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BONE AND KIDNEY EFFECTS FROM CADMIUM EXPOSURE

Dose effect and dose response relationships

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Doctoral thesis
Bone and kidney effects from cadmium exposure – dose effects and dose response relationships.

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Gutta cavat lapidem non vi sed saepe cadendo
(Ovid 47 B.C. – 17 A.D.)

ABSTRACT

Cadmium is a heavy metal that has been dispersed in the environment during the last century due to human activity. It is well known that high cadmium exposure causes renal damage and in severe cases also osteoporosis and osteomalacia.

Osteoporosis is a major cause of morbidity worldwide. A number of risk factors, such as age and gender, are well established, but little is known about the contribution of environmental risk factors.

The aim of the present work was to investigate the effects of low cadmium exposure on bone and kidneys. A cross-sectional study was performed in 520 men and 544 women, aged 16-81, environmentally or occupationally exposed to cadmium. Cadmium in urine as well as in blood was used as the dose estimate, and protein HC was used as a marker of tubular proteinuria, an early sign of renal damage. Bone mineral density (BMD) in the forearm was measured using DXA (dual energy x-ray absorptiometry) technique.

The study revealed that tubular proteinuria occurred in cadmium exposed persons at lower levels of cadmium doses than previously known. At levels between 0.3 and 0.5 nmol cadmium/mmol creatinine in urine, there was a two-fold increased risk of displaying elevated urinary protein HC, compared to the reference (<0.3).

A dose-effect relationship was found between increasing cadmium dose and decreasing BMD for people aged 60 and older. In the older age group, there was a dose-response relationship, showing a three-fold increased risk of low BMD in the group with urinary cadmium higher than 3 nmol/mmol creatinine compared to the lowest dose group. The difference between the age groups may reflect that older bone is more sensitive to cadmium, or that it takes several decades for cadmium to affect bone.

There was also evidence of an increased risk of forearm fractures with increasing cadmium levels. For the population aged 50 and over, the fracture hazard ratio increased by 18% per nmol cadmium/mmol creatinine.

Renal tubular damage was negatively related to bone mineral density and increasing risk of forearm fractures, suggesting that the possible effect of cadmium on the bone may be an indirect effect mediated by the kidneys.

Altogether the thesis reveals relationships between low cadmium doses and early kidney effects, decreased BMD and increased risk of forearm fractures. Although it is difficult to directly compare the cadmium levels in the present study with other groups having other exposure histories, the levels are in the same range as that of many people in the general population. Hereditary, endocrine and life-style factors are probably the primary causes of osteoporosis; however, a comparatively low increase in risk because of cadmium may have a large impact on public health, if a large part of the population has cadmium levels at which there is an increased risk.

LIST OF PUBLICATIONS

The thesis is based on the following papers, referred to in the text by their Roman numerals:

I *Järup L, Alfvén T, Persson B, Toss G, Elinder CG. Cadmium may be a risk factor for osteoporosis. Occup Environ Med, 55:435-439, 1998.*

II *Järup L, Hellström L, Alfvén T, Carlsson D, Grubb A, Persson B, Pettersson C, Schütz A, Spång G, Elinder CG. Low level cadmium exposure and kidney damage - the OSCAR study. Occup Environ Med, 57:668-672, 2000.*

III *Alfvén T, Elinder CG, Carlsson M D, Grubb A, Hellström L, Persson B, Pettersson C, Spång G, Schütz A, Järup L. Low level cadmium exposure and osteoporosis. J Bone Mineral Res, 15:1579-1586, 2000.*

IV *Alfvén T, Järup L, Elinder CG. Cadmium and lead in blood in relation to low bone mineral density and tubular proteinuria. Environ Health Perspect, 110:699-702, 2002*

V *Alfvén T, Elinder CG, Hellström L, Lagarde F, Järup. L. Cadmium exposure and distal forearm fracture. Submitted for publication.*

CONTENTS

1 LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometry
ALP	Alkaline phosphatase
B-Cd	Blood cadmium
B-Pb	Blood lead
BMC	Bone mineral content (g)
BMD	Bone mineral density (g/cm^2)
BMI	Body mass index
CV%	Coefficient of variation %
DXA	Dual energy x-ray absorptiometry
GFR	Glomerular filtration rate
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometry
MT	Metallothionein
OR	Odds ratio
NAG	N-acetyl-β-D-glucosaminidase
PTH	Parathyroid hormone
Protein HC	Human complex forming glycoprotein (also called α_1-microglobulin)
SD	Standard deviation
U-Ca	Urinary calcium
U-Cd	Urinary cadmium
95% CI	95 % confidence interval

Units

1 nmol cadmium / mmol creatinine ≅ 1 μg cadmium / g creatinine

1 nmol cadmium = 0.11 μg cadmium

1 μmol lead = 207 μg lead

2 INTRODUCTION

2.1 BACKGROUND TO THIS THESIS

This thesis focuses on the effects of cadmium on bone and kidneys. Cadmium is a heavy metal that has been dispersed in the environment due to human activities during the last century. In the 1940's the deleterious effects of cadmium on lungs, bones and kidneys were reported in cadmium exposed workers (38;39;98), but the effects were considered to be strictly occupational. However, during the 1950's reports came from Japan about the "Itai-itai disease" (44;45), characterised by renal disease, osteomalacia and osteoporosis. Itai-itai means "ouch-ouch" and refered to the severe pain from multiple fractures due to the damaged bone. The rice in the endemic area had been heavily contaminated with cadmium and after a long controversy it was concluded that cadmium was a necessary cause (77).

The Itai-itai disease showed that cadmium was not merely an occupational health hazard, but also an environmental dilemma. Decades later, in the beginning of the 1990's, it became evident that the general population outside Japan also could be affected by cadmium, as a population in Belgium showed signs of cadmium induced tubular proteinuria, an early sign of renal damage (15). Animal studies (13;14;120;141), in vitro experiments (57;143;145) and human studies (127) during the 1980's and 90's also indicated that cadmium may affect the bone at much lower levels than the Itai-itai patients had encountered.

The combination of increased incidence of osteoporosis-related fractures during the last decades in most parts of the industrialised world and new technology that made it feasible to accurately measure bone mineral density made it highly interesting to study early effects of cadmium exposure on bone. In addition, very little was known about the possible impact of cadmium in the general population in Sweden. Project OSCAR (OSteoporosis – CAdmium as a Risk factor), the base of this thesis, was designed to answer these questions about the early effects of cadmium on bone and kidneys.

This thesis starts by presenting some basic knowledge about bone and kidneys, before a description of cadmium and its health effects. After that the aims of the thesis will be presented before the chapter on subjects and methods. Then the results from the studies included are presented, and the thesis concludes with the discussion about the methods and the results.

2.2 BONE

Without the bone we would not be more than an intelligent jellyfish still flowing around in the sea (108). To meet the requirements of skeletal growth and mechanical function, bone constantly undergoes dynamic remodelling by a coupled process of bone resorption by osteoclasts and reformation by osteoblasts. The osteoblasts synthesize collagen type 1, which comprises 90-95% of the organic matrix of bone. They also produce osteocalcin, the most common non-collagenous protein of bone matrix, and are rich in alkaline phosphatase, an organic phosphate-splitting enzyme. The main mineral component of bone is hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$], which comprises about 25% of the volume and 50% of the mass of normal adult bone. Hormones, growth factors, physical activity, and other stimuli act mainly through osteoblasts to bring about their effects on bone. Bone and calcium metabolism are under the control of several hormones: active vitamin D (1,25-$(OH)_2$-D_3), parathyroid hormone (PTH), calcitonin, oestrogens and different growth factors. The active form of vitamin D is formed in the kidneys and stimulates the gastro-intestinal absorption of calcium and phosphate as well as osteoblastic synthesis of osteocalcin. Parathyroid hormone increases plasma levels of calcium by stimulating bone resorption, activation of vitamin D in the kidneys and tubular reabsorption of calcium. Lack of active vitamin D and excess of PTH lead to bone mineral depletion (26).

The skeleton consists of two macroscopically different tissues: trabecular bone and cortical bone. The trabecular (spongy or cancellous) bone, found mainly in the vertebral bodies, the pelvis and at the ends of the long bones, comprises about 20% of the total bone mass. It is more sensitive to hormonal influence and has a faster rate of metabolism. The cortical (dense or compact) bone is found mainly in long bones (26).

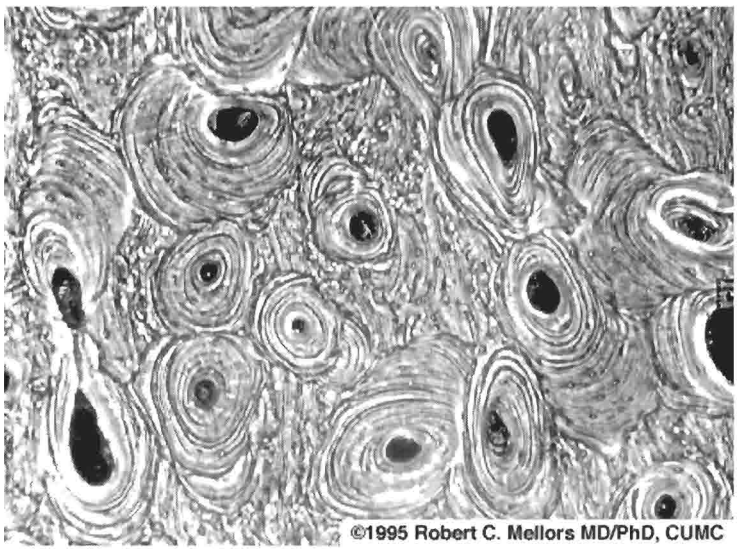

Figure 1 Compact cortical bone and Haversian systems (bone-forming units).

2.2.1 Measurement of bone mineral density

Bone density measurement is a method for determining fragility of bone. During the two last decades many different ways of measuring bone density have been developed, including single photon absorptiometry (SPA), dual photon absorptiometry (DPA), single energy x-ray absorptiometry (SXA), dual energy x-ray absorptiometry (DXA), ultrasound quantitative computed tomography (QCT) and quantitative magnetic resonance (QMR). The most common method today is the DXA, which measures the bone mineral density (BMD), expressed as grams of mineral per area of volume (g/cm^2). BMD can be measured for the whole body or for specific sites, such as the spine, hip and forearm.

By comparing BMD to a reference population, T-scores and Z-scores can be calculated. T-score is used to compare to the mean value in young adults from the same population, and Z-score is used to standardise for age and gender according to the formula:

Z-Score = $(X_u - X_m)/SD$, where
X_u = measured bone density
X_m = group mean for the same age group
SD = standard deviation in the reference population.

In clinical practice T-scores are most often used. However, to be able to compare BMD data between different ages and genders, Z-scores are used. A common definition of low bone mineral density based on Z-score, is a Z-score < -1 (72).

How well can a measurement of BMD predict the risk of a fracture? A meta-analysis reported that all measuring sites had similar predictive abilities (88): a decrease of one SD in BMD resulted in a relative risk of 1.5 (95% CI 1.4, 1.6) for a fracture. Exceptions were spine measurement for predicting vertebral fractures (relative risk 2.3 (95% CI 1.9, 2.8)), and hip measurement for hip fractures (2.6 (95% CI 2.0, 3.5)). Thus a 1 SD decrease in BMD has a better predictive value for a fracture than a 1 SD increase in serum cholesterol has for cardiovascular disease (88).

2.2.2 Osteoporosis

Osteoporosis is defined as "a skeletal disease characterized by compromised bone strength predisposing a person to an increased risk of fracture" (1). Bone strength mainly reflects the integration of BMD and bone quality. Bone quality refers to architecture, accumulation of damage (e.g., microfractures) and mineralisation. The definitions proposed by the WHO, for clinical practice, are the most widely used (146), where osteoporosis is defined as a BMD less than -2.5 SDs compared to young adults from the same population (T-score < -2.5).

Although genetic factors play a large role in the determination of peak bone mass (111;125), 20-30% or more may be explained by environmental factors. Most bone mass is acquired by the end of the second decade of life, even though there may be some continued increase in BMD in the third decade. Between the time peak BMD is attained in the third decade of life and the perimenopausal period for women and late fifth decade for men, little bone is lost, except in the proximal femur, where the BMD loss is approximately 3% per decade (69).

In women, bone loss from most sites begins in the perimenopausal period. Later, when menopause occurs, bone loss is related to oestrogen and androgen concentrations. In the seventh decade bone loss slows, but it still continues. Males also lose bone, but their peak bone mass is higher and their rate of loss is lower (e.g., the rate of loss from the male radius is about half that of women). The mechanisms responsible for the bone loss have not been entirely uncovered.

Many different risk factors for osteoporosis have been suggested (1). Risk factors supported by evidence from large prospective studies include female gender, increased age, oestrogen deficiency, white race, family history of osteoporosis, low intake of calcium and Vitamin D, low weight or low body mass index (BMI) and history of a prior fracture. Use of alcohol, smoking, and caffeine-containing beverages is inconsistently associated with decreased bone mass. On the other hand, some measures of physical activity are associated with an increased BMD. Levels of exercise in childhood and adolescence have an inconsistent relationship to BMD later in life. In women, late menarche, early menopause and low endogenous oestrogen levels are associated with low BMD. Little research has been done on environmental risk factors for osteoporosis; proposed risk factors have been exposure to aluminium (32;42), cadmium, fluorine (24;59;73), lead (42;80;87) and organochlorines (84).

The clinical significance of osteoporosis lies in the fractures that can arise. The most common osteoporosis-related fractures are those of the hip, vertebrae and distal forearm (Colles fracture). A fracture occurs when a force is applied to osteoporotic bone. Most fractures, except for vertebral fractures, result from accidents, usually falls (136). Factors such as sight, balance and muscle strength play important roles in fracture risk (22). Different types of fractures appear with varying frequency at different ages. Forearm and vertebral fractures appear most often in middle-aged women (Type I osteoporosis), and hip fractures are most common among the elderly (Type II osteoporosis) (113).

The prevalence of osteoporosis as well as the incidence of osteoporotic fractures is increasing in the industrialised world (97). Sweden and Norway have the highest incidences of hip fractures in the world (68;93). The fractures cause much morbidity (19) and mortality (20), and the costs for the society are substantial (92;112). The estimated cost of a hip fracture is about 150.000 SEK in Sweden (86).

The lifetime risk of an osteoporotic fracture in Sweden is roughly 50% for women and 25% for men (114). A study from Malmö, in southern Sweden, showed women's lifetime risks of shoulder, forearm, hip and spine fractures as 13%, 22%, 23% and 15%. Corresponding values for men were 4%, 5%, 11% and 9% (114).

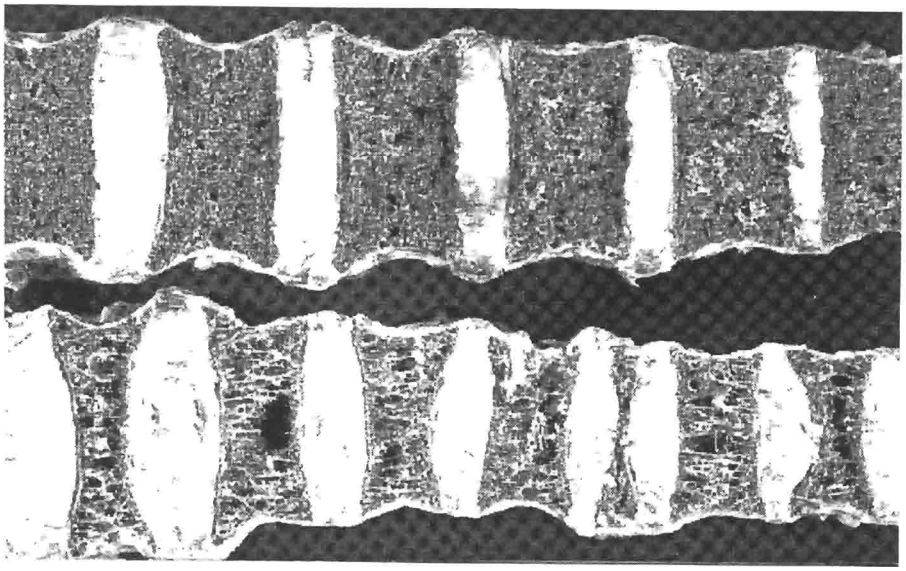

Figure 2. The upper vertebrae are normal and the lower are osteoporotic.

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2.3 KIDNEYS

The kidneys play an essential part in the regulation of the fluid and electrolyte balance in the body and in controlling the blood pressure. The filtration of blood through millions of glomeruli results in the production of about 180 litres a day of primary urine. The primary urine consists of an ultrafiltrate of plasma. In the renal tubules the tubular cells usually reabsorb more than 99.9 % of the substances (e.g., amino acids, small proteins, sugar) and salts. The amount of fluid that the body needs to retain is also reabsorbed. The substances and the fluid that are not reabsorbed are excreted from the body in the secondary urine, usually around 1,5 litres a day (27). The kidneys also conduct important endocrine functions. Angiotensin, which regulates the blood pressure, and erythropoetin, which stimulates the production of red blood cells in the bone marrow, are both synthesised in the kidneys. Active vitamin D (1,25-$(OH)_2$-D_3) is also produced in the kidneys by hydroxylation of 25-OH-D_3, which has already been hydroxylated in the liver (23).

The most serious renal conditions are those, which affect the glomerular filtration rate (GFR). These conditions can finally lead to uraemia if the GFR is very low. The GFR decreases with increasing age. Another type of glomerular damage leads to excessive protein leakage, when large proteins which usually do not pass the glomeruli are not filtered properly. In tubular lesions, the normal capacity of the tubular cells to reabsorb substances from the primary urine is reduced. There are several sensitive methods for determining the tubular function of the kidneys. Analyses of low-molecular plasma proteins, such as β_2-microglobulin, retinol binding protein (RBP), Protein HC (human complex forming glycoprotein, also called α_1-microglobulin) or tubular intracellular enzymes (e.g., NAG (N-acetyl-β-D-glucosaminidase)) are used to detect early tubular damage (27).

The knowledge about causes of renal diseases is limited. Diabetes, arteriosclerosis and various types of autoimmune and hereditary diseases are important causes, but in many cases the aetiology remains unknown. Certain drugs and chemicals, such as phenacetine and ethylene glycol, are well-known to elicit acute and chronic renal failure. There are also a number of chemicals (e.g., polychlorinated volatile hydrocarbons) and heavy metals other than cadmium (e.g., mercury, lead and chromium) that may exert more insidious nephrotoxic effects (27;144).

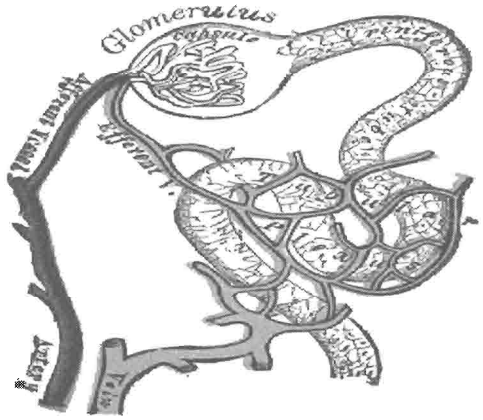

Figure 3. Schematic view of the kidney with the glomerulus, the tubulus and blood vessels.

2.4 CADMIUM

In nature cadmium occurs primarily together with zinc. In its metallic form, it has a silvery appearance. It was discovered in 1817, and industrial use started during the first part of the last century. Cadmium has been found to be a very useful metal: colour pigment (e.g., red like the cover of this theses, although cadmium-free) stabiliser (e.g., in PVC), for soldering, and as a cathode in batteries, among other uses. Ninety per cent of the cadmium used in Sweden today is used in nickel-cadmium batteries (48). If not properly recycled, the used cadmium is then dispersed in the environment. Phosphate fertilizers contain contaminations of cadmium and are a further source of environmental pollution. Sewage sludge can also be an important source of cadmium. Areas in the vicinity of mines, smelters and factories using cadmium in production often show pronounced cadmium contaminations (145).

There has been an increase of cadmium in the Swedish soil during the last century, and cadmium is still increasing in the arable soil (48). Compared to other metals, cadmium is easily available for uptake in vegetables, grain and rice. The uptake by plants from the soil is influenced by a number of factors, e.g., clay and organic content, cat-ion exchange capacity and pH, where a low pH increases the uptake (34;109;145).

2.4.1 Exposure

Food is the main source of cadmium exposure in the non-smoking general population (140;145). Cadmium is present in all types of food, but the concentrations vary to a great extent, depending on the food (70). Highest concentrations are found in shellfish and offal from adult animals. However, staple foods, such as cereals and vegetables, contribute on average more than 75% of total cadmium intake (107). The dietary intake is between 10 - 25 μg cadmium/day in Europe and the US (145), but there is a wide inter-individual variation depending on the type and the amount of food consumed. The variation follows a log-normal distribution, which implies that if the population average intake is 15 μg cadmium/day, individual intake will vary between 4 and 60 μg/day ($\pm$ 2 SD) (30). The intake from water and the exposure from air are low in non-contaminated areas. Smokers face additional exposure.

In the occupational settings, the main route of cadmium exposure is the respiratory system (3). Air concentrations of cadmium fumes or dust vary considerably between different industries, such as smelters, pigment plants and battery factories. In the early years of industrialisation, occupational cadmium exposure could be extensive. Improvements in industrial hygiene have led to continuous decreases in cadmium levels in the workroom air in most Western countries, although the situation is less encouraging in many developing countries (145).

2.4.2 Uptake and distribution

On average less than 5% of the dietary cadmium is absorbed (145), but absorption may be increased several-fold by iron deficiency (5;10;37). The uptake of cadmium following inhalation is much higher, in the order of 10-50%, depending mainly on the particle size and the chemical form of cadmium (145). Individuals smoking 20 cigarettes a day absorb a quantity roughly similar to that absorbed via the food in Europe and the US (145).

After ingestion or inhalation, cadmium is transported to the liver, where metallothionein (MT), a small protein, is synthesized and binds cadmium, preventing cellular toxicity from free cadmium ions. The complex then migrates from the liver into the blood stream. As with other small proteins, it is freely filtered across the glomeruli in the kidneys and then reabsorbed by the proximal tubular cells (103). After reabsorption, cadmium is accumulated in the kidney cortex with a half-life of 10-30 years. Because of this, more than half of the cadmium in the human body is stored in the kidneys and in the liver (145).

2.4.3 Biomarkers of exposure and dose

There is no simple accurate method to measure the whole body burden or concentration of cadmium in different tissues of a living person. To evaluate exposure and accumulation of cadmium, it is usually necessary to study concentrations in easily available indicator media. The two most often used dose estimates for cadmium are blood and urinary cadmium.

Cadmium in blood is primarily localised in the erythrocytes. There are at least two major compartments of cadmium in blood: one which mainly reflects recent exposure with a half-life of 2 to 3 months and the other related to body burden with an approximate half-life of about a decade (67). During high cadmium exposure (e.g., occupational exposure) the cadmium concentration in blood increases rapidly (83); after only a few months, cadmium in blood reaches a concentration that corresponds to the intensity of the exposure. If the exposure stops, the blood cadmium concentration decreases fairly rapidly (28). However, cadmium accumulated in the body will influence the blood cadmium concentration. Therefore, following cessation of exposure, the concentration in blood will not decrease to the pre-exposure level. Also, in the general population, blood cadmium is largely influenced by the body burden of cadmium (10;124). Thus, if there is no recent high cadmium exposure, cadmium in blood may serve as a good estimate of the accumulated body burden of cadmium (66).

Human and animals studies have shown that urinary cadmium concentrations increase in proportion to the amount of cadmium stored in the body, thus reflecting lifelong exposure (12;47;82;83;104;145). The best measure of cadmium in urine is the amount excreted over 24 hours. For practical reasons, 24-hour sampling is rarely feasible, and spot urine samples are commonly used. Spot urine samples vary in composition regarding water and solutes within and between individuals (25). Urinary creatinine or density is used to adjust for this variation (29). If renal tubular damage has occurred, it may lead to an increased cadmium excretion, resulting in urinary cadmium that may not reflect the lifelong exposure (145). This effect has been shown in animal (39;104) and human studies (16;39;83;90).

2.4.4 Health effects

2.4.4.1 Kidney effects

The critical organ for long-term cadmium exposure is considered to be the kidneys. Renal tubular damage, a decreased capacity to reabsorb substances from the primary urine, is the first sign of a toxic effect. The cadmium-MT complex, which is reabsorbed by the tubular cells, is degraded by lysosymes, and cadmium is released into the cytoplasm. Intracellular

cadmium is then bound to MT produced by the tubular cells. It is assumed that the first sign of tubular damage appears when the capacity to produce MT is exceeded, leading to increased excretion of low-molecular-weight proteins in the urine, such as protein HC or β2-microglobulin (40;61;103). NAG, an enzyme localized in lysosomes in the tubular cells, is another urinary marker indicative of tubulotoxic effect (27;61). The tubular proteinuria is an early indicator of toxic effects, but does not in itself give rise to any clinical symptoms. It has been suggested that cadmium induced proteinuria may be reversible if the exposure is substantially decreased (52). However, most studies have shown the tubular proteinuria to be irreversible (17;58;66;115;117). In a large study on environmentally exposed persons in Belgium (the so called Cadmibel-study), a 10% prevalence of tubular proteinuria was observed at cadmium levels equivalent to 2 nmol Cd/mmol creatinine, not adjusting for age (15). No similar data on environmentally exposed people in Sweden or in other countries than Japan or Belgium was known when the OSCAR-study was initiated.

If the cadmium exposure is prolonged, a glomerular damage may also develop, with a decreased glomerular filtration rate (GFR). This has been shown among both occupationally exposed workers (65;110;116) and environmentally exposed populations (99). Uremia was a common cause of death among Japanese farmers suffering from the Itai-itai disease (77). An increased prevalence of renal stones has also been found among cadmium exposed workers (31;40;63;145).

2.4.4.2 Bone effects

The most well known example of cadmium induced bone effect is the Itai-itai disease. Inhabitants in certain areas of the Toyama Prefecture in Japan were affected by renal disease, osteomalacia and osteoporosis. The rice in the area had been heavily contaminated with cadmium due to irrigation of the soil with water contaminated with cadmium from an upstream zinc mine. Most of the Itai-itai patients were women over 40 years of age who had lived in the endemic area for more than 30 years. After further research and debate it was concluded that "Itai-itai disease is caused by chronic cadmium poisoning, on condition of the existence of such inducing factors as pregnancy, lactation, imbalance in internal secretation, aging and deficiency of calcium" (94). At least a couple of hundred cases of Itai-itai disease occurred in Japan. The patients with the disease were exposed to very high levels of cadmium, with mean urinary cadmium excretions of around 30 nmol cadmium / mmol creatinine (39). With decreased exposure to cadmium and improved standards of nutrition, no new cases have been diagnosed in the former endemic area in Japan since the mid 1980's (77).

The mechanisms behind the cadmium induced bone damage are not fully understood; both direct and indirect effects have been proposed: an interference with parathyroid hormone (PTH) stimulation of vitamin D production in the kidneys, reduced activity of kidney enzymes activating vitamin D, increased excretion of calcium in urine, reduced absorption of calcium from the intestines and direct interference with calcium incorporation into bone cells or with collagen production (78).

In vitro experiments have shown that cadmium has a direct effect on both osteoblasts and osteoclasts (145), with decreased activity of ALP, DNA and hydroxyproline in osteoblasts at cadmium levels in the same range as that found in Itai-itai patients (57). Animal experiments have shown that cadmium in the food can affect the bone and calcium metabolism at lower levels than the people in the endemic Itai-itai area were exposed to

(13;14;120;141). For example, experiments on dogs showed that the bone resorption increased significantly 96 hours after exposure to cadmium in the drinking water, resulting in blood-cadmium levels between 27-71 nmol/l, which can be compared to a non-occupationally exposed heavy smoker. No differences in the levels of calciotropic hormones could be detected, indicating a direct effect on bone. Ovariectomised dogs were more sensitive indicating that postmenopausal women may be at increased risk of cadmium induced bone loss (13). Animal experiments have also shown that low calcium intake may increase the toxic effect of cadmium (141) and that cadmium could affect the mechanical properties of bone directly (106).

Studies on the general population in Belgium have shown positive relationships between urinary cadmium and both serum alkaline phosphatase (ALP) (129) and urinary calcium (128), suggesting that calcium metabolism is affected as cadmium accumulates in the body. Dose-response relationships between urinary cadmium and the prevalence of hypercalciuria have also been found in a Chinese study (148). The increased prevalence of renal stones among cadmium exposed workers could be related to the increased excretion of calcium when tubular proteinuria is present (74). Autopsies of Itai-itai patients indicated correlations between the severity of osteomalacia and the degree of damage of the proximal tubuli, suggesting that the osteomalacia is caused by disturbances of the calcium and phosphate regulation in the kidneys. In the studied patients, glomeruli and distal tubuli were only minimally changed (133). Japanese studies have shown decreasing levels of active vitamin D and increasing levels of PTH, with decreasing creatinine clearance in women, exposed to cadmium, implying that changed hormone levels could play a role in the development of cadmium induced bone injury (138). Another Japanese study showed increased serum bone Gla protein levels (an indicator of bone damage), and decreased microdensitometry (an indicator of osteopenia) in a cadmium exposed group compared to a referent group (75). To my knowledge, before this work was begun, the only study comparing BMD between people in a cadmium polluted area and an unpolluted area was done in Japan using ultrasound transmission to measure BMD in the calcaneus in 40 men and 63 women (139). A significant decrease in bone density in cadmium exposed women was noted, but not in men. The possible impact of cadmium on bone in the general population had never been investigated outside Japan before project OSCAR was initiated. In addition, the possible contribution of cadmium induced tubular damage to these effects had not been studied before.

2.4.4.3 Other effects

Very high inhalation exposure to cadmium fumes may cause acute pneumonitis with pulmonary oedema, which may be lethal (41;123). High ingestion of soluble cadmium salts may cause acute gastroenteritis (105). Many other health effects from cadmium have been studied; however, the evidence for other effects than on bone and kidneys is more unclear. IARC (International Agency for Research on Cancer) has classified cadmium as a human carcinogen (group I) on the basis of sufficient evidence in both humans and animals (55), but this has been questioned by newer research (60;61;126). While animal experiments have shown relationships between cadmium and high blood pressure and teratogenic effects (145), human studies have not supported these relationships (61;130).

3 AIMS OF THE THESIS

The primary aim of this thesis was to explore if low-level cadmium exposure could affect bone. A secondary aim was to further evaluate kidney effects. To explore these issues the specific objectives were as follows:

- To evaluate methods for measuring BMD in large epidemiological studies.
- To assess the dose-effect relationships between cadmium and BMD and dose-response relationships between cadmium and low BMD.
- To explore the effects of cadmium on the risk of forearm fractures.
- To assess the dose-effect and dose-response relationships between low-level cadmium dose and renal tubular damage.
- To explore the relationship between cadmium induced tubular damage and bone effects.
- To compare blood and urinary cadmium as different dose estimates in evaluation of cadmium's toxic effects.

4 SUBJECTS AND METHODS

In 1912, production of nickel-cadmium accumulators started in Fliseryd, a village in southeastern Sweden (Figure 4). Initially, the emissions were estimated as 1.5% of the produced mass (8;9), 90% of which was emitted to the river Emån, and the rest to the atmosphere. The total cadmium emissions from this process are estimated as 3.6 tons to air and 32 tons to water. This process changed in 1967, and the total cadmium emission from the new process, until 1974 when the battery plant in Fliseryd was closed, has been calculated to 4.5 tons to air. Lead was also processed (1942-67), and the total lead emission to air was approximately 240 tons (9). For both lead and cadmium, the emissions to the environment and the occupational exposure have decreased with time. However, the concentrations of metals are still elevated in samples 2-5 km from the point source (9). Nowadays the production takes place in a plant in Oskarshamn, where some of the workers from the old plant are still employed. Both the occupational exposure and the emissions to ambient air were substantial in the past but are currently much lower in this plant (2).

In order to obtain a wide range of cadmium exposure in our investigation, we enrolled occupationally exposed workers, environmentally exposed subjects from Fliseryd, and people from a town in the same area, but further away from the battery plants.

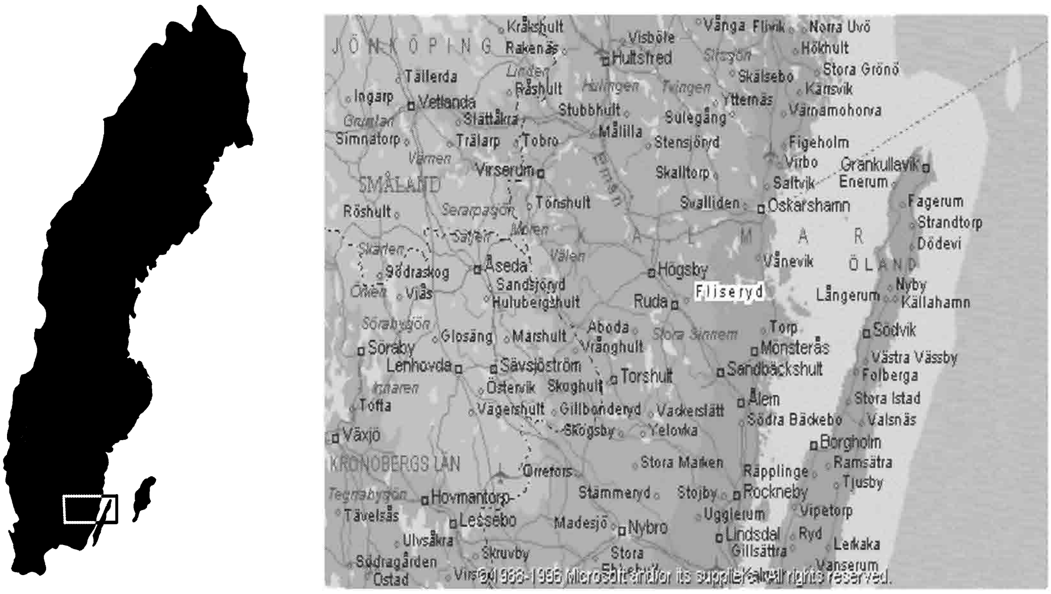

Figure 4. Part of Småland in south-eastern Sweden with Fliseryd marked.

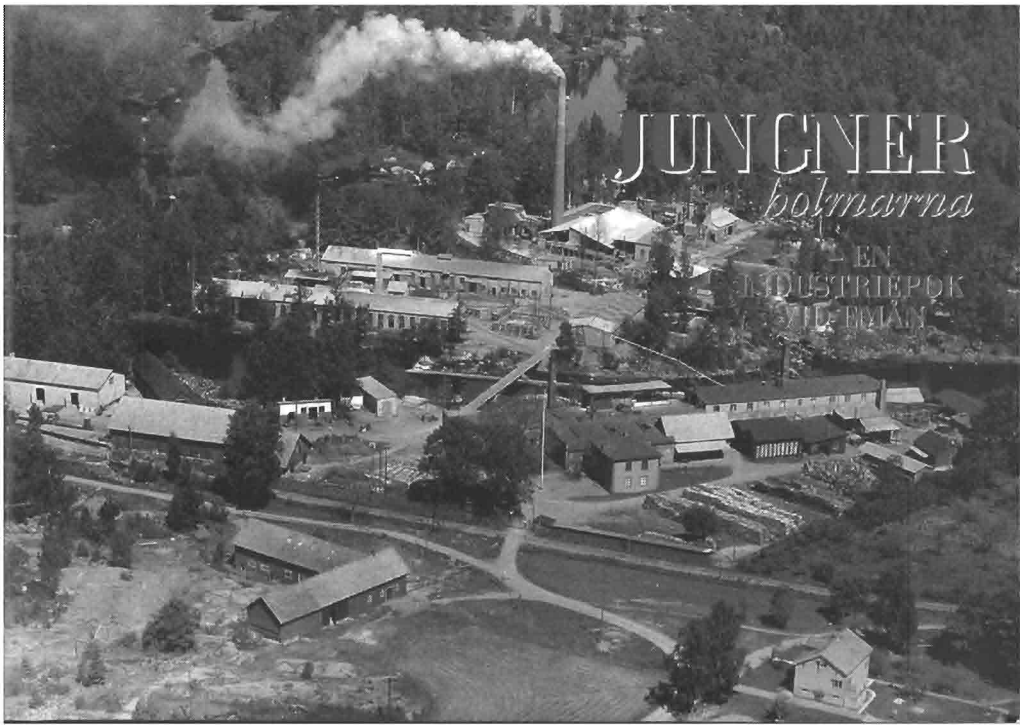

Figure 5. The battery plant in Fliseryd around 1935.

4.1 SUBJECTS

To evaluate the portable BMD-instrument (Osteometer DTX-200), a pilot study was conducted (paper I), including 43 of 46 cadmium exposed solderers from an existing cohort (65;66) at a factory in Linköping. The workers had been exposed to cadmium for at least 5 years before 1978, when cadmium-containing soldering material was abandoned.

Of around 900 workers, that had been employed for at least one year after 1941 at the battery plants, 242 workers still alive, and that had earlier been included in a programme with measurements of cadmium were invited to take part in the present study. Of these, 117 agreed to take part. For these workers measurements of blood and urinary cadmium from the early 1970's until early 1990's were available.

In 1996, at the start of the investigation, about 1500 people resided in Fliseryd near the former nickel cadmium battery plant. Inclusion criteria were age 16 to 80 (born 1916 – 1980) and residency in the area for at least five years between 1910 and 1992. In total, 861 persons fulfilled the inclusion criteria and were invited to participate. In addition, we located 398 people formerly living in Fliseryd, fulfilling the inclusion criteria. Thus 1259 persons were invited, and 768 agreed to participate.

To include subjects with no known environmental cadmium exposure, a referent group from a neighbouring city, Mönsterås, was included. 206 people in the same age range were

randomly selected from a register of a family physician, and 136 of these participated. The numbers of invited and participating people are presented in Table 1.

Table 1. Number of invited persons and participants.

Group	Invited	Participated	Percent
Solderers from Linköping	46	43	93 %
Battery plant workers	242	117	48 %
Residents of Fliseryd	861+398=1259	768	61 %
Referents	206	136	66 %
Total	*1753*	*1064*	*61 %*

All subjects gave informed consent, and the ethical committee at Karolinska Institutet approved the study.

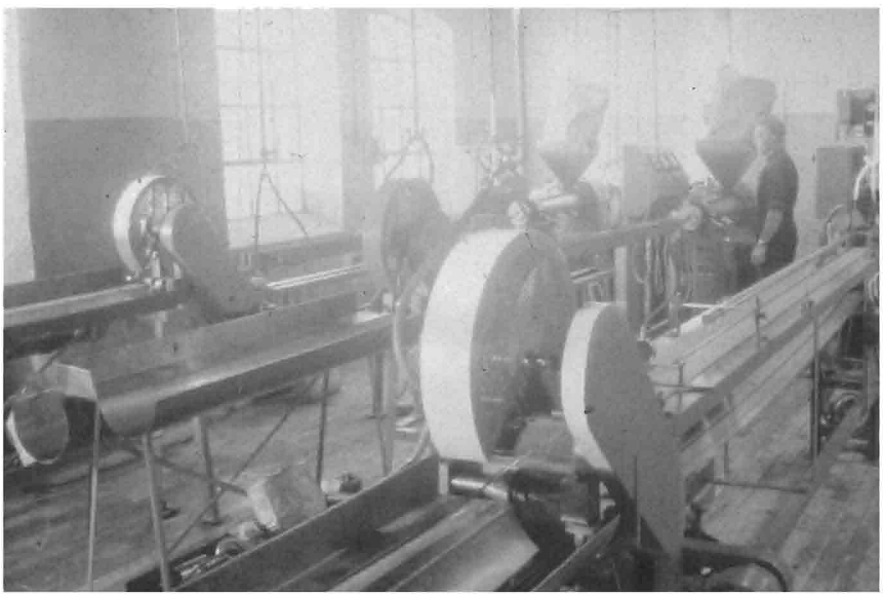

Figure 6. Workers in the battery plant in Oskarshamn during the 1950's.

4.2 METHODS

The study subjects who accepted to participate received a mailed questionnaire and a bottle for collection of first voided morning urine. They handed in the questionnaire, the urine and blood samples were collected and height, weight and BMD were measured by two specially trained nurses at a visit to the local health centre. Blood and urine were kept frozen (-20°C) until analysed.

4.2.1 Questionnaire

Each study subject received a questionnaire including questions about employment, residences, smoking, food habits and medical history, especially fractures, diseases related to osteoporosis and kidney diseases. The questions used in the questionnaires were taken from earlier Swedish studies on osteoporosis, where the questions had been validated. Workers examined in Linköping did not receive the questionnaire. Data on smoking for the workers from Linköping were obtained from an earlier, more limited questionnaire.

Subjects were classified as occupationally exposed if they had worked in either of the battery plants for at least one year. Smokers were classified into never smokers or into former/ current smokers if they had smoked regularly for at least one year. An estimate of dietary calcium intake was calculated from the reported consumption of dairy products (100 ml milk = 120 mg calcium, 1 slice cheese = 87 mg calcium) (54).

Only fractures that occurred at age 20 or later were taken into account, which discriminates between non-adult and adult fractures. All persons aged younger than 20 years were excluded (5 men and 7 women) from the analyses regarding the fractures (paper V). The fractures reported in the questionnaires were validated using x-ray and medical records from the only hospital in the area, where fractures occurring in the region are treated. A random sample of 40 participants who had not reported any fractures was also checked against the medical records. Civic registration numbers (unique for each Swede) were used to trace the patient records.

4.2.2 Analyses

Only a short description of the analytical methods will be described here; for a more thorough description see respectively paper.

4.2.2.1 Metals

Cadmium in urine and cadmium and lead in blood was determined using inductively coupled plasma mass spectrometry (ICP-MS, Fisons VG Plasmaquad PQ2) at the Department of Occupational and Environmental Medicine at the University Hospital in Lund. Each sample was prepared in duplicate. The coefficient of variation (CV%) for the duplicate determinations was 5% for blood cadmium and 8% for urinary cadmium. The accuracy was checked by including commercial reference samples.

For the participants from Linköping, cadmium in urine had been measured earlier at the Department of Occupational Medicine at Linköping University Hospital in 1993, using atomic absorption spectrophotometry (AAS) (65;66).

The earlier measurements of blood and urinary cadmium of the workers at the battery plants had during the 1970's been performed at the Department of Hygiene at Karolinska Institutet, and later at an independent laboratory (Analytica AB) with quality controls fulfilling WHO standards (33;64).

Adjustments for variation in urinary concentrations between individuals were made by dividing the urinary cadmium values by the creatinine concentrations (29).

4.2.2.2 Tubular proteinuria

Urine for determination of Protein HC was stored frozen (-20°C) with a preservative solution (134), until analyses were conducted by the Department of Clinical Chemistry at the University Hospital in Lund. Single radial immunodiffusion was used for the determinations. The limit of detection was 1.7 mg/l and total coefficient of variation (CV%) was 6%.

For the study subjects from Linköping, protein HC was measured in 1993 with zone immunoelectrophoresis (51;66).

The cut-off levels used for tubular proteinuria were 0.8 and 0.6 mg protein HC per mmol creatinine, for men and women respectively. These are the upper 95% limits in a reference population consisting of healthy adults from the city of Lund in southern Sweden (135), 200 kilometres from the study area.

4.2.3 Bone mineral density measurements

Bone mineral density (g/cm^2) was measured in the non-dominant forearm with the patient in a supine position by the ambulant instrument (Osteometer DTX-200). The BMD was measured in the "distal site" of the forearm, defined as the radius and ulnae area from the 8-mm point (where the radius and ulnae are separated by 8 mm) to 24 mm proximal, see Figure 7. The distal site contains 10-20% trabecular bone (121). The internal variation was checked by daily calibration using a phantom.

Z-scores were calculated, comparing the measured BMD to a reference population furnished by the instrument supplier. The reference population consisted of 800 Danes, aged 20-88 years, who were healthy volunteers without any diseases known to influence calcium metabolism. No restrictions were made on smoking or other lifestyle habits. We used a common definition of low bone mineral density which is Z-score < - 1 (72).

In paper I, BMD was also measured in the forearm, lumbar spine and hip (neck and trochanter) with a hospital-based DXA instrument (Hologic QDR 4500). Z-scores were calculated according to reference material provided by the instrument supplier (35).

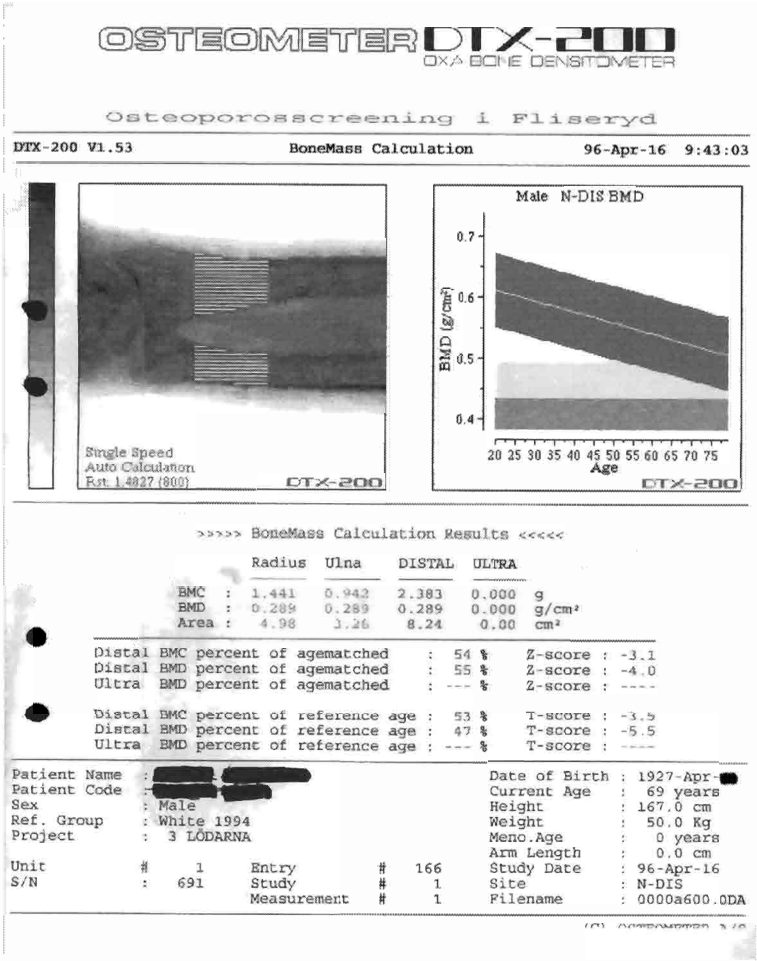

Figure 7. Printout from the Osteometer DTX-200. In the schematic view of the forearm, the dashed area represents the "distal site".

4.2.4 Statistical methods

Two main ways of describing possible relationships between exposure/dose and outcome have been used: dose-effect and dose-response relationships. The dose-effect relationship is used when the effects can be measured quantitatively, and a continuous relationship between dose and effect can be established, for example between urinary cadmium and bone mineral density. The dose-response relationship, when the effects are qualitative and usually dichotomous, for example fracture or not fracture. Continuous effects may be reduced to a dichotomous form by applying a limit above which an individual is considered to have an adverse effect; after this transformation a dose-response analysis can be performed, e.g., for tubular proteinuria.

Variables with a skewed distribution were log transformed (log e) to achieve normal distribution when appropriate.

Multiple regression was used for the multivariate analysis. Odds ratios (ORs) and 95% confidence intervals (95% CI) were computed using logistic regression.

The associations between cadmium dose, lead dose, and tubular proteinuria and the risk of forearm fracture were examined using Cox proportional hazards regression analyses. Current cadmium in urine and blood and lead in blood were used as proxies for the cadmium and lead dose, respectively, at the time of fracture. The time at risk for an adult forearm fracture (i.e., from age 20) was defined as the follow-up period. When a constraint on attained age was implemented, it was from age 50. The follow-up period ended with the first reported forearm fracture, or if no fracture occurred in 1997.

The STATISTICA©, EGRET© (papers I-III) and STATA© 7.0 (papers IV-V and new analyses added to the thesis) software have been used.

4.2.5 General

For the workers from Linköping some data were missing, e.g., blood lead, calcium intake, fractures, etc. In analyses, where these variables were used, these workers have been excluded.

5 RESULTS

According to the questionnaire, 105 of the environmentally exposed subjects had been employed at either battery plant and were therefore re-classified as occupationally exposed. All people not occupationally exposed were then grouped into the same category, environmentally exposed, due to a large overlap of measured cadmium between the group "residences in Fliseryd" and the group "referents". A summary of the study population divided into environmentally exposed and occupationally exposed in the battery plants or Linköping is shown in Table 2.

Table 2. All study subjects categorised according to exposure.

Type of exposure	Examined	Women	Men	Reported in paper
Occupationally exposed (Linköping)	43	2	41	I, III
Occupationally exposed (battery plants)	222	62	160	II, III, IV, V
Environmentally exposed	799	480	319	II, III, IV, V
Total	*1064*	*544*	*520*	

Main characteristics of study population are shown in Table 3. Smokers had an average U-Cd of 1.0 nmol/mmol creatinine (1.3 for men and 0.78 for women) and B-Cd of 10 (12 for men and 8.0 for women). The corresponding data for non-smokers were for U-Cd, 0.70 nmol/mmol creatinine (0.84 for men and 0.59 for women) and for B-Cd, 4.8 (6.4 for men and 3.6 for women).

A telephone survey of a random sample of 5% of the non-participants (both occupationally and environmentally exposed) was performed. It gave no indication that the non-participants differed from the examined group in a systematic way with regard to age, gender, or fracture incidence.

5.1 KIDNEY EFFECTS

A positive, statistically highly significant, linear relation was found between cadmium in urine and urinary protein HC after adjustment for age for both genders. Table 4 shows the regression coefficients for the independent variables age and U-Cd, with protein HC as the dependent variable with all study subjects included. For the environmentally exposed group, there was also a statistically highly significant linear relation between cadmium in urine and urinary protein HC after adjustment for age for both genders, also shown in Table 4.

Table 3. Characteristics of the study population, environmentally exposed, occupationally exposed from the battery plants and occupationally exposed from Linköping. Mean and 10th, 90th percentiles, except for age where the full range is shown.

	Environmentally exposed		Occupationally exposed		Occupationally exposed	
			Battery plants		Linköping	
	Men	Women	Men	Women	Men	Women
Characteristics	(n=319)	(n=480)	(n=160)	(n=62)	(n=41)	(n=2)
Age years (range)	52 (18, 81)	51 (16,81)	58 (24, 81)	57 (25, 77)	59 (40, 78)	43 (42, 43)
Weight (kg) [a]	82 (68, 96)	69 (54, 86)	81 (68, 95)	72 (56, 89)	80 (66, 93)	58 (57, 59)
Calcium intake (g/day) [b]	0.94	0.72	0.84	0.70	n.a.	n.a.
	(0.36, 1.7)	(0.30, 1.2)	(0.25, 1.6)	(0.33, 1.2)		
Smokers (% former or current) [c]	48	42	60	53	64	100
Urinary Cd (nmol/mmol	0.38	0.55	1.7	1.5	3.8	2.6
creatinine) [d]	(0.16, 0.70)	(0.20, 1.0)	(0.34, 4.1)	(0.50, 3,5)	(1.1, 5.9)	(0.60, 4.6)
Blood Cd (nmol/l) [e]	4.0	4.6	15	12	31	11
	(1.2, 9.0)	(1.4, 10)	(2.5, 40)	(3.3, 25)	(8.6, 52)	8.7, 14)
Blood Pb (µmol/l) [f]	0.16	0.11	0.15	0.10	n.a.	n.a.
	(0.080, 0.25)	(0.050, 0.17)	(0.080, 0.25)	(0.05, 0.16)		
Urinary protein HC	0.51	0.46	0.98	0.44	2.1	0.33
(mg/mmol creatinine) [g]	(0.16, 0.96)	(0.15, 0.80)	(0.18, 1,6)	(0.18, 0.83)	(0.29, 6.6)	80.21, 0.45)
BMD (g/cm2)	0.57	0.44	0.55	0.44	0.52	0.50
	(0.47, 0.67)	(0.32, 0.54)	(0.44, 0.66)	(0.31, 0.56)	(0.42, 0.65)	(0.47, 0.54)

n.a. No data available
a) Nine subjects with no measured weight
b) two missing answers
c) eleven missing answers
d) 15 missing laboratory analyses
e) 12 missing laboratory analyses
f) 13 missing laboratory analyses
g) 15 missing laboratory analyses

Table 4. Dose effect relation between urinary cadmium and urinary protein HC for all the study subjects and for the environmentally exposed subgroup, men and women, adjusted for age.

	Total population		Environmentally exposed	
	Men [a]	Women [b]	Men [c]	Women [d]
	Regression Coefficient (95% CI)	Regression Coefficient (95% CI)	Regression Coefficient (95% CI)	Regression Coefficient (95% CI)
Age	0.0098 (0.00092, 0.019)	0.0074 (0.0043, 0.010)	0.0090 (0.0040, 0.014)	0.0047 (0.0012, 0.0081)
Urinary cadmium (nmol/mmol creatinine)	0.45 (0.37, 0.52)	0.11 (0.035, 0.19)	0.37 (0.045, 0.70)	0.40 (0.25, 0.54)

a $R^2 = 0.24$ c $R^2 = 0.078$ b $R^2 = 0.075$ d $R^2 = 0.11$

Another way to examine the relation is to analyse the dose-response relationship between urinary cadmium and tubular proteinuria. A total of 193 persons displayed elevated protein HC concentrations in urine, with a clear dose-response relation between urinary cadmium and the prevalence of increased protein HC in urine as shown in Table 5. The dose-response relation remained even when the occupationally exposed individuals were excluded.

The dose response relationships for elevated urinary protein HC differ between environmentally and occupationally exposed people as can be seen in Figure 8. Confidence intervals are naturally wider when dividing the data into subgroups, but are not included in the figure for the sake of readability. For the environmentally exposed group, an odds ratio of 2.5 is seen in the dose group 0.3-0.5 nmol cadmium/mmol creatinine; in the occupationally exposed group, the same odds ratio is seen first in the dose group 2-3 nmol cadmium/mmol creatinine.

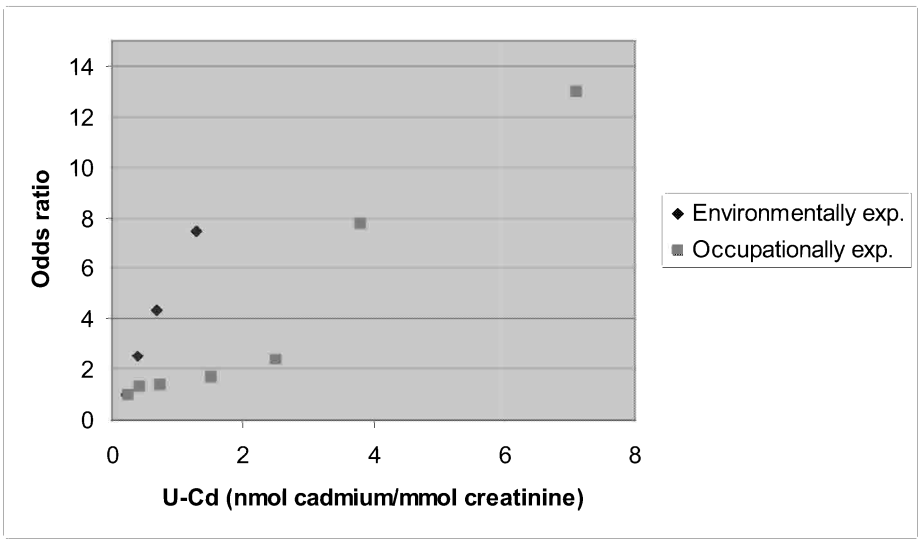

Figure 8. Odds ratios for prevalence of tubular proteinuria related to urinary cadmium for the environmentally and occupationally exposed groups, adjusted for age and gender.

Table 5. Tubular proteinuria (cases defined as urinary protein HC > 0.8 mg/mmol creatinine for men and >0.6 for women mg/mmol creatinine) for the whole study population, as well as for environmentally and occupationally exposed in separate analyses.

U-Cd nmol/mmol creatinine	Mean U-Cd Total population (nmol/mmol creatinine)	Mean U-Cd Environmentally (nmol/mmol creatinine)	Environmentally exposed persons cases/total (prevalence %)	Occupationally exposed persons cases/total (prevalence %)	Total population cases/total (prevalence %)	OR (95% CI)	OR, (95% CI) environmentally exposed
< 0.3	0.21	0.21	12/252 (4.8)	1/14 (7.1)	13/266 (4.9)	1.0	1.0
0.3 - < 0.5	0.38	0.38	33/243 (14)	4/30 (13)	37/273 (14)	1.8 (0.91, 3.6)	2.5 (1.1, 5.5)
0.5 - < 1	0.69	0.68	60/232 (26)	9/70 (13)	69/302 (23)	2.9 (1.5, 5.5)	4.3 (1.9, 11)
1 - < 2	1.4	1.3	22/53 (42)	11/62 (18)	33/115 (29)	3.6 (1.7, 7.4)	7.5 (3.5, 44)
2 - < 3	2.5	2.4	1/1 (100)	8/30 (27)	9/31 (29)	3.7 (1.4, 10)	
3 - < 5	3.8	3.7	0/1 (0)	16/33 (48)	16/34 (47)	8.9 (3.6, 22)	
>= 5	7.1		0	16/22 (73)	16/22 (73)	20 (6.5, 62)	

Table 6. Dose-effect relation between urinary cadmium and bone mineral density (BMD). Multiple linear regression analysis: (BMD) for men and women age 60 and older as a function of age, weight (kg) and urinary cadmium (nmol/mmol creatinine).

	Environmentally exposed		Occupationally exposed		Total	
	Men (n=94)	Women (n=153)	Men (n=94)	Women (n=24)	Men (n=188)	Women (n=177)
	Regression coefficient (95% CI)	Regression coefficient (95% CI)	Regression coefficient (95% CI)	Regression coefficient (95% CI)	Regression coefficient (95% CI)	Regression coefficient (95% CI)
Age	-0.0031 (-0.,0060, -0.00010)	-0.0058 (-0.0079, -0.0037)	-0.0039 (-0.0071, -0.00064)	-0.0037 (-0.011, 0.0044)	-0.0032 (-0.054, -0.0011)	-0.0056 (-0.0076, -0.0036)
Weight	0.0018, (0.00042, 0.0032)	0.0024 (0.0015, 0.0034)	0.0031 (0.0016, 0.0045)	0.0020 (-0.00083, 0.0049)	0.0026 (0.0016, 0.0036)	0.0024 (0.0015, 0.0031)
U-Cd	-0.048 (-0.12, 0.025)	-0.0091 (-0.043, 0.025)	-0.0040 (-0.0097, 0.0016)	-0.026 (-0.060, 0.0082)	-0.0056 (-0.010, -0.00093)	-0.017 (-0.037, 0.0031)

When blood cadmium was used as an estimate of the cadmium dose, there was also a strong positive correlation between cadmium and protein HC, which is shown in Table 2 in Paper IV. The table shows the regression coefficients for the independent variables age, blood cadmium, blood lead, and smoking, with log-transformed protein HC as the dependent variable. No similar effect was seen for blood lead. The results only changed marginally when blood cadmium and blood lead were analysed separately, or smoking was excluded. When the analyses were restricted to environmentally exposed persons, the results remained essentially the same.

Figure 9 shows the dose-response relationship between blood cadmium and tubular proteinuria with odds ratios for tubular proteinuria for different blood cadmium groups in the environmentally and occupationally exposed group, after adjustment for age, gender, and smoking. Exclusion of the study subjects from Linköping yielded a total of 171 cases (128 environmentally and 43 occupationally exposed) with tubular proteinuria. The cut-off level for the lowest dose-group was set at 5 nmol/l, and this group was used as the reference group (n=658). The remaining subjects were divided into five groups where all were of similar size except the highest dose group (n= 84, 93, 80, 66, 28). This resulted in cut-off-points for the different groups of 5, 7, 10, 15 and 30 nmol/l. Excess risks of tubular proteinuria were found for all the environmentally exposed groups exceeding 7 nmol cadmium /l blood. For the occupationally exposed group, excess risks of tubular proteinuria were seen only in the highest dose group, with blood cadmium exceeding 30 nmol /l.

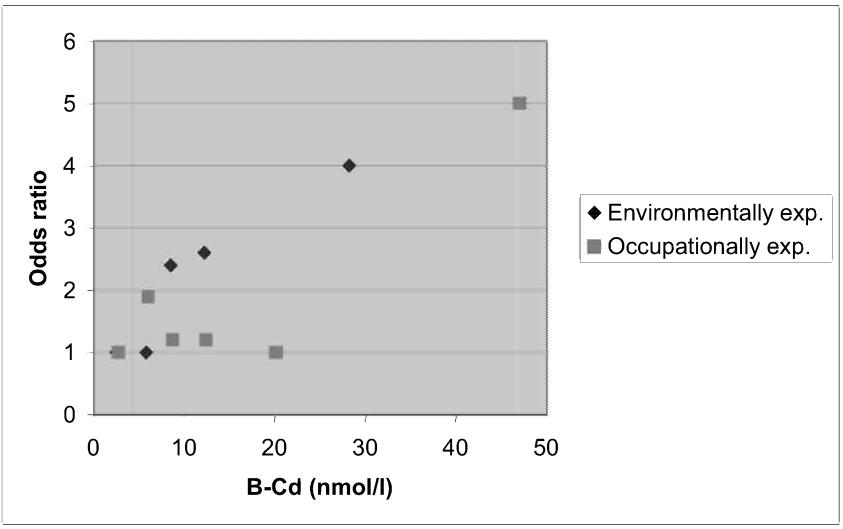

Figure 9. Odds ratios for prevalence of tubular proteinuria related to blood cadmium, adjusted for age, gender and smoking for the environmentally and occupationally exposed groups.

5.2 BONE EFFECTS

The subgroup of solderers from Linköping where the BMD was measured with one hospital based machine (Hologic QDR 4500) and the ambulant instrument (Osteometer DTX-200) was used to validate the ambulant instrument. There was a good correlation between forearm BMD measured with the DTX-200 and the Hologic QDR 4500 (Figure 1 in paper I). The regression coefficient was 1.048, which indicates a very good agreement between the instruments. However, the BMD values resulting from the DTX-200 measurements were in mean 15% lower than the values from the Hologic QDR 4500.

Bone mineral density was negatively associated with age. Figure 1 in paper III shows that BMD decreases more rapidly after 55-60 years of age in both men and women. Therefore, we concentrated the analyses on the older age group (60 years and older).

The dose-effect relationship between internal cadmium dose (expressed as cadmium in urine) and BMD for the subjects aged 60 and over is shown in Table 6. Cadmium dose was negatively related to BMD. In contrast, body weight was positively correlated to BMD. Table 6 includes the statistically significant explanatory variables for BMD for men and women, among environmentally and occupationally exposed individuals, as well as for the total older age group. When smoking status (never-smokers versus ever-smokers) was added to the regression, there was no change in the regression coefficients. Smoking was therefore not included in the further analyses.

We also analysed the dose-response relationships between urinary cadmium and low bone mineral density, using Mantel-Haenszel method. The cut-off point used to define low bone mineral density was < -1 Z-score, and groups are stratified by age and gender; the results are presented in Table 5 paper III. There was a marked dose-response relation for low bone mineral density in men over 60 years of age, with an odds ratio of 2.2 (95% CI 1.0 – 4.8) for the group with U-Cd between 0.5 and 3 nmol/mmol creatinine and an odds ratio of 5.3 (95 % CI 2.0 - 14) in the highest dose category. In the stratified analysis of the whole group, adjusting for gender, age group (< 60 years or >= 60 years) and weight group (<=64 kg, 65-74 kg, 75-83 kg, >=84kg) the odds ratio for low bone mineral density in the highest dose group was 2.5 (95% CI 1.2 – 2.5).

Using logistic regression analysis to analyse the dose-response relationship, the results remain similar, as shown in Table 7. The Table shows a logistic regression model including urinary cadmium and weight. There was a three times (OR=3.2, 95% CI: 1.7 – 5.9) higher risk of low bone mineral density in the group having urinary cadmium levels over 3 nmol/mmol creatinine, compared with the lowest dose category.

Table 7. Logistic regression model for low bone mineral density (Z-Score < -1), including urinary cadmium (nmol/mmol creatinine) as a categorical variable, and weight as a continuous variables.

Variable	Odds ratio	95 % CI
U-Cd < 0.5	1	-
U-Cd >=0.5 and <3	1.12	0.81 – 1.56
U-Cd >=3	3.2	1.72 – 5.9
Weight	0.931	0.918 – 0.947

The dose-effect relationship between blood cadmium and BMD is presented in Table 3 in paper IV. The Table shows a multiple linear regression for the sub-group aged 60 and older, with distal BMD as the dependent variable, and age, weight, blood cadmium, blood lead, and smoking, as independent variables. In both the whole group and the older subgroup there was a negative correlation between age and BMD and a positive correlation between weight and BMD. There was a negative correlation between cadmium dose for both men and women in the older age group (significant for women and of borderline significance for men). In contrast, no significant trends were observed for lead. In the whole group (all ages), no significant correlations could be found between blood cadmium, lead, and BMD. Smoking did not alter the analyses. The results only changed marginally if blood cadmium and blood lead were examined in separate analyses.

Dose-response relationships using blood as the dose estimate are displayed in Table 4 in paper IV. The table shows the odds ratios (with 95% confidence intervals) for low bone mineral density (Z-score <-1) for three blood cadmium groups for people over 60 years of age, adjusted for weight and smoking. The cut-off level for the lowest dose-group was set at 5 nmol/l as above. The remaining subjects were divided into two groups of similar size (n=177, 174). This resulted in the cut-off-points for the different groups at 5 and 10 nmol/l. Statistically significant differences were seen at blood cadmium levels over 5 nmol/l, and in the group with blood cadmium over 10 nmol/l the OR was 2.9 (95% C.I. 1.4, 5.8).

Table 8 shows Z-score, as the dependent variable, as a function of weight and urinary protein HC, for men and women. The results show that protein HC is associated with a decrease in Z-score for men, which is statistically significant; no association was found for women.

Table 8. Dose-effect relation between urinary excretion of protein HC and degree of osteoporosis (Z-score). Multiple linear regression analysis; degree of osteoporosis (Z Score) for men and women, age 60 and older, as a function of weight (kg) and urinary protein HC (mg/mmol creatinine).

	Men [a] (n=196)		Women [b] (177)	
	Regression coefficient	95% CI	Regression coefficient	95% CI
Weight	0.048	0.032, 0.065	0.037	0.022, 0.053
U-Protein HC	-0.14	-0.24, -0.037	-0.012	-0.27, 0.24

a $R^2 = 0.18$ b $R^2 = 0.11$

Fractures

Three study participants did not report whether they had had a fracture or not and were therefore excluded from the fracture analyses. 113 study subjects reported a forearm fracture. Sixty-eight of these fractures were found in medical records, 56 of these were confirmed to be forearm fractures. The 12 other fractures were 9 fractures of the carpus or metacarpus, two of the elbow, and one fissure in the forearm. For 38 of the 45 forearm fractures not found in the medical records, the year of the fracture had been given in the questionnaire. Seven subjects for whom it was not possible to find the year of the forearm fracture were excluded from the analyses. Thus, it was possible to obtain information about the year of the fracture for a total of 94 forearm fractures. Sixty-three of the subjects

had their forearm fracture when they were adults (age at fracture >=20) and 43 of these were confirmed in the medical records. In addition there were also 16 hip fractures (8 men and 8 women) and 20 vertebral fractures (9 men and 11 women) reported in the questionnaires. Three of the reported hip fractures were confirmed in the medical records, one was a knee fracture, seven coxarthrosis and five were not found in the medical records. Only four of the vertebral fractures, were validated as vertebral fractures; the other were not found in the medical records. Because of the poor validity of reported hip and vertebrae fractures, only forearm fractures were used in the analyses concerning fractures.

Table 1 in paper V shows characteristics of the study population aged 20 and older with information divided according to history of forearm fracture. The mean age for having a forearm fracture was 46 years (youngest 21, oldest 69). The sub-population of persons aged 50 years or older is presented in Table 2 in paper V. Twenty-eight of these 32 forearm fractures that occurred when the patients were 50 years or older were confirmed by the medical records. In this subgroup, the mean age for having a forearm fracture was 58 years. No one who had a forearm fracture at age 50 or later had had a forearm fracture earlier. Generally, both men and women with forearm fractures were older, and had higher levels of urinary cadmium, blood cadmium and protein HC. In the study population aged 20 and older the mean blood cadmium for men without forearm fractures were 7.6 and for women 5.4, compared to 10 and 6.4 in the group with forearm fractures. The corresponding numbers for the sub-population of persons aged 50 were 8.9 for men and 6.0 for women without forearm fractures and 17 and 6.6 for men and women with forearm fractures.

Table 3 in paper V presents the unadjusted and the adjusted hazard ratios for forearm fracture at age 50 and over. The adjusted hazard ratio for forearm fracture was 1.18 (CI 1.01, 1.37) per unit nmol cadmium/mmol creatinine. Using Body Mass Index (BMI) instead of body weight did not affect the results. Including age at time of cadmium measurement did not change the adjusted fracture hazard ratio in relation to urinary cadmium.

For forearm fractures before the age of 50, no associations were observed in relation to cadmium dose. Therefore, the cadmium related fracture hazard ratios for different levels of cadmium dose would not be constant over the follow-up time, as required in Cox regression analyses, without implementing a restriction on age at risk. For age at risk beyond age 50, the proportional hazard assumption was not violated.

Using blood cadmium instead of urinary cadmium as the dose estimate adjusting for the same variables as in Table 3 showed a hazard ratio for forearm fracture of 1.02 (95% CI 0.99, 1.05) for an increase of 1 nmol/l cadmium. Because the units for urinary and blood cadmium are different, it is preferable to compare the increase in risk for e.g. one inter-quartile range, equal to 0.41 nmol cadmium/mmol creatinine for U-Cd and 4.53nmol/l for B-Cd. The increase in risk for one inter quartile range was 1.09 for blood cadmium and 1.07 for urinary cadmium.

The hazard fracture ratio in relation to urinary protein HC was 1,22 (95% CI 1.05, 1.40) per increase of 1 mg/mmol creatinine urinary protein HC adjusted for the same variables as in Table 3 in paper V.

No similar relationships were found between blood lead and the risk of forearm fracture.

5.3 BLOOD AND URINARY CADMIUM AS ESTIMATES OF CADMIUM BODY BURDEN

In Figure 10 log-transformed blood cadmium as a function of log-transformed urinary cadmium is shown. As can be seen in the figure there is a strong correlation between the two different dose estimates of cadmium used in this thesis, with a correlation coefficient of 0.79.

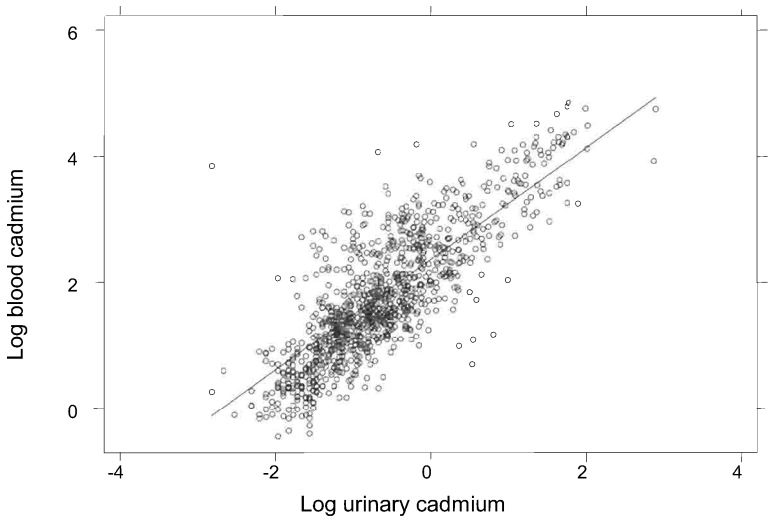

Figure 10. Log-transformed B-Cd as a function of log-transformed U-Cd, for the whole study population. (n=1035, 31 missing records).

Before project OSCAR no earlier measurements of cadmium of the environmentally exposed group had been performed. In contrast, the workers at the battery plants had been followed from the early 1970's to the mid 1990's. These data were used to study how blood and urinary cadmium levels had changed over time and the relationship between the measurements made in 1997 in this study and earlier ones. For all the workers except one, we had between one and 45 measurements of blood cadmium and between one and 20 measurements of urinary cadmium. These measurements were used to calculate a mean for every worker in five-year intervals, beginning in 1970 for blood cadmium and 1975 for urinary cadmium. The means for the workers for the different time intervals were then used to compute a mean for the study group in five-year intervals as shown in Figures 11 and 12. As can be seen in Figure 11, the mean urinary cadmium was almost the same from 1976 to 1985 as it was in 1997. However, most workers with high cadmium levels in 1997 had higher levels during the period 1976-85, and workers with low urinary cadmium in 1997 had lower levels earlier (Figure 13). Altogether the mean has not changed. Figure 12 shows that the blood cadmium levels have decreased continuously since the measurements began, from 75 nmol cadmium/l in 1970-75 to 14 nmol cadmium/l in 1997, with a faster decrease in the beginning of the measurement.

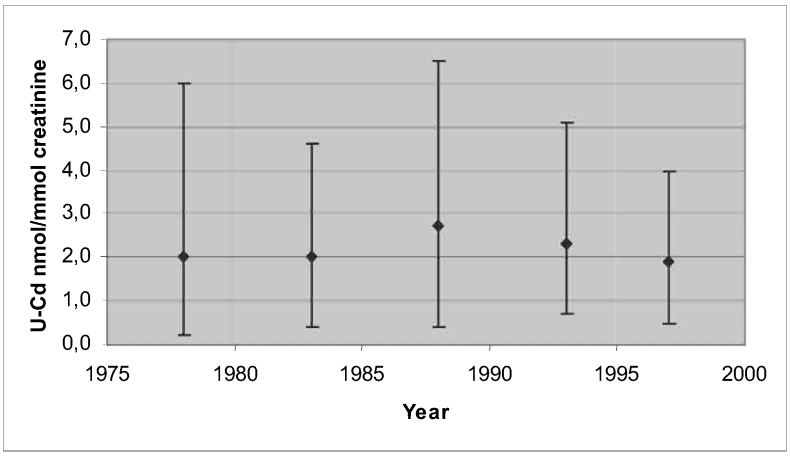

Figure 11. Mean urinary cadmium with 10 and 90% percentiles, for the workers from the battery plants, 1976-1997. (1976-80 n=95, 1981-85 n=80, 1986-90 n=62, 1991-95 n=48).

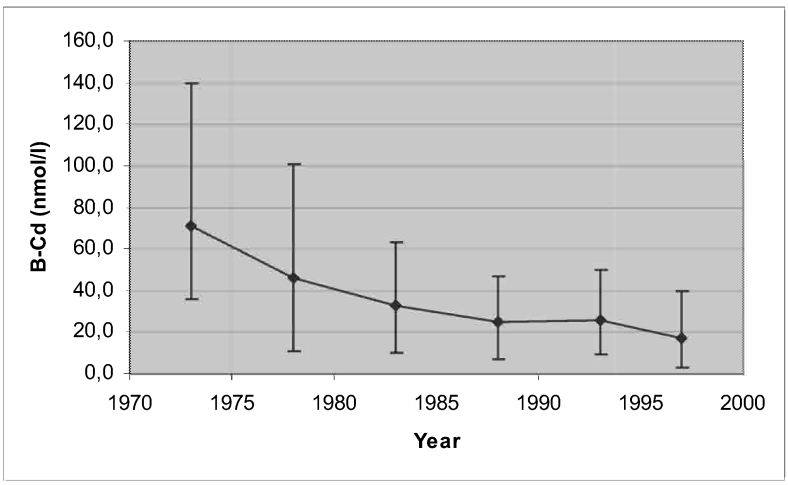

Figure 12. Mean blood cadmium with 10 and 90% percentiles, for the workers from the battery plants, 1970-1997. (1970-75 n=36, 1976-80 n=96, 1981-85 n=90, 1986-90 n=78, 1991-95 n=54).

Figure 13 shows urinary cadmium measured in 1997 as a function of urinary cadmium measured between 1976 and 1980. There was a correlation between the earlier and the recent measured cadmium with a correlation coefficient of 0.67. A urinary cadmium of 4 nmol/mmol creatinine in 1997 corresponds to roughly the double of twenty years earlier. The urinary cadmium data are not normally distributed, but the results do only change marginally if using a non-parametric test, with Spearman's rank correlation coefficient of 0.65 ($p<0.0001$). Figure 14 shows the same relationship for blood cadmium. There is also a correlation between the earlier and the recent measured cadmium with a correlation coefficient of 0.72, with Spearman's rank correlation coefficient of 0.69 ($p<0.0001$). A blood cadmium of 20 nmol/l in 1997 corresponds to roughly 50 twenty years earlier. The workers with high urinary and blood cadmium levels during the end of the 1970's still had

high cadmium levels, although lower, and the workers with low cadmium levels still had low levels.

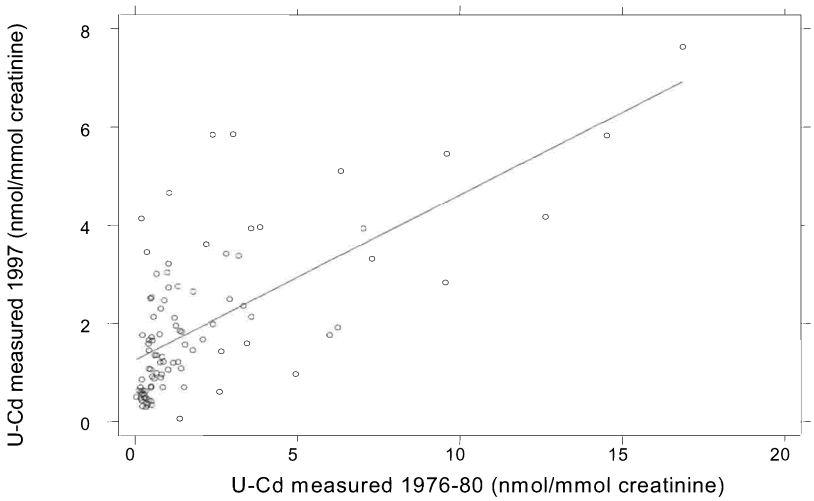

Figure 13. Urinary cadmium measured in 1997 as a function of urinary cadmium measured between 1976 and 1980. (n=94)

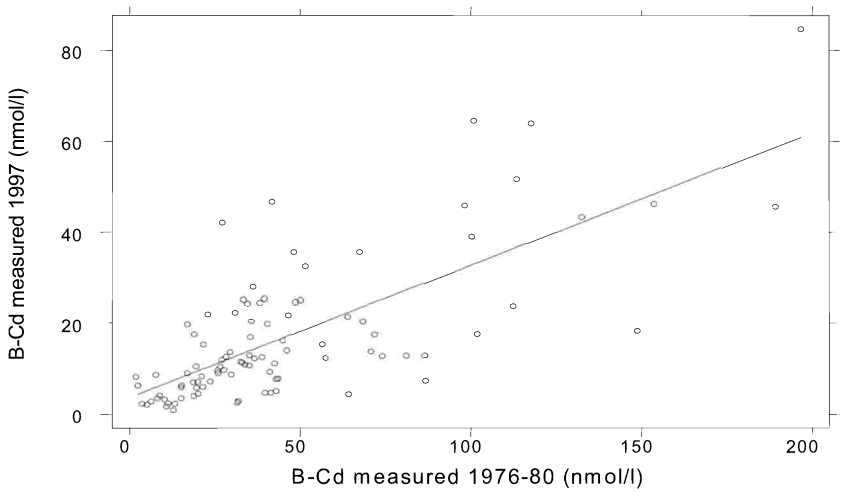

Figure 14. Blood cadmium measured in 1997 as a function of blood cadmium measured between 1976 and 1980. (n=96)

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Epidemiology commonly examines associations between different factors and diseases; i.e., exposures and outcomes. A set of different study designs exists, but the most often used are cohort, case-control or cross-sectional designs, all having both advantages and disadvantages. As some of the difficulties are general for all epidemiological research and some differ between study methods, certain issues of general importance will be discussed initially, after which special emphasis will be put on the cross-sectional study design, used in project OSCAR. Thereafter the project specific questions will be discussed.

The overall goal of an epidemiological study is accuracy in measurement: to estimate the value of the parameter with little error. Or as Rothman points out, "Every epidemiological study should be viewed as a measurement exercise" (118). Sources of error in measurement may be classified as either random (caused by chance) or systematic (caused by biases). To minimise error, one should try to get the best precision (lack of random error or sometimes called reliability) and validity (lack of systematic error) possible.

Precision corresponds to the reduction of random error. Precision can be improved in two ways. The primary method is to increase the size of the study, but it can also be improved by modifying the design of the study to increase the efficiency with which information is obtained from a given number of study subjects. For example, the proportion of subjects exposed, the proportion of subjects who have or will develop disease, and the distribution of subjects according to key variables that must be controlled for in the analysis, will all affect the efficiency and in turn the precision of the study.

Validity of a study is usually separated into two parts. The external validity, the validity of the inferences as they pertain to people outside the study population, and the internal validity, the validity of inferences drawn as they pertain to the actual subjects in the study. The latter is a prerequisite for the former. Internal validity implies an accurate measurement apart from random errors; however, various biases can detract the results. There are three major categories of biases: selection bias, information bias and confounding. These categories are not always clearly demarcated. A useful distinction between confounding and other biases is to consider a bias to be confounding if it, at least theoretically, can be controlled for in the data analysis.

Selection bias is associated with how the study subjects are recruited to the study. The common element of such biases is that the relation between exposure and disease is different for those who participate and those who would theoretically be eligible for the study but do not participate.

Information bias deals with exposure measurements and classification of individuals as healthy or diseased. If non-differential (random) the results will most often be diluted; any association between exposure and outcome will be underestimated. On the other hand, if misclassification is differential (systematic), the distortion of the results may take any direction.

Confounding is a systematic error introduced when the exposed and unexposed subjects in a study differ with respect to other factors (confounders) that influence the risk of obtaining the outcome under study. The confounding factor must be associated with both the exposure and the disease under study. If the confounder is identified and measured, it is

possible to adjust for it in the analyses. If unidentified or unadjusted, the results may be distorted.

C*ross sectional studies* include all persons in the population, or a representative sample of all such persons, at the time of ascertainment, including also those who have the disease. Usually the exposure information is collected at the same time as the disease information, so that different exposure subpopulations may be compared with respect to their disease prevalence (119). One of the main problems particularly associated with the cross-sectional approach is that one most often measures current exposure, which may have little relation to exposure during the etiologically relevant period. The induction time between relevant exposure and disease occurrence is often unknown regardless of study design In addition, in cross-sectional studies the time from disease occurrence until the time of the study is also unknown. However, in the present studies we have used urinary and blood cadmium as markers of the total body burden. Both these dose estimates have, as discussed in the introduction, been shown to be good proxies for the total body burden. Another problem with the cross-sectional study design is that one most often measure prevalence and not incidence of the disease under study. If the duration of the disease differs depending on the exposure this can cause bias. This is, however, not likely to affect the results of the present investigation as tubular proteinuria and low BMD are most probably irreversible and we measured incidence of fractures.

6.1.1 Study population

If the relation between exposure and disease differed between those who participated and those who did not, a selection bias could be introduced. In this study we had a participation rate of 60%, which is considered good for this type of study, which includes blood and urinary samples as well as BMD measurements. The Cadmibel study (131) had a similar participation rate. Still, a selection bias may be present. For instance, maybe some of the heavily exposed workers were not able to participate because of hip fractures? This would cause an underestimation of the observed effect between cadmium exposure and osteoporosis. Or the other way round, if only heavily exposed workers with a history of fractures and/or kidney disease participated, the effect may be overestimated. However, in the present investigation we attempted to rule out this possibility through a telephone survey of 5% of the non-participants. It gave no indication that the non-participants differed from the examined group regarding exposure and effect.

The lowest participation rate was among the battery workers. A common reason for not participating was that they were tired of studies. The high participation rate in Linköping may be partly explained by a participation re-imbursement.

Throughout the study, results both on group and individual levels have been presented to the participants. Everyone who wanted this was given his/her cadmium and BMD results, and at the time of the first official report of the results (62) all the participants were invited to information meetings. People with osteoporosis received treatment when appropriate. As been pointed out in the literature (89), it is important that people who participate in studies are informed about the results.

6.1.2 Exposure assessment

Ideally, to establish dose-effect and dose-response relationships between cadmium exposure and bone and kidney effects, we would measure the internal dose of cadmium at the time of causal action. This period is however not known and we do not have any means to measure cadmium retrospectively. Although this may seem more obvious concerning the fracture outcome, the other outcomes suffer from the same limitations. On the other hand, cadmium has a very long half-life, which means that the present levels may serve as good estimates of the total body burden (145). This was confirmed by the results of the previous measurements of cadmium in the workers from the battery plants. The good correlation between the earlier measured cadmium concentrations and the measurements in 1997 showed that the dose estimates used in the present investigation are good proxies for the biologically relevant dose. However, the levels of both blood and urinary cadmium for the occupationally exposed subjects have decreased during the last decades. This may also be the case, but less pronounced, for the environmentally exposed subjects living close to the battery plant. This decrease can lead to a differential misclassification, indicating stronger dose-effect relationships than the true ones and a shift of the dose-response curves to the left.

Another possible difficulty with the exposure assessment of cadmium is that different methods were used to measure urinary cadmium for the workers in Linköping (AAS) and the other study subjects (ICP-MS)(paper III). However, a comparison between the two types of methods has earlier showed a very good agreement (R^2=1.0). Only at very low cadmium concentration (< 0.25 μg/l) the method used in Linköping shows somewhat lower results (4). Since the workers from Linköping had relatively high cadmium levels the different methods used should not pose a problem.

Almost no epidemiological studies have perfect dose estimates; blood and urinary cadmium are no exceptions. In addition to the above-mentioned possible differential misclassifications, there may also be other misclassification of the dose estimates. However, we have no reason to believe that this misclassification differs in a systematic way between the different exposure groups, so the misclassification will be non-differential. This means that a possible relationship will most likely be diluted and the effect more difficult to find.

Cadmium in urine is widely used as an indicator of cadmium body burden (18;145), and has been shown to have a close relationship to total body burden. However, once tubular proteinuria is present, the kidney damage may cause increased excretion, which eventually may cause decreased kidney cadmium, followed by decreased cadmium excretion. In this situation, blood cadmium may be a better dose estimate when tubular proteinuria is present (66). Available data on blood cadmium suggest that there are at least two major compartments, one related to recent exposure and the other related to body burden, probably reflecting redistributed cadmium (10;29;39;66;83;124). In this study there was a good correlation between urinary cadmium and blood cadmium. To determine that the relationship between cadmium and tubular proteinuria and low bone mineral density was not only caused by an increased excretion of cadmium, due to either decreased re-absorption from primary urine or to increased losses of stored kidney cadmium, once tubular proteinuria was present, blood cadmium was also used as a dose estimate in paper IV. The results remained essentially the same regardless of dose estimate; thus a causal association is highly likely.

Lead is often and most easily measured in blood, and it is a commonly used indicator of the total body burden (147). However, the half-life of lead in blood is only approximately a month (147), so it typically represents mostly, but not only, relatively recent exposure. Lead in blood is derived from levels in the environment and from lead stored in tissues, mostly bone, that re-enters the blood.

6.1.3 Outcome assessment

Measurements of outcome may also give rise to misclassification. The same technique has been used for all subjects, regardless of exposure, minimizing the risk of any differential misclassification of the outcome in the study. The only exception to this procedure was the analysis of protein HC, where a different technique was used for the workers from Linköping. However, no large systematical differences are known for different methods of analysing protein HC (43) and it was only in paper III the two different methods were used together.

There are many different markers for tubular proteinuria; most previous studies have used $ß_2$-microglobulin as a marker of tubular dysfunction. A major problem when using $ß_2$-microglobulin as a biomarker in large epidemiological studies is the instability of the protein in acidic urine. Protein HC has been shown to be a sensitive and reliable marker of early tubular damage, not suffering from the instability in acidic protein (36;137). The reference interval and the cut-off points for tubular dysfunction were taken from a rather limited reference material, based on a population living in a region not far from the study area (135). It should be noted that the cut-off points correspond to the 95th percentile in the reference population, and thus 5% of the study population should be expected to have values exceeding the cut-off point.

Of the several different techniques available for assessing BMD, X-Ray based methods are most commonly used. Present X-Ray based measuring techniques have a precision in vivo of 1.5% or better. Both the stationary hospital based instrument (Hologic QDR 4500) and the ambulant one (Osteometer DTX-200) claim to have a precision of 1% or better. Nevertheless, when we started using the ambulant instrument, it had not been on the market for a very long time. Therefore, it was of great importance to validate the instrument against an established hospital based one. The regression coefficient (1.048) indicates a very good agreement between the instruments (Figure 1, paper I). The BMD values resulting from the DTX-200 measurements were on average about 15% lower than the values obtained with the Hologic QDR 4500. It is well-known that different bone mineral devices may yield different results (up to 20% variation) in the same bone (46). It is not recommended to use results from different devices to compare BMD, without proper calibration (114). The differences may be due to slightly different measuring positions or other differences between the devices. However, it was concluded that the DTX-200 showed valid forearm BMD data and that the data could be used with confidence in our studies.

6.1.4 Possible confounding

Two common confounders in epidemiological studies are age and gender. It is well-known that cadmium levels increase with age and that the risks for tubular proteinuria, low BMD

and fractures increase with age. Gender influences the uptake of cadmium (4), and women have normally lower BMD and greater fracture risk then men (21). Therefore, the analyses were adjusted for age and gender, when genders were not analysed separately. Smoking increases the cadmium dose and has in some studies shown to be associated with reduced BMD (122). However, adjusting for smoking only changed our estimates marginally.

Other factors in the environment that have been proposed to be risk factors for osteoporosis are lead (42), aluminium (42) and fluorine (42) and organochlorines (84). The battery plant in Fliseryd had also produced lead batteries, and the exposure to lead may have been extensive for some of the workers and people living close to the factory. Nevertheless, including blood lead in the analyses did not change the risk estimate. According to the water supplying company in the area, concentrations of fluorine in the drinking water did not differ between different water resources in the area (96). A paper mill, where organochlorines could have polluted the environment, was in use earlier in one of the neighbouring villages to Fliseryd. If organochlorines affect the bone, this exposure may have had an effect on the BMD on the study population. However, a possible confounder must be associated with both the exposure and the disease. There is no reason to believe that any exposure to organochlorines was associated with cadmium exposure as the sources of exposure were separately situated. There are several other suggested predictive factors for osteoporosis that were not adjusted for in the analyses, e.g., alcohol consumption, physical activity, some medications. In line with the possible impact of organochlorines, there is no reason to believe that there is an association between these factors and the cadmium exposure.

Weight was not considered a confounder, as there is no known association between weight and cadmium dose. However, it is well-known that body weight affects BMD and fracture risk and therefore, the analyses concerning BMD and fracture risk were also adjusted for weight.

There is also a risk of misclassification of confounders. Age and gender are quite easy to measure; more difficult are, for example, smoking, calcium intake and body burden of lead. The smoking variable used was crude (never or former/current), maybe the impact of smoking had been different if a more detailed smoking variable had been used. As discussed above, blood lead is not a very good estimate of body burden; there is still a risk of possible confounding from lead. We cannot rule out that the results would have been different if the estimate of the total body burden of lead hade been better. Methods for detecting low lead levels in bone with in vivo XRF have been developed (53;81), but they are not easily available.

6.1.5 Random error

The possibilities of random errors should always be kept in mind. Although the study included more than 1000 people, many stratified analyses suffered from a limited number of people included in the different sub-groups. Apart from limiting the possibilities of stratifications and the complexity of regression models, the limited number in sub-group analyses leaves greater margin for random variation as reflected in the sometimes wide confidence intervals.

6.2 CAUSATION AND CAUSAL INFERENCE

In this thesis, associations between cadmium exposure and tubular proteinuria, low BMD and the risk of forearm fractures have been revealed, at lower levels than previously observed. But can we know that there is a causal inference between cadmium exposure and these outcomes? In the field of epidemiology, several attempts have been made to establish causal criteria. The most widely cited and used are the criteria presented by Hill 1965 (50). Hill suggested that the following aspects of an association should be considered in attempt to distinguish causal from non-causal associations: strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence and analogy. The criteria about the specificity has been questioned (118), and others overlap each other. Today the most often used criteria are consistency, biological gradient, plausibility and temporality (142). The criteria should not be used as a checklist, and Hill warned against using them too rigidly. However, they can be used as a base for a discussion, which will be done here.

Consistency of the described associations is explained as, "Has it been repeatedly observed by different persons, in different places, circumstances and times?" (50). In other parts of the world, where cadmium has contaminated the environment, associations between cadmium and renal tubular effects have been recorded among residents (40;61;76;102;145). During the last decade, several studies of both occupationally and environmentally exposed populations have shown that tubular proteinuria occurs at urine cadmium doses of 2 to 4 nmol/mmol creatinine (15;64;149). In the Cadmibel-study, a 10% prevalence of tubular proteinuria was observed at 2 nmol cadmium/mmol creatinine, without adjusting for age (15). A recent study on the general population in America showed dose-effect relationships between cadmium and alanine glucosaminidase (AAP) and NAG, markers of tubular damage at urinary cadmium levels below 2 nmol cadmium/mmol creatinine (102). Our findings are in agreement with these results, but a 10% prevalence of tubular proteinuria is already found at 1.0 nmol cadmium/ mmol creatinine, calculated from the mean age of the study population (53 years).

Up to now, few studies involving measurements of BMD have been made on cadmium exposed individuals. In Japan, Tsuritani et al found a lower BMD in the calcaneus of 35 inhabitants in a cadmium-polluted area than in 68 controls (using ultrasound technique) (139). Staessen et al found a negative correlation between urinary cadmium and bone mineral density and an increased fracture risk with increasing cadmium levels in postmenopausal women (but not in men) in a study of environmentally exposed individuals in Belgium (the Pheecad study) (132). These findings are in agreement with ours. As in the Pheecad study, cadmium's effect on BMD is more clearly expressed in women, when blood cadmium was used as the dose estimate. In contrast to the Belgian findings, however, the effect was, more clearly expressed in men when urinary cadmium was used. The reason for this difference may be that the study base included more occupationally exposed workers, most of whom were men. The range of cadmium dose was also wider in our study, which facilitates the detection of an association between dose and effect.

Biological gradient refers to the presence of dose-response relationships. Marked dose-response relationships have been presented for the association between urinary cadmium and tubular proteinuria in the present study, both for the environmentally and occupationally exposed subgroups (Table 5 and Figures 8-9). The dose-response

relationship was, however, steeper for the environmentally exposed subgroup. A possible explanation for this could be a "healthy worker effect"; relatively healthy people become or remain workers in the battery plants. In the general population there are most probably a higher prevalence of diabetes and other diseases that can affect the kidneys and maybe exacerbate cadmium's toxic effects. Another possibility could be that the health effects of cadmium varies depending of route of exposure, for occupationally exposed subjects mostly via inhalation and for environmentally exposed subjects mostly via food. There were also dose-response relationships between cadmium dose and the risk of low BMD (Table 7, Table 5 in Paper III and Table 4 in paper IV) and forearm fractures (Figure 1 in Paper V).

Plausibility refers to the biological plausibility of the hypothesis. The possible mechanisms behind cadmium's effects on bone are still unclear, but several potential mechanisms have been suggested (78). One proposed mechanism is that a reduced activity of renal enzymes hydroxylating 25-hydroxicalcitriol to 1,25-dihydroxicalcitriol may lead to demineralisation of the skeleton. This mechanism has been noted in Itai-itai patients and in persons with cadmium induced tubular lesions (6;100;101;138). Therefore, the association that we found in men between urine protein HC and BMD is reasonable. It has also been suggested from animal data that bone lesions may occur independently of the renal damage (106;120) and that bone mineral loss may precede the occurrence of renal dysfunction (120). These experiments have shown that bone resorption due to cadmium exposure can occur at B-Cd concentrations similar to those reported persons occupationally exposed to cadmium and for heavy smokers (27-80 nmol/l). Experiments in vitro have also shown a direct effect of cadmium on bone tissue (14;71;85;95;143).

In the present investigation, associations between cadmium dose, measured in urine and blood, and BMD and the risk for forearm fractures were shown among the elderly, but not among the younger study subjects. This may reflect that older bone is more sensitive to cadmium. Another possible explanation is that it takes several decades for cadmium to affect the bone. The Itai-itai disease in Japan was mostly found in older women (39). An earlier Swedish study on cadmium exposed battery workers has shown that elderly people are more likely to have tubular proteinuria at a certain cadmium level: a 10% prevalence of tubular proteinuria was shown at urinary cadmium levels of 5.0 nmol/mmol creatinine in the people under 60 years of age and at 1.5 nmol/mmol creatinine in those over 60 years of age (64).

Temporality refers to the necessity that the cause precedes the effect in time. This is as Rothman says a "Sine qua non" (118): if the exposure does not precede the outcome, there is no causal inference between the two. In cross-sectional studies both exposure and outcome are measured at the same time, so there can always be some doubt about the temporality. On the other hand, we know that the cadmium exposure started in the beginning of the 20th century in the study area and that cadmium has a very long half-life in the human body. The risk that the outcome would precede the exposure is in this case likely small, although a cross-sectional study design has been used.

6.3 GENERAL SUMMARY

It was already known before this work that high cadmium exposure could cause kidney and bone damage. Paracelsus, a Swiss alchemist born 1493, said that everything is toxic; it all depends on the dose (11). This thesis has demonstrated that cadmium at lower levels than previously anticipated can affect the kidneys, shown as tubular proteinuria. It also seems like as that cadmium at relatively low levels may affect the bone; both a lower bone mineral density and a higher risk for forearm fractures were found with increasing cadmium dose. There was also a relationship between the excretion of tubular proteinuria and lower BMD and the risk of forearm fractures indicating that the kidneys may play a role in the pathogenesis of cadmium's effects on bone.

Both blood and urinary cadmium levels in our study population were relatively low, close to that in populations not considered to be cadmium exposed (61;145). Also, the occupationally exposed subgroup in this study had relatively low concentrations of urinary cadmium. The mean urinary cadmium in the study group was 0.86 nmol/mmol creatinine (90% CI 0.2 – 1.75), which is much lower than that of the Itai-itai patients (mean urinary cadmium excretions of around 30 nmol cadmium/mmol creatinine) (39), but only marginally higher than the mean urinary cadmium concentrations in non-smokers in the general Swedish population (0.02 to 0.7 nmol cadmium / mmol creatinine) (61). However, it is important to remember that it is not possible to directly compare the cadmium levels in our study to other groups with other exposure histories. Most occupationally exposed workers, and most probably, many of the environmentally exposed people in our study had a much more pronounced exposure earlier when the battery plant still was operating.

The existence of any clinical effects of early tubular damage is still debated. However, two recently published studies in Japan have revealed increased mortality of inhabitants with cadmium induced tubular proteinuria (7;91). In addition, a recent study from the same area as this study shows an age and gender adjusted rate ratio of 1.8 (95% CI 1.3-2.3) for End Stage Renal Disease in a cadmium exposed population, compared to an unexposed group (49). Associations have also been found in Japan between the cadmium intake and mortality in polluted areas (56;79). In this thesis I have also shown that there is a relationship between tubular proteinuria, low BMD and an increased risk of forearm fractures.

Although it is clear that hereditary, endocrine and life-style factors have a major role for the development of osteoporosis (21), cadmium may also play a role. A comparatively low increased risk caused by cadmium may have a pronounced impact on public health, if a large part of the population has cadmium levels at which there is an increased risk. From a public health point of view it is therefore important to minimise the cadmium dispersion to the environment, although further studies are needed to further confirm the relationships between low level cadmium exposure and bone effects.

It will be of great interest to continue following up the OSCAR-cohort, by doing so some of the difficulties with a cross-sectional study will be avoided. As the cohort ages, the number of hip fractures will increase, making it possible to study the association between cadmium exposure and incidence of hip fractures. Using stored blood and urine, new analyses can also easily be made, e.g., studies on the gene-environment interactions. It would also be useful to further explore the potential etiologic association between cadmium and osteoporosis in a pan-European study. Also of interest are studies in low-

income countries, where the cadmium exposures at some places are still much higher than that in the industrialised parts of the world.

I would like to finish with another quotation from Rothman, "All of the fruits of scientific work, in epidemiology or other disciplines, are at best only tentative formulations of a description of nature, even when the work itself is carried out without mistakes. The tentativeness of our knowledge does not prevent practical applications, but it should keep us skeptical and critical, not only of everyone else's work but of our own as well" (119).

6.4 COMMENTARY AND CORRECTIONS TO PUBLISHED PAPERS

In paper III data on regression analyses are presented as "regression coefficients"; instead they should properly have been called "standardised regression coefficients". These values are referred to as "BETA"-values in Statistica. However, it is more common to use un-standardised regression coefficients. Therefore the regression coefficients are presented as un-standardised in this thesis.

In Table 6 in paper III the dose response relationship between urinary cadmium and low BMD are adjusted for age, gender and weight. All this adjustments are, however, not necessary as a Z-score in itself is already adjusted for age and gender. The correct analysis is presented in Table 7, where the dose-response relationship is adjusted for weight only. The results remain mostly the same, but the dose-response relationship is somewhat stronger if correctly adjusted.

7 CONCLUSIONS

Tubular proteinuria, an early sign of renal dysfunction, occurred in cadmium exposed people at low levels. At urine cadmium concentrations between 0.3 and 0.5 nmol cadmium/mmol creatinine in urine, there was a two-fold increased risk of tubular proteinuria, as evidenced by elevated urinary protein HC, compared to the reference (<0.3).

A dose-effect relationship was found between cadmium dose and BMD for people aged 60 and older. In the older age group, there was a dose-response relationship, showing a three-fold increased risk of low BMD in the group with urinary cadmium higher than 3 nmol/mmol creatinine compared to the lowest dose group. The difference between the age groups may reflect that older bone is more sensitive to cadmium, or that it takes several decades for cadmium to affect bone

There was evidence of an increased risk of forearm fractures with increasing cadmium levels. For the population aged 50 and over the fracture hazard ratio increased by 18% per nmol cadmium/mmol creatinine.

Urinary cadmium is commonly used as a dose estimate reflecting cadmium body burden. However, elevated levels of cadmium in urine may reflect not only high levels of cadmium dose but also renal dysfunction. Using blood cadmium as an alternative dose estimate, the associations remained essentially the same; possible confounding effect from elevated urinary cadmium levels due to tubular damage did not have any impact on the analyses.

Evidence of renal tubular damage, in the form of elevated urine protein HC, was negatively related to BMD, and increased risk of forearm fractures, suggesting that the possible effect of cadmium on the bone may be an indirect effect mediated by the kidneys.

8 RÉSUMÉ (Summary in French)

Le cadmium est un métal lourd qui a été dispersé dans l'environnement tout au long du vingtième siècle. Il est bien connu qu'une forte exposition au cadmium cause des dommages rénaux et, dans les cas les plus sévères, de l'ostéoporose et de l'ostéomalacie. L'ostéoporose est l'une des principales causes de morbidité à travers le monde. Certains facteurs de risques, comme l'âge et le sexe, sont bien établis, mais on connait peu de choses sur ceux liés à l'environnement.

Le but du présent travail était d'étudier les effets sur les os et les reins lies aux expositions relativement faible de cadmium. Une étude transversale a été réalisée sur 520 hommes et 544 femmes, âgés de 16 à 81 ans, exposés au cadmium dans leur environnement et dans leur milieu du travail. Le cadmium, aussi bien dans l'urine que dans le sang, a été utilisé comme dose d'estimation, et la protéine HC comme marqueur de protéinurie tubulaire et de signe annonciateur de dommage rénal. La densité minérale de l'os (DMO) dans l'avant-bras a été mesurée à l'aide de la technique DXA.

L'étude révèle que chez ceux exposé dans leur environnement, un excès de protéinurie tubulaire pourrait être lies a des faibles taux cumulés de cadmium. Aux niveaux situés entre 0.3 et 0.5 nmol cadmium/mmol créatinine dans l'urine, le risque d'un taux élevé de protéines HC dans l'urine était plus que doublé.

De plus, des relations "dose-effet" et "dose réponse" ont été trouvées entre la dose de cadmium et une DMO décroissante chez les personnes âgée de 60 ans et plus. Chez les sujets âgés, il y avait trois fois plus de risques de basse DMO dans le groupe avec du cadmium dans l'urine dépassant 3 nmol cadmium/mmol créatinine, comparé au groupe avec la dose la plus basse. La modification de l'effet avec l'âge des groupes pourrait refléter le fait qu'un os plus âgé est plus sensible au cadmium, ou bien qu'il faut plusieurs décénnies au cadmium pour affecter l'os.

L'augmentation du niveau de cadmium était associée à une augmentation du risque de fracture de l'avant-bras. Pour la population âgée de 50 ans ou plus, le taux relatif de fractures augmentait de 18% par nmol cadmium/mmol créatinine.

Une association négative était présente entre le dommage tubulaire rénal et la densité minérale de l'os, suggérant que l'effet du cadmium sur l'os pourrait être un effet indirect implicant la mediation par les reins

L'ensemble de cette thèse met en évidence des relations entre d'une part, des taux de cadmium relativement faibles et d'autre part une atteinte rénale précoce, une diminution de la DMO et une augmentation du risque fracturaire des os de l'avant-bras. Bien que difficiles à comparer directement avec les taux de cadmium d'autres groupes exposés différemment, les taux retrouvés ici sont ceux habituellement rencontrés dans la population générale. Même si les facteurs de risque liés à l'hérédité, à l'endocrinologie et aux modes de vie sont probablement primordiaux dans la genèse de l'ostéoporose, une élévation relativement faible du risque lié au cadmium peut avoir d'importants effets sur la santé publique si une grande partie de la population y est soumise.

9 SAMMANFATTNING (Summary in Swedish)

Kadmium är en tungmetall som till följd av det moderna industrisamhället kommit att spridas i biosfären och därigenom orsaka exponering av människor. Det är väl känt att kraftig exponering för kadmium kan leda till njurskador och vid mycket höga nivåer också orsaka benskörhet och svåra skelettskador.

Benskörhetsrelaterade frakturer är ett folkhälsoproblem med långvarigt lidande för de drabbade och stora kostnader för samhället. Riskfaktorer, som t.ex. kvinnligt kön, hög ålder och låg fysisk aktivitet är välkända. Eventuella samband mellan benskörhet och olika miljöfaktorer har dock inte tidigare studerats i någon högre utsträckning.

Huvudfrågeställningen i avhandlingen är huruvida långvarig exponering för låga kadmium-nivåer i miljön kan påverka njurar och skelett. Totalt undersöktes 520 män och 544 kvinnor i åldrarna 16-81, miljö- eller yrkesexponerade. Som mått på exponering (dos) analyserades kadmium i blod och urin. Tubulära njurskador identifierades genom ökad utsöndring av protein-HC i urinen. Bentätheten undersöktes med hjälp av röntgenteknik (DXA).

Resultaten visar tydliga samband mellan kadmiumdos och förekomsten av tidiga njurskador, så kallad tubulär proteinuri. Hos miljöexponerade individer uppträdde detta redan vid betydligt lägre nivåer än vad man tidigare visat. Vid nivåer mellan 0,3 och 0,5 nmol kadmium/mmol kreatinin förelåg en fördubblad risk för en ökad utsöndring av protein HC, jämfört med referensnivån (<0,3).

Vidare visar studierna att bentätheten hos dem som är 60 och äldre är lägre hos de med högre nivåer kadmium än de med lägre. I den äldre åldersgruppen fanns en tre gånger ökad risk för låg bentäthet hos de med kadmiumnivåer i urinen över 3 nmol/mmol kreatinin jämfört med dem med lägst nivåer. Skillnaderna mellan de olika åldersgrupperna kan antingen bero på att skelettet blir känsligare för kadmium när det åldras eller att det tar några decennier för kadmium att påverka skelettet.

De med högre kadmium- nivåer hade dessutom en ökad risk att drabbas av handledsfrakturer. För de som var 50 och äldre ökade risken att bryta handleden med 18% för varje nmol kadmium/mmol kreatinin.

Det fanns även ett samband mellan tidiga njurskador och lägre bentäthet och ökad risk för handledsfrakturer, vilket talar för att kadmium påverkar skelettet indirekt via njurarna.

Sammanfattningsvis visar således avhandlingen samband mellan exponering för jämförelsevis låga kadmiumnivåer och tidig njurpåverkan, nedsatt bentäthet och ökad risk för handledsfrakturer. Nivåerna är i närheten av dem som många i den allmänna befolkningen har. Trots att genetiska, hormonella och livsstilsfaktorer sannolikt är de viktigaste riskfaktorerna för benskörhet, bör det noteras att även en förhållandevis liten riskökning på grund av kadmiumexponering, kan ha avsevärd betydelse för folkhälsan, om en stor del av befolkningen har kadmiumhalter i kroppen vid vilka riskökning föreligger.

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