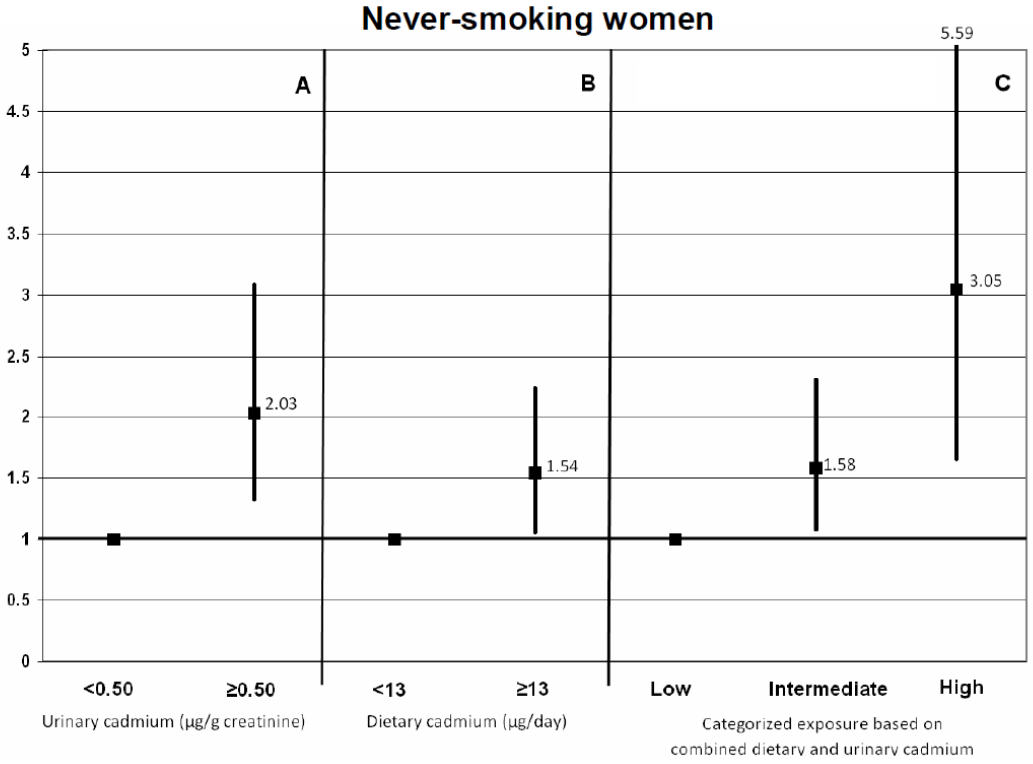

Figure 15. Multivariable-adjusted odds ratio and 95% CI of any first incident fracture among never-smoking women. Urinary cadmium (A) as categorized into below (low) or above (high) 0.50µg/g creatinine; estimated dietary cadmium (B) as categorized into below (low) or above (high) the median, 13µg/day and (C) with combined high urinary cadmium (≥0.50µg/g creatinine) and dietary cadmium (≥13µg/day), as compared to the reference category (<0.50µg/g creatinine and <13µg/day). From 'Cadmium as a Risk Factor for Osteoporosis and Fractures in Women.' A Engström.[176]

6.2.4 Cadmium and chronic kidney disease

This large prospective population-based cohort study is the first to assess the association between dietary cadmium exposure and CKD. We found no evidence of an association between dietary cadmium exposure and an increased risk of CKD either before or after stratification by smoking status. Our results are consistent with the findings of a prospective nested case-referent study which showed no association between erythrocyte cadmium and CKD at similar exposures.[84]

With an average dietary cadmium exposure of 13μg/d in women and 19μg/d in men our study populations are at the lower end of the exposure gradient. Exposures in our cohorts are therefore not comparable with exposures seen in the occupationally exposed or those living in areas with significant contamination.

The exceptionally high exposures seen in Toyama Prefecture and also among occupationally exposed groups are not, however, comparable with the relatively low-level exposure experienced across most of the World. Recent studies have reported reduced glomerular function in relation to cadmium in low exposure areas,[78,79,82] however, as previously discussed (see section 2.4.1) the interpretation and clinical significance of these findings have been questioned. By using dietary cadmium as our exposure metric we are able to avoid the potential issues with co-excretion that make the results of biomarker studies difficult to interpret.

Smoking as a confounder

Smoking is an important source of cadmium exposure and is associated with an increased risk of CKD,[177] it therefore a potential confounder. In our study we both adjusted for pack-years of cigarette use and stratified for smoking status.

Previous biomarker studies have reported decreased GFR in relation to low-level cadmium exposure. While many of these studies adjust for smoking status, residual confounding cannot be ruled out. A study by Åkesson et al reported a decreased glomerular filtration rate (GFR) (as estimated by cystatin C in serum) and decreased creatinine clearance (as assessed by serum creatinine), in relation to both blood and urinary cadmium in a Swedish population.[78] However, only the association between blood cadmium and creatinine clearance persisted among never smokers. This may imply that the other observed associations resulted from confounding by a non-cadmium dependent effect of smoking. In addition, as the biomarkers of dose and effect, as measured in blood, are likely to be similarly effected by GFR the observed association between blood cadmium and creatinine clearance may also indicate confounding rather than any toxic effect.

Chronic kidney disease and cardiovascular disease

CKD is associated with an increased risk of cardiovascular disease (CVD) and conversely hypertension is a risk factor for CKD. Given this inter-relationship we might therefore expect to see the results for CKD reflected in those for CVD in relation

to dietary cadmium exposure. Previous studies carried out to assess the relationship between dietary cadmium exposure and CVD (myocardial infarction, ischemic and haemorrhagic stroke) incidence and mortality, in the SMC and COSM cohorts, found no association.[37,38] Within other populations, studies using biomarkers of exposure have shown inconsistent results in terms of CVD.[39,40,178,179]

Limitations and possible explanations for a lack of an observed association between cadmium and chronic kidney disease.

There are several limitations to consider which may account for the lack of an observed association. Firstly, our dietary cadmium exposure estimates are based on self-report, and on the average cadmium content in each reported food; this inevitably leads to some degree of measurement error. We cannot, therefore exclude the possibility of exposure misclassification. This may in part account for the rather low correlation between FFQ-estimated dietary cadmium and urinary cadmium concentrations ($r=0.2$)[144] which suggests that the dietary cadmium estimates may not fully reflect concentrations in the kidney. Misclassification is most likely non-differential and would, therefore, lead to an attenuation of the true association. Secondly, the range of exposures in our study population is relatively narrow and may not provide sufficient differentiation between high and low exposures for an effect to be identified. In this study we had sufficient power (>80%) to detect increased HR for CKD of 1.30 in men and 1.48 in women. Thirdly, the exposure levels in our populations may simply not have been high enough. The estimated dietary cadmium exposure in our study populations were in the same range as those observed in other areas of Europe and the US with no significant sources of contamination (see section 5.3). These exposures represent the lower end of the exposure gradient and are not comparable with exposures seen in the occupationally exposed or those living in areas with significant contamination. In our study populations, the EFSA TWI (see section 2.4.2) was exceeded in 3.3% of men and 1.6% of women. Among a subsample of 2,688 women from the SMC cohort, the median urinary cadmium concentration was 0.34 μg/g creatinine,[129] with only 1.7% having concentrations >1 μg/g creatinine – the reference level set by EFSA. This indicates that exposure levels in our populations are below those at which detrimental effects on the kidney would be expected based on current knowledge.

6.2.5 Cadmium and kidney stone incidence

This study is the first to prospectively assess the risk of kidney stones associated with cadmium exposure in a non-occupationally exposed population. In this large population-based cohort we observed no increased risk of kidney stones in relation to dietary cadmium exposure in men or women.

Kidney stone composition

Our hypothesis is that cadmium exposure may increase the risk of calcium stone formation through increased calcium excretion. Hypercalciuria is a known risk factor for calcium stone formation, however, kidney stones may be composed not only of calcium oxalate and calcium phosphate but also uric acid or cystine. We do not have any information on the composition of the stones in our study population, however, an analysis of stone material collected from 3,176 men, treated with extracorporeal shockwave lithotripsy in Stockholm County, found that 95.6% of stones contained calcium oxalate and in 92.6% it was the dominant component (H-G Tiselius, personal communication). It is thus reasonable to assume that at least 90 % of the stones in our study population were composed primarily of calcium oxalate (Tiselius 1996).

Cadmium and calcium metabolism

The skeleton and kidneys both play a key role in calcium homeostasis (**Figure 16**). Serum calcium is closely regulated and is maintained within a tight range by balance between intestinal absorption of calcium, the excretion of calcium in urine and calcification and decalcification of bone – around 99% of the calcium within the human body is contained within the skeleton. The major fluxes of calcium are regulated by parathyroid hormone (PTH), which increases renal tubular reabsorption and bone reabsorption; calcitonin, which inhibits bone reabsorption and vitamin D, which augments intestinal absorption of calcium. An imbalance in one part of the system can have consequences elsewhere in the system. For example those with hyper absorption are at an increased risk of kidney stone formation and those who have had kidney stones are more likely to have a fracture.[180]

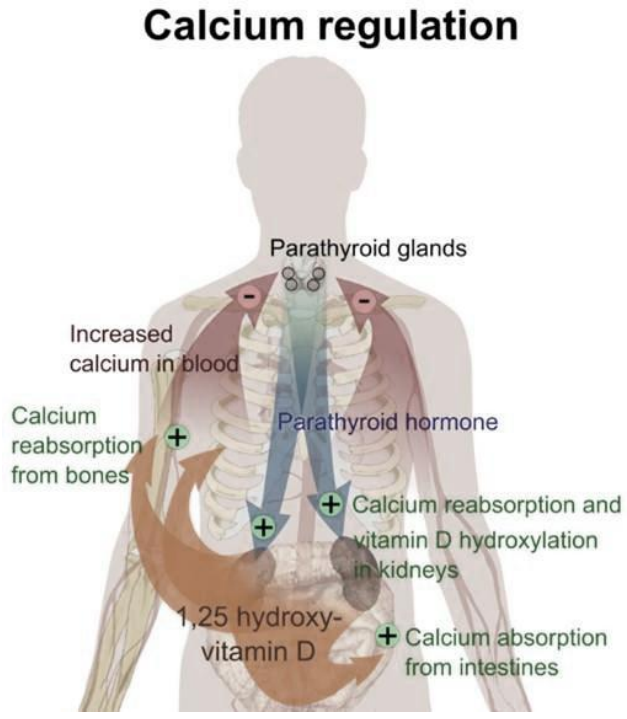

Figure 16. Calcium regulation in the human body

Cadmium may interfere with calcium homeostasis in a number of ways. Firstly, it may increase calcium excretion as a result of damage to the kidney tubule. Secondly, it may increase bone resorption by stimulating osteoclasts. Thirdly, it may interfere with the activation of vitamin D and fourthly it may reduce cadmium absorption from the intestines.

Positive associations have previously been observed between urinary cadmium on the one hand and bone resorption markers[132,133] and calcium excretion[129,133] on the other. In view of our observed associations between dietary cadmium and reduced bone mineral density and increased fracture risk,[129,152] (**Paper V**), this may indicate an effect of dietary cadmium exposure on increased bone resorption in our study populations which may translate into increased urinary calcium excretion.

Other dietary factors related to kidney stones

Oxalate is an important component of the majority of kidney stones in Sweden. The analysis of oxalate in food is problematic and there is currently insufficient, reliable data on the oxalate content of various foods. We were therefore unable to adjust for dietary oxalate in our analysis and this represent a potential source of confounding.

Kidney stone recurrence

Kidney stones are highly recurrent and by excluding those with a previous diagnosis of stones we may be excluding those who are most susceptible as well as reducing the power of the study. In the case of kidney stones it is particularly important to make these pre-baseline exclusions since patients are likely to modify their diet either based on the recommendations from the doctor or others.

6.2.6 Cadmium and fractures

Our findings indicate that dietary cadmium exposure, at levels seen in the general population, is associated with an increased risk of fractures. In this large population-based study of men, we show for the first time, an association between dietary cadmium exposure and higher rates of both any fracture and hip fracture; these findings were independent of tobacco smoking. This study provides the first data on hip fracture rates in relation to cadmium exposure and is the first to report an excess risk of any fracture associated with long-term low-level cadmium exposure in men. Our prospective results in men are similar to those observed in our cohort of Swedish women[129,152] (see **figure 15**). They are also consistent with previous findings in other populations and, in addition, show a clear statistically significant increased rate of all fractures.

Smoking as a confounder

Smoking is potentially an important confounder when investigating the association between cadmium exposure and bone effects. As previously discussed (see section 2.1.2), smoking is an important source of cadmium exposure. Smoking has also been shown to increase fracture risk.

Data from the SMC and COSM cohorts allow us to adjust for smoking status (never, past, current) and/or for pack years – a measure of smoking intensity. While this represents good data on smoking habits it may not fully capture lifetime cumulative exposure to tobacco smoke. For example, exposure at an individual level will depend on inhalation frequency and depth; cigarette brand and smoking patterns.

We found a stronger association between dietary cadmium exposure and hip fractures among never smokers than among ever smokers (**Paper V**). We were able to show, for the first time, the independent effect of dietary cadmium on fracture risk, as well as the combined effect of both tobacco smoking and dietary cadmium exposure. Our findings provide further evidence that the observed associations between cadmium exposure and bone effects are not simply driven by the effect of smoking, and are consistent with those of Engström et al, 2012.[152] These data support the causality of our findings and help to characterize the risk associated with combined exposure to dietary cadmium and tobacco smoke.

Low impact fractures

Osteoporotic fractures are usually defined as a fracture associated with minimal trauma such as a fall from standing height. While several studies have shown that osteoporotic individuals are more likely to suffer a fracture irrespective of the cause of injury[181,182] inclusion of both high and low-impact fractures will lead to a lower risk estimate than for minimal-trauma fractures alone.

Fruit and vegetables

In the COSM cohort, fruit and vegetables account for 15% of dietary cadmium exposure. These foods are also high in a number of number of vitamins and minerals that have previously been linked to higher bone mass, such as vitamin C,[183] zinc, potassium, and magnesium.[184] In addition, the alkalizing effect of fruit and vegetables may promote the retention of bone mass by helping to maintain the acid-base balance, thereby avoiding the alkali-access effect on bone.[184,185]

Since those in the highest tertile of cadmium exposure reported a higher intake of these foods, we conducted an analysis adjusting for fruit and vegetable consumption. This analysis showed that those in the lowest tertile of fruit and vegetable consumption and the highest tertile of dietary cadmium exposure had a 41% higher rate of fracture compared with those with low cadmium exposure and high fruit and vegetable consumption. These results suggest that cadmium is counteracting the protective effects of these foods.

Aetiological fraction and economic costs

The calculation of the aetiological fraction and associated costs requires some assumptions to be made. Firstly, we assume that the observed association between dietary cadmium exposure and fracture risk is causal. While causality cannot be established based on the current evidence, the consistency of findings, from both

experimental and epidemiological studies, is suggestive of a causal association between cadmium and bone effects. Secondly, that the relationship between dietary cadmium exposure and fracture risk is linear. Our spline analysis supports a linear relationship.

There is currently insufficient data to perform a meta-analysis on the association between dietary cadmium exposure and fracture risk. Such an analysis would provide a more robust point estimate and further studies in this area would therefore be beneficial.

In our Cox proportional hazards model, we include only the first fracture occurring after baseline. This could result in an underestimation of the true proportion of fractures occurring as a consequence of high dietary cadmium intake, since it does not allow for multiple fractures (and associated costs) occurring in the same individual. Another potential limitation is that our calculations do not take account of fractures occurring in the under 50s.

The proportion of fractures that are due to elevated dietary cadmium exposure is estimated at 7% for men and 13% for women. Data from the health-economic study shows that the economic costs of fractures in people over the age of 50 are SEK 39 billion per year in Sweden. Based on this, we estimate that the economic cost of fractures caused by elevated cadmium concentrations in food is roughly SEK 4.2 billion per year. Health care costs total almost SEK 1.5 billion, while other costs are due to reduced quality of life and premature death occurring as a consequence of the fracture. These results show the high economic costs that may be incurred as a result of the cadmium in our diet, and highlight the need to reduce exposure.

7 CONCLUSIONS

- The urinary cadmium concentrations in our population sample were in the same range as those where associations with kidney and/or bone effects have been observed previously.

- Modelled air cadmium concentrations were found to be a significant predictor of urinary cadmium concentrations suggesting that emissions from the smelter have contributed to exposure in the local population. Dose–response relationships between U-Cd and U-NAG support the need for measures to reduce environmental cadmium exposure.

- Using an NMR-based approach, we have demonstrated the capacity, in principle, of metabolic profiling to characterize the metabolic consequences of exposure to environmental toxicants, such as Cd and tobacco smoke, in a human population.

- Our results do not support a role of dietary cadmium exposure in the development of CKD at these low exposure levels.

- Our null findings do not support a strong association between dietary cadmium and kidney stone risk at the exposure levels seen in the general population. This may suggest that any effect of cadmium exposure on kidney stone risk is only relevant at occupational exposure levels.

- Our results indicate that dietary cadmium exposure is associated with an increased rate of fractures among men even at relatively low levels of exposure from dietary sources. This association was independent of smoking and was most pronounced among men with low fruit and vegetable consumption. At a population level, the attributable risk may be considerable as a result of the high prevalence of the disease and the large number of people exposed.

The results of this thesis suggest that the toxic effects of cadmium exposure around the Avonmouth smelter may be detected in urinary biomarkers. In addition, the results of our prospective studies do not support a role of dietary cadmium exposure, at the level seen in the general population, in the development of CKD or kidney stones. However, our results do provide further evidence of increase fracture risk in relation to cadmium exposure. In conjunction with recent findings, the results of this thesis suggest that bone may be a more sensitive target of cadmium toxicity than the kidney in terms of clinically relevant outcomes.

8 FUTURE RESEARCH

The results presented in this thesis, contribute to the body of scientific evidence relating to the effects of low-level cadmium exposure on the bone and kidneys. Further research in this area should include:

- The application of metabonomic techniques to human biological samples has great potential for characterising the effects of exposure to toxins in the diet and environment. More studies in this area are required.

- No studies have so far investigated whether the excretion of U-NAG is effected by normal inter-individual variation in the same way as low molecular weight proteins such as B2M. Such information would help to determine whether observed associations between urinary cadmium and U-NAG, at low levels of exposure, could be accounted for by co-excretion.

- Our data suggests that the bone may be a more sensitive target of cadmium toxicity than the kidney. While previous risk assessments have acknowledged the evidence relating to bone effects, the data has been considered insufficient and too heterogeneous for a risk assessment to be performed with this data. Further research in this area is therefore needed.

- Currently, much of the data on cadmium exposure and kidney stones comes from small occupational studies. A nested case-control study of cadmium exposure and kidney stones would help to determine whether there is a significantly increased risk of kidney stones at environmentally relevant levels of exposure. Such a study would benefit from urinary measurements of calcium, phosphate, citrate, uric acid as well as cadmium.

9 SAMMANFATTNING (SUMMARY IN SWEDISH)

Kadmium (Cd) är giftig metall som inte har någon biologisk funktion hos djur eller människa. Spridningen av Cd till miljön, bland annat via industriella processer och jordbruksnäringen, har lett till att människans exponering har ökat under det senaste århundradet. Kadmium tas upp av växter vilket leder till att kosten är den i särklass största exponeringsvägen och exponeringen är dessutom livslång. Rökare får även i sig kadmium via tobaksrök. För yrkesexponerade och boende i områden nära industriella utsläpp av Cd kan kontakten med den förorenade miljön också vara av betydelse för exponeringen. Inälvsmat, vissa skaldjur och fröer kan innehålla höga halter av kadmium, men den huvudsakliga exponeringen sker genom bröd och andra spannmålsprodukter, rotfrukter inklusive potatis och grönsaker. Kadmium utsöndras långsamt och ansamlas därför främst i njuren och det är sedan länge känt att metallen kan orsaka njurskador. Kadmium kan även skada skelettet. Det är dock fortfarande osäkert vid vilka exponeringsnivåer dessa toxiska effekter på njuren och skelettet uppstår. Dessutom är den kliniska relevansen av vissa väldigt tidiga effekter på till exempel njuren vid en låg exponering fortfarande oklar.

Syftet med denna avhandling var att: 1) mäta kroppsbelastningen av kadmium och tidiga tecken på njurpåverkan i en population boende i ett industriellt kontaminerat område samt att utveckla och validera en luftspridningsmodell för dessa utsläpp, 2) identifiera biomarkörer i urin med hjälp ”metabonomics” som kan kopplas till kadmiumexponering, 3) utvärdera sambandet mellan långsiktig, låg kadmiumexponering från kosten och risken att drabbas av kronisk njursvikt, njursten och frakturer i två stora populationsbaserade kohorter av svenska kvinnor och män.

I ett populationsbaserat urval av 180 individer boende nära ett zinksmältverk i Avonmouth i Sydvästra England uppmättes medianhalten av kadmium i urin till 0,22 nmol Cd/mmol kreatinin. Tre procent av individerna uppvisade halter över 1 nmol Cd/mmol kreatinin (~1 μg/g kreatinin) vilket är den referenspunkt som den europeiska livsmedelsmyndigheten EFSA har satt som gränsvärde i sin riskbedömning från 2009 för att undvika effekter på njuren. De modellerade utsläppshalterna av kadmium till luft från smältverket korrelerade starkt med de uppmätta halterna från övervakningsstationerna ($r = 0,84$) och var dessutom ett viktigt instrument för att kunna uppskatta kroppsbelastningen av kadmium, dvs. halterna i urin ($p = 0,04$). I en tvärsnittsanalys förelåg ett signifikant dos-responsförhållande mellan kadmiumhalterna i urin och en biomarkör för tidig påverkan på njuren. Med hjälp av metabonomics identifierades sex metaboliter i urin som var kopplade till kadmiumhalterna i urin. Dessa metaboliter var antigen relaterade till den mitokondriella metabolismen eller till enkolsmetabolismen.

Två stora populationsbaserade kohorter av kvinnor och män från Mellansverige användes för att undersöka sambandet mellan kostens innehåll av kadmium och förekomsten av kronisk njursvikt, njursten och frakturer. Kadmiumintaget var i genomsnitt 19 μg/dag hos män och 13 μg/dag hos kvinnor. Under en uppföljningstid på i genomsnitt 12 till 13 år identifierades 599 fall av kronisk njursvikt och 707 fall av

njursten bland männen och 253 respektive 209 fall bland kvinnorna. Det förelåg inget samband mellan kadmiumintaget via kosten och risken att drabbas av njursvikt eller njursten hos vare sig män eller kvinnor.

Under en 10-års uppföljning av 22 173 svenska män inträffade totalt 2 183 fall av frakturer varav 374 fall utgjordes av höftfrakturer. Sambandet mellan kadmium från kosten och risken att drabbas av en fraktur visade på en statistiskt säkerställd ökad risk. Männen med det högsta kadmiumintaget hade 19 procents högre risk att drabbas av frakturer jämfört med männen med det lägsta intaget. I relation till höftfrakturer observerades en 70 procentig ökad risk hos personer som aldrig rökt. Denna studie är den första som studerat risken för höftfrakturer i relation till kadmiumexponering och den första att rapportera en ökad risk för frakturer kopplade till kadmiumintaget hos män.

Sammantaget tyder resultaten från denna avhandling på att de toxiska effekterna av exponering för kadmium hos boende runt ett smältverk i Avonmouth kan spåras med hjälp av olika biomarkörer i urin. Baserat på fynden från kohortstudierna förefaller kadmiumexponeringen via kosten – vid de nivåer som ses i den allmänna befolkningen exponerade för förhållandevis låga halter – öka risken för frakturer men inte för njursvikt eller njursten. Tillsammans med nyligen publicerade resultat från andra studier talar dessa fynd för att effekten på ben kan vara mer betydelsefull ur ett kliniskt perspektiv och kan uppstå vid lägre nivåer än motsvarande effekter på njuren.

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11 REFERENCES

1. WHO. *Environmental Health Criteria 134: Cadmium.* Geneva1992. Available at: http://www.inchem.org/documents/ehc/ehc/ehc134.htm, 134.

2. WHO. *Health risks of heavy metals from long-range transboundary air pollution. Joint WHO/Convention Task Force on the Health Aspects of Air Pollution, Copenhagen.*2007. Available at: http://www.euro.who.int/__data/assets/pdf_file/0007/78649/E91044.pdf.

3. AEAT. *UK Emissions of Air Pollutants 1970 to 2008*2010. AEAT/ENV/R/3036. Available at: http://www.airquality.co.uk/reports/cat07/1009030925_2008_Report_final270805.pdf.

4. V. Vestreng, Breivik, K., Adams, M., Wagner, A., Goodwin, J., Rozovskaya, O., Pacyna, J. M.,. *Inventory Review 2005. Emission Data reported to LRTAP Convention and NEC Directive. Initial review for HMs and POPs*: Oslo, Meteorological Synthesizing Centre – West;2005. Available at: http://www.emep.int/publ/reports/2005/emep_technical_1_2005.pdf.

5. J. Hogervorst, M. Plusquin, J. Vangronsveld, et al. House dust as possible route of environmental exposure to cadmium and lead in the adult general population. *Environ Res.* Jan 2007;103(1):30-37.

6. European Commission. *A possible EU wide charge on cadmium in phosphate fertilisers: Economic and environmental implications. Final report to the European Commission. Report number E-00/02.* 2000.

7. A Andersson. *Trace elements in agricultural soils - fluxes, balances and background values.*1992.

8. J Eriksson, A Andersson, R. Andersson. *Tillståndet i svensk åkermark (The state of Swedish arable land)*: Swedish Environmental Protection Agency Report 4778.;1997.

9. EFSA. Cadmium in food. Scientific Opinion of the Panel on Contaminants in the Food Chain. *The EFSA Journal.* 2009; 980:1-139.

10. C. G. Elinder, B. Lind, T. Kjellstrom, L. Linnman, L. Friberg. Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. Estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. *Arch Environ Health.* Nov-Dec 1976;31(6):292-302.

11. EC. *European Union Risk Assessment Report. Cadmium oxide and cadmium metal.* 2007. CAS No: 1306-19-0 and 7440-43-9 EINECS No: 215-146-2 and 231-152-8. Available at: http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/cdmetal_cdoxideENVreport302.pdf.

12. U. Nilsson, A. Schutz, S. Skerfving, S. Mattsson. Cadmium in kidneys in Swedes measured in vivo using X-ray fluorescence analysis. *Int Arch Occup Environ Health.* 1995;67(6):405-411.

13. T. M. Leazer, Y. Liu, C. D. Klaassen. Cadmium absorption and its relationship to divalent metal transporter-1 in the pregnant rat. *Toxicol Appl Pharmacol.* Nov 15 2002;185(1):18-24.

14. J. Tallkvist, C. L. Bowlus, B. Lonnerdal. DMT1 gene expression and cadmium absorption in human absorptive enterocytes. *Toxicol Lett.* Jun 20 2001;122(2):171-177.

15. Y. Lind, A. Wicklund Glynn, J. Engman, L. Jorhem. Bioavailability of cadmium from crab hepatopancreas and mushroom in relation to inorganic cadmium: a 9-week feeding study in mice. *Food Chem Toxicol.* Aug 1995;33(8):667-673.

16. D. W. Kim, K. Y. Kim, B. S. Choi, et al. Regulation of metal transporters by dietary iron, and the relationship between body iron levels and cadmium uptake. *Arch Toxicol.* May 2007;81(5):327-334.

17. A. Åkesson, M. Berglund, A. Schutz, P. Bjellerup, K. Bremme, M. Vahter. Cadmium exposure in pregnancy and lactation in relation to iron status. *Am J Public Health.* Feb 2002;92(2):284-287.
18. M. Berglund, A. Akesson, B. Nermell, M. Vahter. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ Health Perspect.* Dec 1994;102(12):1058-1066.
19. M. Vahter, A. Akesson, C. Liden, S. Ceccatelli, M. Berglund. Gender differences in the disposition and toxicity of metals. *Environ Res.* May 2007;104(1):85-95.
20. H. M. Meltzer, A. L. Brantsaeter, B. Borch-Iohnsen, et al. Low iron stores are related to higher blood concentrations of manganese, cobalt and cadmium in non-smoking, Norwegian women in the HUNT 2 study. *Environ Res.* Jul 2010;110(5):497-504.
21. P. G. Reeves, R. L. Chaney. Bioavailability as an issue in risk assessment and management of food cadmium: a review. *Sci Total Environ.* Jul 15 2008;398(1-3):13-19.
22. A. M. Wing. The effects of whole wheat, wheat bran and zinc in the diet on the absorption and accumulation of cadmium in rats. *Br J Nutr.* Jan 1993;69(1):199-209.
23. Y. Lind, J. Engman, L. Jorhem, A. W. Glynn. Accumulation of cadmium from wheat bran, sugar-beet fibre, carrots and cadmium chloride in the liver and kidneys of mice. *Br J Nutr.* Aug 1998;80(2):205-211.
24. G. Eklund, K. P. Grawe, A. Oskarsson. Bioavailability of cadmium from infant diets in newborn rats. *Arch Toxicol.* Nov 2001;75(9):522-530.
25. L. Jarup, A. Rogenfelt, C. G. Elinder, K. Nogawa, T. Kjellstrom. Biological half-time of cadmium in the blood of workers after cessation of exposure. *Scand J Work Environ Health.* Aug 1983;9(4):327-331.
26. B. Amzal, B. Julin, M. Vahter, A. Wolk, G. Johanson, A. Akesson. Population toxicokinetic modeling of cadmium for health risk assessment. *Environ Health Perspect.* Aug 2009;117(8):1293-1301.
27. C. Orlowski, J. K. Piotrowski, J. K. Subdys, A. Gross. Urinary cadmium as indicator of renal cadmium in humans: an autopsy study. *Hum Exp Toxicol.* Jun 1998;17(6):302-306.
28. M. Akerstrom, L. Barregard, T. Lundh, G. Sallsten. The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. *Toxicol Appl Pharmacol.* May 1 2013;268(3):286-293.
29. B. G. Armstrong, G. Kazantzis. A problem in looking for relationships between concentrations of urinary components. *Br J Ind Med.* Jan 1985;42(1):70-71.
30. G. Johansson, S. Bingham, M. Vahter. A method to compensate for incomplete 24-hour urine collections in nutritional epidemiology studies. *Public Health Nutr.* Dec 1999;2(4):587-591.
31. Y. Suwazono, A. Akesson, T. Alfven, L. Jarup, M. Vahter. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers.* Mar-Jun 2005;10(2-3):117-126.
32. G. B. Forbes, G. J. Bruining. Urinary creatinine excretion and lean body mass. *Am J Clin Nutr.* Dec 1976;29(12):1359-1366.
33. K. M. Davies, R. P. Heaney, K. Rafferty. Decline in muscle mass with age in women: a longitudinal study using an indirect measure. *Metabolism.* Jul 2002;51(7):935-939.
34. L. Jarup, M. Berglund, C. G. Elinder, G. Nordberg, M. Vahter. Health effects of cadmium exposure--a review of the literature and a risk estimate. *Scand J Work Environ Health.* 1998;24 Suppl 1:1-51.
35. G. F. Nordberg. Cadmium and health in the 21st century--historical remarks and trends for the future. *Biometals.* Oct 2004;17(5):485-489.
36. L. Jarup, A. Akesson. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol.* Aug 1 2009;238(3):201-208.
37. B. Julin, A. Wolk, L. D. Thomas, A. Akesson. Exposure to cadmium from food and risk of cardiovascular disease in men: a population-based prospective cohort study. *Eur J Epidemiol.* Aug 24 2013.

38. B Julin, C Bergkvist, A Wolk, A Åkesson. Exposure to cadmium from food and risk of cardiovascular disease in women - a population-based prospective cohort study. *Epidemiology.* 2013;Accepted.
39. M. Tellez-Plaza, E. Guallar, B. V. Howard, et al. Cadmium exposure and incident cardiovascular disease. *Epidemiology.* May 2013;24(3):421-429.
40. B. Fagerberg, G. Bergstrom, J. Boren, L. Barregard. Cadmium exposure is accompanied by increased prevalence and future growth of atherosclerotic plaques in 64-year-old women. *J Intern Med.* Dec 2012;272(6):601-610.
41. IARC. *Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 58. Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry* 1993. Available at: http://monographs.iarc.fr/ENG/Monographs/vol58/index.php.
42. E. Kellen, M. P. Zeegers, E. D. Hond, F. Buntinx. Blood cadmium may be associated with bladder carcinogenesis: the Belgian case-control study on bladder cancer. *Cancer Detect Prev.* 2007;31(1):77-82.
43. R. M. Park, L. T. Stayner, M. R. Petersen, M. Finley-Couch, R. Hornung, C. Rice. Cadmium and lung cancer mortality accounting for simultaneous arsenic exposure. *Occup Environ Med.* May 2012;69(5):303-309.
44. T. Nawrot, M. Plusquin, J. Hogervorst, et al. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. *Lancet Oncol.* Feb 2006;7(2):119-126.
45. B. Julin, A. Wolk, J. E. Johansson, S. O. Andersson, O. Andren, A. Akesson. Dietary cadmium exposure and prostate cancer incidence: a population-based prospective cohort study. *Br J Cancer.* Aug 21 2012;107(5):895-900.
46. A. Åkesson, B. Julin, A. Wolk. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a population-based prospective cohort study. *Cancer Res.* Aug 1 2008;68(15):6435-6441.
47. J. A. McElroy, M. M. Shafer, A. Trentham-Dietz, J. M. Hampton, P. A. Newcomb. Cadmium exposure and breast cancer risk. *J Natl Cancer Inst.* Jun 21 2006;98(12):869-873.
48. B. Julin, A. Wolk, L. Bergkvist, M. Bottai, A. Akesson. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. *Cancer Res.* Mar 15 2012;72(6):1459-1466.
49. B. Julin. *Dietary Cadmium Exposure and the Risk of Hormone-Related Cancers* [PhD Thesis]. Stockholm: Institute of Environmental Medicine, Karolinska Institutet; 2012.
50. WHO/FAO. *Evaluation of certain food additives and contaminants: Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives.* 2011. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_960_eng.pdf.
51. T. C. Turin, M. Tonelli, B. J. Manns, P. Ravani, S. B. Ahmed, B. R. Hemmelgarn. Chronic kidney disease and life expectancy. *Nephrol Dial Transplant.* Aug 2012;27(8):3182-3186.
52. D. Hamilton, P. Riley, U. Miola, D. Mousa, W. Popovich, A. al Khader. Total plasma clearance of 51Cr-EDTA: variation with age and sex in normal adults. *Nucl Med Commun.* Feb 2000;21(2):187-192.
53. G. S. Grewal, G. M. Blake. Reference data for 51Cr-EDTA measurements of the glomerular filtration rate derived from live kidney donors. *Nucl Med Commun.* Jan 2005;26(1):61-65.
54. E. D. Poggio, A. D. Rule, R. Tanchanco, et al. Demographic and clinical characteristics associated with glomerular filtration rates in living kidney donors. *Kidney Int.* May 2009;75(10):1079-1087.
55. A. S. Levey, L. A. Stevens, C. H. Schmid, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* May 5 2009;150(9):604-612.
56. R. Hojs, S. Bevc, R. Ekart, M. Gorenjak, L. Puklavec. Serum cystatin C-based formulas for prediction of glomerular filtration rate in patients with chronic kidney disease. *Nephron Clin Pract.* 2010;114(2):c118-126.
57. V. Jha, G. Garcia-Garcia, K. Iseki, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* Jul 20 2013;382(9888):260-272.

58. M. Kerr, B. Bray, J. Medcalf, D. J. O'Donoghue, B. Matthews. Estimating the financial cost of chronic kidney disease to the NHS in England. *Nephrol Dial Transplant.* Oct 2012;27 Suppl 3:iii73-80.
59. A. P. Grollman. Aristolochic acid nephropathy: Harbinger of a global iatrogenic disease. *Environ Mol Mutagen.* Jan 2013;54(1):1-7.
60. J. Wijkstrom, R. Leiva, C. G. Elinder, et al. Clinical and Pathological Characterization of Mesoamerican Nephropathy: A New Kidney Disease in Central America. *Am J Kidney Dis.* Jul 10 2013.
61. F. Thevenod. Nephrotoxicity and the proximal tubule. Insights from cadmium. *Nephron Physiol.* 2003;93(4):p87-93.
62. O. W. Moe. Kidney stones: pathophysiology and medical management. *Lancet.* Jan 28 2006;367(9507):333-344.
63. E. M. Worcester, F. L. Coe. Clinical practice. Calcium kidney stones. *N Engl J Med.* Sep 2 2010;363(10):954-963.
64. H. G. Tiselius. A hypothesis of calcium stone formation: an interpretation of stone research during the past decades. *Urol Res.* Aug 2011;39(4):231-243.
65. C. Y. Pak. Kidney stones. *Lancet.* Jun 13 1998;351(9118):1797-1801.
66. L. D. Thomas, C. G. Elinder, H. G. Tiselius, A. Wolk, A. Akesson. Ascorbic Acid supplements and kidney stone incidence among men: a prospective study. *JAMA Intern Med.* Mar 11 2013;173(5):386-388.
67. C. M. Johnson, D. M. Wilson, W. M. O'Fallon, R. S. Malek, L. T. Kurland. Renal stone epidemiology: a 25-year study in Rochester, Minnesota. *Kidney Int.* Nov 1979;16(5):624-631.
68. K. K. Stamatelou, M. E. Francis, C. A. Jones, L. M. Nyberg, G. C. Curhan. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. *Kidney Int.* May 2003;63(5):1817-1823.
69. G. C. Curhan. Epidemiology of stone disease. *Urol Clin North Am.* Aug 2007;34(3):287-293.
70. G. F. Nordberg. Historical perspectives on cadmium toxicology. *Toxicol Appl Pharmacol.* Aug 1 2009;238(3):192-200.
71. C. G. Elinder, C. Edling, E. Lindberg, B. Kagedal, O. Vesterberg. Assessment of renal function in workers previously exposed to cadmium. *Br J Ind Med.* Nov 1985;42(11):754-760.
72. L. Jarup, B. Persson, C. G. Elinder. Decreased glomerular filtration rate in solderers exposed to cadmium. *Occup Environ Med.* Dec 1995;52(12):818-822.
73. H. Nakagawa, M. Nishijo, Y. Morikawa, et al. Urinary cadmium and mortality among inhabitants of a cadmium-polluted area in Japan. *Environ Res.* Mar 2006;100(3):323-329.
74. M. Nishijo, Y. Morikawa, H. Nakagawa, et al. Causes of death and renal tubular dysfunction in residents exposed to cadmium in the environment. *Occup Environ Med.* Aug 2006;63(8):545-550.
75. M. Nishijo, H. Nakagawa, Y. Morikawa, et al. Mortality in a cadmium polluted area in Japan. *Biometals.* Oct 2004;17(5):535-538.
76. K. Nogawa, E. Kobayashi, Y. Okubo, Y. Suwazono. Environmental cadmium exposure, adverse effects and preventive measures in Japan. *Biometals.* Oct 2004;17(5):581-587.
77. L. Hellstrom, C. G. Elinder, B. Dahlberg, et al. Cadmium exposure and end-stage renal disease. *Am J Kidney Dis.* Nov 2001;38(5):1001-1008.
78. A. Åkesson, T. Lundh, M. Vahter, et al. Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. *Environ Health Perspect.* Nov 2005;113(11):1627-1631.
79. P. M. Ferraro, S. Costanzi, A. Naticchia, A. Sturniolo, G. Gambaro. Low level exposure to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-2006. *BMC Public Health.* 2010;10:304.
80. Y. Hwangbo, V. M. Weaver, M. Tellez-Plaza, E. Guallar, B. K. Lee, A. Navas-Acien. Blood cadmium and estimated glomerular filtration rate in Korean adults. *Environ Health Perspect.* Dec 2011;119(12):1800-1805.
81. Y. Kim, B. K. Lee. Associations of blood lead, cadmium, and mercury with estimated glomerular filtration rate in the Korean general population: Analysis

of 2008-2010 Korean National Health and Nutrition Examination Survey data. *Environ Res.* Oct 2012;118:124-129.

82. A. Navas-Acien, M. Tellez-Plaza, E. Guallar, et al. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol.* Nov 1 2009;170(9):1156-1164.

83. J. Lin, E. L. Knight, M. L. Hogan, A. K. Singh. A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. *J Am Soc Nephrol.* Oct 2003;14(10):2573-2580.

84. J. N. Sommar, M. K. Svensson, B. M. Bjor, et al. End-stage renal disease and low level exposure to lead, cadmium and mercury; a population-based, prospective nested case-referent study in Sweden. *Environ Health.* Jan 23 2013;12(1):9.

85. E. A. Butler, F. V. Flynn. The proteinuria of renal tubular disorders. *Lancet.* Nov 8 1958;2(7054):978-980.

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

87. T. Jin, M. Nordberg, W. Frech, et al. Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad). *Biometals.* Dec 2002;15(4):397-410.

88. T. Jin, G. Nordberg, T. Ye, et al. Osteoporosis and renal dysfunction in a general population exposed to cadmium in China. *Environ Res.* Nov 2004;96(3):353-359.

89. J. P. Buchet, R. Lauwerys, H. Roels, et al. Renal effects of cadmium body burden of the general population. *Lancet.* Sep 22 1990;336(8717):699-702.

90. L. Jarup, L. Hellstrom, T. Alfven, et al. Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup Environ Med.* Oct 2000;57(10):668-672.

91. C. de Burbure, J. P. Buchet, A. Leroyer, et al. 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.* Apr 2006;114(4):584-590.

92. P. Hotz, J. P. Buchet, A. Bernard, D. Lison, R. Lauwerys. Renal effects of low-level environmental cadmium exposure: 5-year follow-up of a subcohort from the Cadmibel study. *Lancet.* Oct 30 1999;354(9189):1508-1513.

93. Y. Liang, L. Lei, J. Nilsson, et al. Renal function after reduction in cadmium exposure: an 8-year follow-up of residents in cadmium-polluted areas. *Environ Health Perspect.* Feb 2012;120(2):223-228.

94. A. Chaumont, M. Nickmilder, X. Dumont, T. Lundh, S. Skerfving, A. Bernard. Associations between proteins and heavy metals in urine at low environmental exposures: evidence of reverse causality. *Toxicol Lett.* May 5 2012;210(3):345-352.

95. N. Haddam, S. Samira, X. Dumont, et al. Confounders in the assessment of the renal effects associated with low-level urinary cadmium: an analysis in industrial workers. *Environ Health.* May 14 2011;10(1):37.

96. M. Akerstrom, G. Sallsten, T. Lundh, L. Barregard. Associations between urinary excretion of cadmium and proteins in a nonsmoking population: renal toxicity or normal physiology? *Environ Health Perspect.* Feb 2013;121(2):187-191.

97. R. Scott, P. J. Patterson, R. Burns, et al. Hypercalciuria related to cadmium exposure. *Urology.* May 1978;11(5):462-465.

98. G. Kazantzis. Renal tubular dysfunction and abnormalities of calcium metabolism in cadmium workers. *Environ Health Perspect.* Feb 1979;28:155-159.

99. R. G. Adams, J. F. Harrison, P. Scott. The development of cadmium-induced proteinuria, impaired renal function, and osteomalacia in alkaline battery workers. *Q J Med.* Oct 1969;38(152):425-443.

100. M. J. Thun, A. M. Osorio, S. Schober, W. H. Hannon, B. Lewis, W. Halperin. Nephropathy in cadmium workers: assessment of risk from airborne occupational exposure to cadmium. *Br J Ind Med.* Oct 1989;46(10):689-697.

101. L. Jarup, B. Persson, C. G. Elinder. Blood cadmium as an indicator of dose in a long-term follow-up of workers previously exposed to cadmium. *Scand J Work Environ Health.* Feb 1997;23(1):31-36.
102. L. Jarup, C. G. Elinder. Incidence of renal stones among cadmium exposed battery workers. *Br J Ind Med.* Jul 1993;50(7):598-602.
103. P. M. Ferraro, M. Bonello, A. C. Frigo, A. D'Addessi, A. Sturniolo, G. Gambaro. Cadmium Exposure and Kidney Stone Formation in the General Population-An Analysis of the National Health and Nutrition Examination Survey III Data. *J Endourol.* May 2011;25(5):875-880.
104. J. A. Kanis, O. Johnell, A. Oden, C. De Laet, D. Mellstrom. Epidemiology of osteoporosis and fracture in men. *Calcif Tissue Int.* Aug 2004;75(2):90-99.
105. A. C. Looker, H. W. Wahner, W. L. Dunn, et al. Updated data on proximal femur bone mineral levels of US adults. *Osteoporos Int.* 1998;8(5):468-489.
106. J. A. Kanis, C. C. Gluer. An update on the diagnosis and assessment of osteoporosis with densitometry. Committee of Scientific Advisors, International Osteoporosis Foundation. *Osteoporos Int.* 2000;11(3):192-202.
107. D. Marshall, O. Johnell, H. Wedel. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ.* May 18 1996;312(7041):1254-1259.
108. WHO. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser.* 1994;843:1-129.
109. J. A. Kanis, L. J. Melton, 3rd, C. Christiansen, C. C. Johnston, N. Khaltaev. The diagnosis of osteoporosis. *J Bone Miner Res.* Aug 1994;9(8):1137-1141.
110. F. Borgstrom, P. Sobocki, O. Strom, B. Jonsson. The societal burden of osteoporosis in Sweden. *Bone.* Jun 2007;40(6):1602-1609.
111. C. Cooper, E. J. Atkinson, S. J. Jacobsen, W. M. O'Fallon, L. J. Melton, 3rd. Population-based study of survival after osteoporotic fractures. *Am J Epidemiol.* May 1 1993;137(9):1001-1005.
112. SBU. *Osteoporos - prevention, diagnostik och behandling.* Stockholm2003.
113. H. Olofsson, L. Byberg, R. Mohsen, H. Melhus, H. Lithell, K. Michaelsson. Smoking and the risk of fracture in older men. *J Bone Miner Res.* Jul 2005;20(7):1208-1215.
114. H. D. Nelson, M. C. Nevitt, J. C. Scott, K. L. Stone, S. R. Cummings. Smoking, alcohol, and neuromuscular and physical function of older women. Study of Osteoporotic Fractures Research Group. *JAMA.* Dec 21 1994;272(23):1825-1831.
115. M. Lorentzon, C. Swanson, N. Andersson, D. Mellstrom, C. Ohlsson. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. *J Bone Miner Res.* Aug 2005;20(8):1334-1341.
116. P. R. Ebeling. Osteoporosis in men. New insights into aetiology, pathogenesis, prevention and management. *Drugs Aging.* Dec 1998;13(6):421-434.
117. J. K. Lambert, M. Zaidi, J. I. Mechanick. Male osteoporosis: epidemiology and the pathogenesis of aging bones. *Curr Osteoporos Rep.* Dec 2011;9(4):229-236.
118. B. Gullberg, O. Johnell, J. A. Kanis. World-wide projections for hip fracture. *Osteoporos Int.* 1997;7(5):407-413.
119. I. Elffors, E. Allander, J. A. Kanis, et al. The variable incidence of hip fracture in southern Europe: the MEDOS Study. *Osteoporos Int.* Sep 1994;4(5):253-263.
120. O. Johnell, B. Gullberg, E. Allander, J. A. Kanis. The apparent incidence of hip fracture in Europe: a study of national register sources. MEDOS Study Group. *Osteoporos Int.* Nov 1992;2(6):298-302.
121. A. A. Ismail, S. R. Pye, W. C. Cockerill, et al. Incidence of limb fracture across Europe: results from the European Prospective Osteoporosis Study (EPOS). *Osteoporos Int.* Jul 2002;13(7):565-571.
122. J. A. Kanis, O. Johnell, C. De Laet, B. Jonsson, A. Oden, A. K. Ogelsby. International variations in hip fracture probabilities: implications for risk assessment. *J Bone Miner Res.* Jul 2002;17(7):1237-1244.

123. O. Strom, F. Borgstrom, J. A. Kanis, et al. Osteoporosis: burden, health care provision and opportunities in the EU: a report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos.* Dec 2011;6(1-2):59-155.

124. J. A. Staessen, H. A. Roels, D. Emelianov, et al. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Public Health and Environmental Exposure to Cadmium (PheeCad) Study Group. *Lancet.* Apr 3 1999;353(9159):1140-1144.

125. C. M. Gallagher, J. S. Kovach, J. R. Meliker. Urinary cadmium and osteoporosis in U.S. Women >or= 50 years of age: NHANES 1988-1994 and 1999-2004. *Environ Health Perspect.* Oct 2008;116(10):1338-1343.

126. H. Wang, G. Zhu, Y. Shi, et al. Influence of environmental cadmium exposure on forearm bone density. *J Bone Miner Res.* Mar 2003;18(3):553-560.

127. T. Alfven, C. G. Elinder, M. D. Carlsson, et al. Low-level cadmium exposure and osteoporosis. *J Bone Miner Res.* Aug 2000;15(8):1579-1586.

128. T. Alfven, C. G. Elinder, L. Hellstrom, F. Lagarde, L. Jarup. Cadmium exposure and distal forearm fractures. *J Bone Miner Res.* Jun 2004;19(6):900-905.

129. A. Engstrom, K. Michaelsson, Y. Suwazono, A. Wolk, M. Vahter, A. Akesson. Long-term cadmium exposure and the association with bone mineral density and fractures in a population-based study among women. *J Bone Miner Res.* Mar 2011;26(3):486-495.

130. M. H. Bhattacharyya. Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. *Toxicol Appl Pharmacol.* Aug 1 2009;238(3):258-265.

131. T. Nawrot, P. Geusens, T. S. Nulens, B. Nemery. Occupational cadmium exposure and calcium excretion, bone density, and osteoporosis in men. *J Bone Miner Res.* Jun 2010;25(6):1441-1445.

132. A. Åkesson, P. Bjellerup, T. Lundh, et al. Cadmium-induced effects on bone in a population-based study of women. *Environ Health Perspect.* Jun 2006;114(6):830-834.

133. R. Schutte, T. S. Nawrot, T. Richart, et al. Bone resorption and environmental exposure to cadmium in women: a population study. *Environ Health Perspect.* Jun 2008;116(6):777-783.

134. J. K. Nicholson, J. C. Lindon, E. Holmes. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica.* Nov 1999;29(11):1181-1189.

135. J. A. Bonnell. Lead smelting at Avonmouth. *Br J Ind Med.* Apr 1973;30(2):199-201.

136. Pollution Inventory. 2007; www.environment-agency.gov.uk/business/444255/446867/255244/.

137. A. Colgan, P. K. Hankard, D. J. Spurgeon, C. Svendsen, R. A. Wadsworth, J. M. Weeks. Closing the loop: a spatial analysis to link observed environmental damage to predicted heavy metal emissions. *Environ Toxicol Chem.* May 2003;22(5):970-976.

138. CEH. *Heavy metals in European Mosses: 2000/2001 survey*: UNECE ICP Vegetation. Centre for Ecology and Hydrology;2003.

139. A. Norman, R. Bellocco, F. Vaida, A. Wolk. Total physical activity in relation to age, body mass, health and other factors in a cohort of Swedish men. *Int J Obes Relat Metab Disord.* May 2002;26(5):670-675.

140. Baker SJ. *Trace and Major Elements in the Atmosphere at Rural Locations in the UK: Summary of Data for 1999*.2001. AEAT/R/ENV/0264 Issue 2. Available at: http://www.airquality.co.uk/archive/reports/empire/Rural_Trace_Metals_2000_Report.pdf.

141. M. Messerer, S. E. Johansson, A. Wolk. The validity of questionnaire-based micronutrient intake estimates is increased by including dietary supplement use in Swedish men. *J Nutr.* Jul 2004;134(7):1800-1805.

142. N. Orsini, R. Bellocco, M. Bottai, et al. Validity of self-reported total physical activity questionnaire among older women. *Eur J Epidemiol.* 2008;23(10):661-667.
143. W. Willett, M. J. Stampfer. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* Jul 1986;124(1):17-27.
144. B. Julin, A. Wolk, L. Bergkvist, M. Bottai, A. Akesson. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study [Supplementary Material]. *Cancer Res.* Mar 15 2012;72(6):1459-1466.
145. J. Tencer, H. Thysell, A. Grubb. Analysis of proteinuria: reference limits for urine excretion of albumin, protein HC, immunoglobulin G, kappa- and lambda-immunoreactivity, orosomucoid and alpha 1-antitrypsin. *Scand J Clin Lab Invest.* Dec 1996;56(8):691-700.
146. T. W. M. Fan. Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. *Progress in Nuclear Magnetic Resonance Spectroscopy.* 1996;28(2):161-219.
147. O. Cloarec, M. E. Dumas, A. Craig, et al. Statistical total correlation spectroscopy: an exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. *Anal Chem.* Mar 1 2005;77(5):1282-1289.
148. J. F. Ludvigsson, E. Andersson, A. Ekbom, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health.* 2011;11:450.
149. F. Dieterle, A. Ross, G. Schlotterbeck, H. Senn. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. *Anal Chem.* Jul 1 2006;78(13):4281-4290.
150. Swedish Chemical Agency. *Economic cost of fractures caused by dietary cadmium exposure*2013. Available at: http://www.kemi.se/Documents/Publikationer/Trycksaker/Rapporter/Rapport-4-13-cadmium.pdf.
151. WHO. Air quality guidelines for Europe. *WHO Regional Publications, European series No 91. World Health Organisation,Geneva.* 2000.
152. A. Engstrom, K. Michaelsson, M. Vahter, B. Julin, A. Wolk, A. Akesson. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone.* Jun 2012;50(6):1372-1378.
153. A. B. Hill. The Environment and Disease: Association or Causation? *Proc R Soc Med.* May 1965;58:295-300.
154. M. Dosemeci, S. Wacholder, J. H. Lubin. Does nondifferential misclassification of exposure always bias a true effect toward the null value? *Am J Epidemiol.* Oct 1990;132(4):746-748.
155. SBU. *Methods to Estimate and Measure Renal Function (Glomerular Filtration Rate)*2012. Available at: http://www.sbu.se/en/Published/Yellow/Methods-to-Estimate-and-Measure-Renal-Function-Glomerular-Filtration-Rate/.
156. O. Johnell, B. Gullberg, J. A. Kanis. The hospital burden of vertebral fracture in Europe: a study of national register sources. *Osteoporos Int.* 1997;7(2):138-144.
157. E. Holmes, R. L. Loo, J. Stamler, et al. Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature.* May 15 2008;453(7193):396-400.
158. J. L. Griffin, L. A. Walker, J. Troke, D. Osborn, R. F. Shore, J. K. Nicholson. The initial pathogenesis of cadmium induced renal toxicity. *FEBS Lett.* Jul 28 2000;478(1-2):147-150.
159. J. L. Griffin, L. A. Walker, R. F. Shore, J. K. Nicholson. Metabolic profiling of chronic cadmium exposure in the rat. *Chem Res Toxicol.* Oct 2001;14(10):1428-1434.
160. J. K. Nicholson, D. P. Higham, J. A. Timbrell, P. J. Sadler. Quantitative high resolution 1H NMR urinalysis studies on the biochemical effects of cadmium in the rat. *Mol Pharmacol.* Sep 1989;36(3):398-404.
161. S. H. Oh, S. C. Lim. A rapid and transient ROS generation by cadmium triggers apoptosis via caspase-dependent pathway in HepG2 cells and this is inhibited

through N-acetylcysteine-mediated catalase upregulation. *Toxicol Appl Pharmacol.* May 1 2006;212(3):212-223.

162. E. A. Belyaeva, D. Dymkowska, M. R. Wieckowski, L. Wojtczak. Mitochondria as an important target in heavy metal toxicity in rat hepatoma AS-30D cells. *Toxicol Appl Pharmacol.* Aug 15 2008;231(1):34-42.

163. R. Talhout, T. Schulz, E. Florek, J. van Benthem, P. Wester, A. Opperhuizen. Hazardous compounds in tobacco smoke. *Int J Environ Res Public Health.* Feb 2011;8(2):613-628.

164. L. L. Hamm. Renal handling of citrate. *Kidney Int.* Oct 1990;38(4):728-735.

165. F. Cardellach, J. R. Alonso, S. Lopez, J. Casademont, O. Miro. Effect of smoking cessation on mitochondrial respiratory chain function. *J Toxicol Clin Toxicol.* 2003;41(3):223-228.

166. O. Miro, J. R. Alonso, D. Jarreta, J. Casademont, A. Urbano-Marquez, F. Cardellach. Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes. *Carcinogenesis.* Jul 1999;20(7):1331-1336.

167. L. L. Hamm, K. S. Hering-Smith. Pathophysiology of hypocitraturic nephrolithiasis. *Endocrinol Metab Clin North Am.* Dec 2002;31(4):885-893, viii.

168. K. S. Engstrom, M. Vahter, G. Johansson, et al. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine. *Free Radic Biol Med.* May 1 2010;48(9):1211-1217.

169. J. M. Llobet, G. Falco, C. Casas, A. Teixido, J. L. Domingo. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J Agric Food Chem.* Jan 29 2003;51(3):838-842.

170. G. Ysart, P. Miller, M. Croasdale, et al. 1997 UK Total Diet Study--dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Addit Contam.* Sep 2000;17(9):775-786.

171. D. L. MacIntosh, J. D. Spengler, H. Ozkaynak, L. Tsai, P. B. Ryan. Dietary exposures to selected metals and pesticides. *Environ Health Perspect.* Feb 1996;104(2):202-209.

172. K. W. Thomas, E. D. Pellizzari, M. R. Berry. Population-based dietary intakes and tap water concentrations for selected elements in the EPA region V National Human Exposure Assessment Survey (NHEXAS). *J Expo Anal Environ Epidemiol.* Sep-Oct 1999;9(5):402-413.

173. E. H. Larsen, N. L. Andersen, A. Moller, A. Petersen, G. K. Mortensen, J. Petersen. Monitoring the content and intake of trace elements from food in Denmark. *Food Addit Contam.* Jan 2002;19(1):33-46.

174. S. Sand, W. Becker. Assessment of dietary cadmium exposure in Sweden and population health concern including scenario analysis. *Food Chem Toxicol.* Jan 3 2012.

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

176. A. Engstrom. *Cadmium as a risk factor for osteoporosis and fractures in women.* [PhD Thesis]. Stockholm: Institute of Environmental Medicine, Karolinska Institutet; 2011.

177. S. R. Orth, S. I. Hallan. Smoking: a risk factor for progression of chronic kidney disease and for cardiovascular morbidity and mortality in renal patients--absence of evidence or evidence of absence? *Clin J Am Soc Nephrol.* Jan 2008;3(1):226-236.

178. A. Menke, P. Muntner, E. K. Silbergeld, E. A. Platz, E. Guallar. Cadmium levels in urine and mortality among U.S. adults. *Environ Health Perspect.* Feb 2009;117(2):190-196.

179. M. Tellez-Plaza, A. Navas-Acien, A. Menke, C. M. Crainiceanu, R. Pastor-Barriuso, E. Guallar. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. *Environ Health Perspect.* Jul 2012;120(7):1017-1022.

180. K. Sakhaee, N. M. Maalouf, R. Kumar, A. Pasch, O. W. Moe. Nephrolithiasis-associated bone disease: pathogenesis and treatment options. *Kidney Int.* Feb 2011;79(4):393-403.
181. D. C. Mackey, L. Y. Lui, P. M. Cawthon, et al. High-trauma fractures and low bone mineral density in older women and men. *JAMA.* Nov 28 2007;298(20):2381-2388.
182. K. M. Sanders, J. A. Pasco, A. M. Ugoni, et al. The exclusion of high trauma fractures may underestimate the prevalence of bone fragility fractures in the community: the Geelong Osteoporosis Study. *J Bone Miner Res.* Aug 1998;13(8):1337-1342.
183. K. Michaelsson, L. Holmberg, H. Mallmin, et al. Diet and hip fracture risk: a case-control study. Study Group of the Multiple Risk Survey on Swedish Women for Eating Assessment. *Int J Epidemiol.* Aug 1995;24(4):771-782.
184. K. L. Tucker, M. T. Hannan, H. Chen, L. A. Cupples, P. W. Wilson, D. P. Kiel. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. *Am J Clin Nutr.* Apr 1999;69(4):727-736.
185. S. A. New. Intake of fruit and vegetables: implications for bone health. *Proc Nutr Soc.* Nov 2003;62(4):889-899.