



Full Length Article



Low-level cadmium exposure is associated with decreased cortical thickness, cortical area and trabecular bone volume fraction in elderly men: The MrOS Sweden study

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ABSTRACT

It is well known that high-level exposure to cadmium can cause bone disease such as osteoporosis, osteomalacia and fractures. However, the effect of low-level exposure, as found in the general population (mainly derived from diet and smoking), has only been assessed recently. The aim of this study was to examine if cadmium exposure in the general Swedish population causes other bone changes than decreased areal bone mineral density as measured by traditional DXA technology, e.g. changes in microstructure and geometry, such as cortical thickness or area, cortical porosity and trabecular bone volume. The study population consisted of 444 men, aged 70–81 years at inclusion year 2002–2004, from the Swedish cohort of the Osteoporotic Fractures in Men Study (MrOS). Cadmium was analyzed in baseline urine samples (U–Cd). Different parameters of bone geometry and microstructure were measured at the distal tibia at follow-up in 2009, including examination with high-resolution peripheral quantitative computed tomography (HR-pQCT). Associations between bone parameters and U–Cd in tertiles were estimated in multivariable analyses, including potential confounding factors (age, smoking, BMI, and physical activity). We found significant associations between U–Cd and several bone geometry or microstructure parameters, with 9% lower cortical thickness ($p = 0.03$), 7% lower cortical area ($p = 0.04$), and 5% lower trabecular bone volume fraction ($p = 0.02$) in the third tertile of U–Cd, using the first tertile as the reference. Furthermore, significant negative associations were found between log-transformed U–Cd and cortical thickness, cortical area, trabecular number and trabecular bone volume fraction, and a significant positive association with trabecular separation. The results indicate that low-level Cd exposure in the general population has negative effects on both cortical and trabecular bone.

1. Introduction

Osteoporosis was originally seen mainly as a disorder that affects women, but in recent years it has become clear that it is also a major health concern for men. One of three fragility fractures occurs in a man, and men have higher mortality than women after a hip fracture [1,2].

WHO has defined osteoporosis as a condition with bone mineral

density (areal bone mineral density; aBMD) measured by DXA (dual energy X-ray absorptiometry) at least 2.5 standard deviations below the mean for a young adult woman [3]. However, low aBMD as measured by DXA is a relatively rough measure and only one of several possible indicators of impaired bone quality and increased risk of fractures [4]. New techniques now enable us to study bone microstructure and geometry in detail, e.g. high resolution peripheral quantitative computed

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tomography (HR-pQCT) which may improve prediction of fracture risk [5–7].

The heavy metal cadmium is a wide-spread pollutant in many parts of the world. It occurs in air, water and soil both naturally and as a result of the use of cadmium-containing fertilizers and pollution from industrial activities, and is absorbed by growing crops [8]. In the general population, the main sources of cadmium are diet and cigarette smoking [8]. It has long been known that exposure to high levels of cadmium can cause osteoporosis, osteomalacia and increase the risk of fractures [9,10]. The effects of exposure to low-level cadmium, as found in the general population in many countries, have not been as clear. However, recent studies conducted in Sweden, Belgium and the United States have shown associations also between low-level cadmium exposure and increased risk of osteoporosis and fractures [11–22], although some studies showed no association [23,24]. In a meta-analysis 2016, the authors found that high cadmium exposure might be a risk factor for any fracture (pooled relative risk for the highest versus the lowest category of cadmium concentration 1.30 (95% CI 1.13–1.49)), though they underline that the result should be interpreted with caution due to heterogeneity among studies and possible publication bias [22].

The mechanisms behind the effects of cadmium on bone are still not fully clear, but both indirect and direct effects on bone have been suggested [25]. A possible indirect mechanism is decreased formation of active vitamin D (1,25(OH)₂D) in the kidney, resulting in decreased calcium uptake from the intestine, while suggested direct effects mainly focus on the stimulation of osteoclast formation and/or activity, resulting in increased bone resorption, as has been shown in experimental studies, mainly on rats [26,27]. A few experimental studies have used histological methods or micro-computed tomography to study the effects of cadmium on bone microstructure, showing mainly decreased trabecular bone volume fraction and trabecular number, and increased trabecular separation, but also decreased cortical bone area [28–30].

The main sources of cadmium in the general population are diet and cigarette smoking. It is present in most food items, but agricultural crops such as wheat and potatoes usually account for the main part of the intake [31]. Cadmium is absorbed in the lungs or in the gut and accumulates mainly in the kidney. It is excreted in urine, and smokers have about twice as high levels of cadmium in the kidney and in urine as never-smokers [9,32]. Cadmium has a very long half-life (10–30 years) and since the excretion is proportional to the cadmium levels in the kidney, U–Cd can be used as a biomarker for the body burden of cadmium [33].

In a previous study on the men in the Osteoporotic Fractures in Men (MrOS) cohort, we found that those with higher cadmium in urine at baseline had an increased risk of osteoporosis-related fractures [21]. However, low bone mineral density measured by DXA could not explain the entire increase in fracture risk. The aim of the present study was to examine the effects of low-level cadmium exposure on other indicators of impaired bone quality, such as cortical and trabecular bone parameters measured by HR-pQCT in a cohort of elderly men in Sweden.

2. Methods

2.1. Study population

The study population consists of 444 men from Gothenburg, who were part of the Swedish cohort in the MrOS study, which is a multinational epidemiologic study of risk factors for osteoporosis and fractures in older men. The Gothenburg cohort included 1010 men with a mean age of 75 years at baseline (2002–2004). They were randomly selected from national population registers and contacted by telephone

and letters. To be included in the study, they had to be able to walk without aid, sign an informed consent and complete a questionnaire. The inclusion rate was 45% for the Swedish cohort at baseline. In 478 of the 600 men who participated in the 5-year follow-up in 2009, bone geometry and microstructure was studied using HR-pQCT. Of the 478 images, 22 were excluded due to low quality or misplaced scout views. In 444 of these men, urine cadmium (U–Cd) at baseline had also been analyzed.

The study was approved by the Ethics Committee at the University of Gothenburg and conducted in accordance with the Declaration of Helsinki.

2.2. Assessment of bone geometry and microstructure

Bone geometry and microstructure were measured at the distal tibia using a high-resolution 3D peripheral quantitative computed tomography (HR-pQCT) device (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland) as has been described previously [7]. The following parameters were obtained after processing of the images: cortical thickness (mm), cortical cross-sectional area (mm²), trabecular bone volume fraction (%), trabecular number (mm⁻¹), trabecular thickness (μm), and trabecular separation (mm). Coefficients of variation ranged from 0.1%–1.6% [34]. Image quality was graded ranging from grade 1 (highest quality) to grade 5 (unacceptable), using the recommendations from the manufacturer (Scanco Medical AG). A preliminary quality grading was made directly after the measurement was finished, and repeated measurements were made for all scans with insufficient quality (grade 4 or 5). Only images with acceptable quality (grade 1–3) were included in the study. All measurements and grading of the quality were performed by the same two operators. Cortical porosity was calculated by the formula: cortical porosity (%) = cortical pore volume / (cortical pore volume + cortical bone volume), as described by Sundh et al. [7]. The algorithm has previously been published by Burghardt et al. [35].

2.3. Assessment of covariates

At baseline, information about smoking habits, medical history, medication, calcium intake, physical activity and other possible covariates was collected using a standardized questionnaire. Pack-years were estimated as the mean number of packs of cigarettes smoked per day multiplied by the number of years the person had been a smoker. The variable physical activity was the total daily walking distance (km/day), which was a combination of the self-reported distance walking outdoors in daily life and walking as a means of exercise. Body mass index (BMI) was calculated as the weight in kilograms divided by height in square meters (kg/m²), as measured at baseline. Calcium intake in mg/day was estimated from questions about the weekly intake of a number of calcium-containing foods and supplements. Blood samples were collected in order to assess kidney function (eGFR, estimated from cystatin C), total and free testosterone, total and free estradiol, and sex hormone binding globulin (SHBG), as has been previously described [36,37].

2.4. Urine samples

At baseline, morning urine was collected and frozen for later analyses. In 2012, the urine samples were analyzed for cadmium (by inductively coupled mass spectrometry, ICP-MS) at the Department of Occupational and Environmental Medicine, Lund University Hospital, as previously described [21]. The urine samples were diluted ten times with an alkaline solution and corrected for molybdenum oxide-based

Table 1

Characteristics of the study cohort at baseline and bone microstructure at follow-up, stratified by tertiles of urinary cadmium. Data, except for U–Cd, are presented as mean (range). U–Cd = cadmium in urine; BMI = body mass index; eGFR = glomerular filtration rate estimated from cystatin C; SHBG = sex hormone binding globulin. ANOVA *p*-value is given for difference between tertiles.

Characteristics	All (N = 444)	Tertile 1 (N = 152)	Tertile 2 (N = 142)	Tertile 3 (N = 150)	<i>p</i> -Value
U–Cd (µg/g creatinine) [mean (median)]	0.31 (0.24)	0.14 (0.15)	0.24 (0.24)	0.55 (0.44)	
U–Cd tertile limits (µg/g creatinine)		0.01–0.18	0.19–0.30	0.31–6.75	
Age (years)	74.5 (70.5–81.0)	74.6 (70.5–80.9)	74.8 (70.5–81.0)	74.2 (70.5–80.8)	0.273
BMI (kg/m ²)	26.2 (17.0–39.3)	26.3 (19.3–37.3)	26.1 (19.2–33.9)	26.1 (17.0–39.3)	0.918
Pack-years (number)	12.5 (0–93.0)	4.1 (0–70.5)	9.5 (0–93.0)	23.9 (0–84.0)	<0.001
Physical activity (km/day) ^a	4.3 (0–20.0)	4.1 (0–16.0)	4.8 (0–16.0)	4.1 (0–20.0)	0.117
Calcium intake (mg/d)	937.5 (0–2919)	939 (0–2236)	920 (182–2919)	953 (198–2051)	0.789
eGFR (mL/min/1.73 m ²)	73.6 (26.0–142.0)	74.2 (32.0–121.0)	73.4 (35.0–142.0)	73.3 (26.0–116.0)	0.890
Total testosterone (nM)	16.1 (0.09–43.9)	16.1 (0.49–43.9)	16.4 (0.31–37.3)	15.9 (0.09–32.0)	0.774
Free testosterone (nM)	0.28 (0.001–1.04)	0.28 (0.004–1.04)	0.28 (0.003–0.72)	0.28 (0.001–0.66)	0.965
Total estradiol (pM)	80.8 (3.7–202.6)	84.6 (10.4–202.6)	79.8 (3.7–157.2)	78.0 (3.7–202.6)	0.145
Free estradiol (pM)	1.4 (0.04–3.7)	1.4 (0.1–3.5)	1.3 (0.04–3.2)	1.3 (0.05–3.7)	0.229
SHBG (nM)	46.0 (3.3–170.0)	45.9 (3.3–102.9)	46.8 (3.6–144.5)	45.2 (12.8–170.0)	0.827
Cortical thickness (mm)	0.99 (0.09–1.74)	1.06 (0.32–1.65)	0.96 (0.09–1.59)	0.93 (0.26–1.74)	<0.001
Cortical area (mm ²)	119.3 (12.1–203.9)	127.5 (41.5–202.7)	117.7 (12.1–198.7)	112.6 (34.3–203.9)	0.001
Cortical porosity (%)	11.8 (3.8–25.4)	11.2 (4.3–25.4)	12.0 (4.2–23.8)	12.2 (3.8–25.4)	0.089
Cortical density (mg/cm ³)	777.4 (359.5–919.1)	790.5 (359.5–919.1)	772.5 (483.8–902.8)	768.7 (563.9–912.7)	0.028
Trabecular bone volume fraction (%)	15.0 (7.7–23.5)	15.6 (8.4–23.5)	14.9 (7.9–22.9)	14.4 (7.7–21.7)	0.001
Trabecular number (mm ⁻¹)	1.98 (1.2–2.9)	2.02 (1.18–2.84)	1.97 (1.41–2.85)	1.94 (1.20–2.73)	0.067
Trabecular thickness (µm)	76 (42–107)	77 (51–107)	76 (42–105)	74 (44–106)	0.082
Trabecular area (mm ²)	817.0 (474.9–1309.7)	791.1 (474.9–1309.7)	836.4 (523.5–1229.0)	824.9 (514.4–1202.6)	0.021
Trabecular separation (mm)	0.44 (0.28–0.77)	0.43 (0.28–0.77)	0.44 (0.28–0.65)	0.46 (0.30–0.77)	0.013

^a Physical activity is the daily walking distance, calculated as the combination of self-reported walking outdoors in daily life and walking as a means of exercise.

interference. In order to assess the precision, the samples were prepared in duplicate and the coefficient of variation (CV), was found to be 4.4% for duplicate preparations. The limit of detection (LOD) for U–Cd was 0.05 µg/L (calculated as three times the standard deviation of the blank).

Three quality control samples were used. Creatinine concentrations in urine were analyzed using the Jaffé method with a COBAS 6000 instrument from Roche Diagnostics (Rotkreuz, Switzerland), with an LOD of 0.1 mmol/L.

Table 2

Associations between bone microstructure and urinary cadmium (*N* = 444). Data are presented as mean values of bone microstructure parameters for tertiles of urinary cadmium. *P*-value for effect of cadmium on bone microstructure parameters, with tertile 1 (T1) as the reference category, is given within parentheses.

Mean U–Cd (range) (µg/g creatinine)	T1 (N = 152)	T2 (N = 142)	T3 (N = 150)	<i>p</i> -Value for linear trend
	0.14 (0.01–0.18)	0.24 (0.19–0.30)	0.55 (0.31–6.75)	
Cortical thickness (mm)				
Model 1	1.07	0.97 (<0.01) ^a	0.92(<0.01) ^a	<0.01 ^a
Model 2	1.05	0.96 (0.02) ^a	0.96 (0.03) ^a	0.02 ^a
Cortical area (mm ²)				
Model 1	127.6	118.4 (0.02)	111.8 (<0.01) ^a	<0.01 ^a
Model 2	125.0	117.5 (0.06)	116.0 (0.04) ^a	0.03 ^a
Cortical porosity (%)				
Model 1	11.2	12.0 (0.12)	12.3 (0.02) ^a	0.02 ^a
Model 2	11.3	11.9 (0.22)	12.2 (0.09)	0.09
Cortical density (mg/cm ³)				
Model 1	790.9	774.3 (0.051)	766.5 (<0.01) ^a	<0.01 ^a
Model 2	787.4	773.9 (0.12)	771.7 (0.10)	0.09
Trabecular bone volume fraction (%)				
Model 1	15.6	14.9 (0.047) ^a	14.3 (<0.01) ^a	<0.01 ^a
Model 2	15.4	14.9 (0.07)	14.6 (0.02) ^a	0.01 ^a
Trabecular number (mm ⁻¹)				
Model 1	2.02	1.97 (0.14)	1.94 (0.03) ^a	0.03 ^a
Model 2	2.01	1.97 (0.20)	1.96 (0.15)	0.13
Trabecular thickness (µm)				
Model 1	78	76 (0.31)	74 (0.02) ^a	0.02 ^a
Model 2	77	76 (0.33)	75 (0.08)	0.08
Trabecular area (mm ²)				
Model 1	791.0	836.0 (<0.01) ^a	825.3 (0.04) ^a	0.04 ^a
Model 2	789.5	835.9 (<0.01) ^a	824.4 (0.07)	0.052
Trabecular separation (mm)				
Model 1	0.43	0.44 (0.13)	0.46 (<0.01) ^a	<0.01 ^a
Model 2	0.43	0.44 (0.17)	0.45 (0.052)	0.049 ^a

^a *p* < 0.05. Model 1 is a crude model with age, and model 2 is adjusted for age, BMI, smoking (pack-years) and physical activity (daily walking distance), in a multiple regression model (general linear model, least squares means).

Table 3

Associations between bone microstructure and urinary cadmium in never-smokers ($N = 177$). Data are presented as mean values of bone microstructure parameters for tertiles of urinary cadmium (based on all 444 men). Model 1 is a crude model with age, and model 2 is adjusted for age, BMI, and physical activity (daily walking distance) in a multiple regression model (general linear model, least squares means). P -values for effect of cadmium on bone quality, with tertile 1 (T1) as the reference category, are given within parentheses.

Mean U–Cd (range) ($\mu\text{g/g}$ creatinine)	T1 ($N = 93$) 0.14 (0.07–0.18)	T2 ($N = 58$) 0.24 (0.19–0.30)	T3 ($N = 26$) 0.44 (0.31–1.94)	p-Value for linear trend
Cortical thickness (mm)				
Model 1	1.05	1.01 (0.43)	0.99 (0.36)	0.29
Model 2	1.04	1.02 (0.60)	1.00 (0.49)	0.45
Cortical area (mm^2)				
Model 1	126.2	123.3 (0.62)	120.7 (0.48)	0.43
Model 2	125.0	124.5 (0.92)	122.0 (0.69)	0.71
Cortical porosity (%)				
Model 1	11.5	11.4 (0.88)	11.7 (0.85)	0.92
Model 2	11.5	11.4 (0.79)	11.6 (0.95)	0.96
Cortical density (mg/cm^3)				
Model 1	789.1	785.5 (0.74)	772.7 (0.27)	0.30
Model 2	788.2	786.1 (0.85)	774.7 (0.36)	0.41
Trabecular bone volume fraction (%)				
Model 1	15.4	15.2 (0.65)	14.8 (0.35)	0.35
Model 2	15.3	15.3 (0.89)	14.9 (0.50)	0.54
Trabecular number (mm^{-1})				
Model 1	2.01	1.98 (0.56)	1.99 (0.70)	0.59
Model 2	2.00	2.00 (0.96)	2.00 (0.95)	0.94
Trabecular thickness (μm)				
Model 1	77	77 (0.96)	75 (0.38)	0.46
Model 2	77	77 (0.82)	75 (0.33)	0.38
Trabecular area (mm^2)				
Model 1	794.6	822.8 (0.24)	832.4 (0.24)	0.16
Model 2	791.9	825.3 (0.17)	836.5 (0.16)	0.09
Trabecular separation (mm)				
Model 1	0.43	0.44 (0.52)	0.44 (0.48)	0.40
Model 2	0.43	0.44 (0.96)	0.44 (0.75)	0.78

2.5. Statistical analysis

As we assumed a nonlinear relation between U–Cd and bone geometry and microstructure, associations were first evaluated with U–Cd in tertiles using general linear models (least squares means). Associations between U–Cd at baseline and bone geometry and microstructure variables were analyzed in two different multiple regression models. Model 1 was a crude model including only U–Cd and age, while model 2 also included the co-variables BMI, smoking (pack-years) and physical activity (daily walking distance). Associations were also examined with U–Cd as a continuous variable by multiple linear regression (with the same covariates as in model 2). Since U–Cd levels were skewed, this was performed both with untransformed and log transformed U–Cd. We also performed multiple linear regression analyses of bone geometry and microstructure parameters as a function of age, BMI, pack-years, and physical activity, i.e. without U–Cd in the model, in order to study the risk for over-adjustment when both cumulative smoking and U–Cd are included in the model. Statistical analyses were performed using the SAS software package (version 9.4).

3. Results

3.1. Baseline characteristics

The main characteristics of the study cohort are shown in Table 1. The mean U–Cd level was 0.31 (median 0.24, range 0.01–6.75) $\mu\text{g/g}$ creatinine. The mean number of pack-years was 12.5 in all men (range 0–93.0), with a mean of 4.1 in the lowest (first) tertile and 23.9 in the third tertile of U–Cd. The mean daily walking distance was 4.3 km (range 0–20). The mean calcium intake was 938 mg/day (range

0–2919). Sex hormone levels were relatively similar, as well as eGFR, over U–Cd tertiles.

Only 30 men were current smokers, and 414 were non-smokers (including 237 former smokers and 177 never-smokers). Mean U–Cd was 0.63 (median 0.48, range 0.16–2.55) $\mu\text{g/g}$ creatinine in current smokers and 0.29 (median 0.23, range 0.01–6.75) $\mu\text{g/g}$ creatinine in non-smokers. In former smokers, the median time since smoking cessation was 29 years (data not shown).

3.2. Associations between U–Cd at baseline and bone geometry and microstructure at follow-up

Bone geometry and microstructure variables measured at follow-up stratified by tertiles (T1 – T3) of U–Cd levels at baseline are shown in Table 1. In a crude regression model adjusted only for age, cortical thickness, cortical area, cortical density, trabecular bone volume fraction, trabecular number, and trabecular thickness were significantly lower in T3 (U–Cd >0.3 $\mu\text{g/g}$ creatinine) using T1 as reference (Table 2). Cortical porosity, trabecular separation and trabecular area were significantly higher in T3. When we included age, BMI, smoking (pack-years) and physical activity in the model, we found significantly lower cortical thickness, cortical area and trabecular bone volume fraction in T3. The mean cortical thickness, adjusted for covariates, was 0.09 mm (9%) lower in the highest tertile of U–Cd (T3) as compared to the lowest tertile (T1), and the mean cortical area was 9 mm^2 (7%) lower in T3 (Table 2). As for trabecular bone volume fraction, the difference was 0.8 percentage (5% lower in T3 than in T1). The associations with U–Cd remained when current smoking was included in the model (data not shown).

In addition, when men with low kidney function were excluded

Table 4

Multiple linear regression analyses of bone microstructure parameters as a function of log (base e, natural logarithm) urinary cadmium, age, BMI, pack-years, and physical activity. Beta coefficients with *p*-values within parentheses are presented. *N* = 431 (cortical porosity tibia *N* = 429).

Variable, beta coefficient (<i>p</i> -value)	Cortical thickness (mm)	Cortical area (mm ²)	Cortical porosity (%)	Cortical density (mg/cm ³)	Trabecular bone volume fraction (%)	Trabecular number (mm ⁻¹)	Trabecular thickness (μm)	Trabecular area (mm ²)	Trabecular separation (mm)
Log U-Cd (μg/g creatinine)	-0.06 (0.02) ^a	-6.9 (0.02) ^a	0.34 (0.36)	-7.8 (0.24)	-0.75 (<0.01) ^a	-0.06 (0.02) ^a	-1.0 (0.15)	14.7 (0.27)	0.02 (<0.01) ^a
Age (years)	-0.02 (<0.01) ^a	-2.5 (<0.01) ^a	0.30 (<0.01) ^a	-7.3 (<0.01) ^a	-0.02 (0.61)	0.01 (<0.01) ^a	-0.6 (<0.01) ^a	2.6 (0.27)	-0.003 (0.01) ^a
BMI (kg/m ²)	0.02 (<0.01) ^a	2.7 (<0.01) ^a	-0.06 (0.38)	1.9 (0.11)	0.22 (<0.01) ^a	0.04 (<0.01) ^a	-0.3 (0.14)	5.8 (0.01) ^a	-0.01 (<0.01) ^a
Smoking (pack-years)	-0.002 (0.01) ^a	-0.31 (<0.01) ^a	0.01 (0.40)	-0.44 (0.06)	-0.01 (0.13)	-0.0004 (0.62)	-0.05 (0.18)	-0.04 (0.93)	0.0002 (0.49)
Physical activity (km/day)	0.0003 (0.95)	0.10 (0.84)	0.009 (0.88)	0.49 (0.65)	0.04 (0.34)	0.0007 (0.87)	0.2 (0.36)	-0.30 (0.89)	-0.0006 (0.63)
R-squared	0.11	0.15	0.06	0.11	0.10	0.18	0.04	0.02	0.17
Adj. R-squared	0.10	0.14	0.05	0.10	0.09	0.17	0.03	0.01	0.16

^a *p* < 0.05.

(eGFR <60 mL/min; *N* = 100), the results were very similar, and the trabecular area was highest, and the association with U-Cd also significant, in T3 (data not shown).

In never-smokers, cortical thickness, cortical area and cortical density were lower in the third tertile of U-Cd, but the difference was not significant, neither in the crude regression model with age, nor in the model including age, BMI and physical activity (Table 3). In the 177 never-smokers there were only 26 men in T3.

When we calculated separate tertiles for U-Cd in the 177 never-smokers, cortical thickness was significantly lower in both T2 and T3, but lowest in T2 in a crude model. For the other bone geometry and microstructure variables, there were no significant differences between tertiles. In the multivariate model (including age, BMI and physical activity), cortical thickness was significantly lower in T2 (*p* < 0.01) but not in T3.

In a multiple linear regression model adjusted for age, BMI, smoking (pack-years) and physical activity including all 444 subjects, log-transformed U-Cd (lnU-Cd) was significantly associated with cortical thickness, cortical area, trabecular bone volume fraction, trabecular number and trabecular separation (Table 4). When the variable pack-years was replaced by current number of cigarettes/day, the results were almost identical (data not shown). We found no significant associations between untransformed U-Cd as a continuous variable and bone geometry and microstructure in the same multivariate model (Supplemental Table S1). However, when two individuals with U-Cd > 2 μg/g creatinine were excluded, U-Cd was significantly associated with trabecular bone volume fraction (β -1.81, *p* = 0.01) and trabecular separation (β 0.05, *p* = 0.01) in the same model (data not shown).

When U-Cd was excluded from the model in the multiple linear regression analyses, the beta estimates for effect of cumulative smoking on cortical area, cortical density, trabecular bone volume fraction and trabecular number further decreased, and increased for trabecular separation (Supplemental Table S2).

4. Discussion

To our knowledge, this is the first study to show associations between cadmium in urine and impaired bone microarchitecture in humans. In elderly men from the general population, with relatively low cadmium levels, we found statistically significant effects on both cortical and trabecular bone (lower cortical thickness, cortical area and trabecular bone volume fraction).

In a previous study from the same cohort of elderly men, cortical area was a predictor of incident fractures independently of aBMD [6]. In this study, we found that the mean cortical area, adjusted for covariates, was 9 mm² (7%) lower in the highest tertile of U-Cd (T3) as compared to the lowest tertile (T1). As cortical area in our study decreased by 2.5 mm²

per year, a difference in 9 mm² between T1 and T3 corresponds to about four years of ageing. In a recent study on elderly women in Sweden (age 75–80 years), those with prevalent hip fracture had 16 mm² (12%) lower cortical area as compared to the controls, measured at distal tibia (*p* < 0.001) [38]. This indicates that a decrease in cortical area of the magnitude seen in the present study may be of clinical significance.

As most of the body burden of cadmium in smokers usually is derived from tobacco, including smoking as a covariate in the multivariate model may result in over-adjustment. As we adjusted for cumulative smoking (pack-years) in this study, we would focus on the effect of cadmium from diet on bone. The effect of smoking on bone geometry and microstructure may however be partly mediated by cadmium. This is suggested by a further decrease in the beta estimate for pack-years for several bone parameters when U-Cd was excluded from the multiple linear regression analyses. In a review of the endocrine and metabolic effects of smoking cessation, the author concluded that it was associated with an increased body weight, but also that the risk of osteoporosis was lower in former smokers [39]. Furthermore, in a recently published study by our research group, we investigated the hypothesis that part of the negative effects of smoking on bone are mediated via cadmium in tobacco smoke, using mediation analysis [40]. We found significant inverse associations between smoking and BMD, and the indirect effects of cadmium were estimated to be 43% of the total smoking effect on whole body BMD, 59% of the effect on total hip BMD and 70% of the effect on trochanter BMD. Smoking was also associated with higher fracture risk, and the indirect effect of cadmium was at least 50% of the effect of smoking in non-vertebral osteoporosis fractures, including hip fractures.

When we analyzed never-smokers separately, the tendency was partly the same as for the whole group, with lower cortical thickness and cortical area in the third tertile of U-Cd. However, the results for never-smokers were not statistically significant, probably because there were so few never-smokers in the highest tertile of U-Cd (T3; *N* = 26), and a larger study on never-smokers would contribute valuable information.

There was a tendency toward a dose-response relationship between U-Cd and bone geometry and microstructure parameters in the multiple regression model with U-Cd in tertiles, with lower cortical thickness, cortical area and trabecular bone volume fraction both in T3 and T2, however significant also in T2 only for cortical thickness. It is somewhat surprising to see this possible effect of cadmium already at those low levels of U-Cd (0.19–0.30 μg/g creatinine in T2). This suggests a curvilinear relationship, which is supported by the results from the multiple linear regression analyses with log-transformed U-Cd. However, we consider that a linear relationship between U-Cd and bone parameters is biologically more reasonable than a relationship with log-transformed U-Cd, since the latter would mean that the impact per increase of U-Cd is strongest at low U-Cd.

In a review of cadmium toxicity in experimental animals, the author concluded that long-term dietary cadmium exposure in rats results in decreased mineral density and increased bone fragility at low levels that corresponded to those seen in many countries following environmental exposure [25]. In an *in vitro* study, the authors found that Cd inhibited the viability and activity of osteoblasts, and increased the number of osteoclasts, indicating that Cd inhibits bone formation at high levels and increases bone resorption at low dose levels [41]. In another experimental study, the authors found effects on the histological structure of compact bone (mainly fewer and smaller osteons), but no effect on cortical thickness, after subchronic oral exposure to Cd in rats [42]. In a study on growing rats, exposure to high doses of Cd led to a reduced trabecular number, which resulted in decreased trabecular volume in the tibia [43]. In an experimental study conducted in 2017, rats exposed to Cd in drinking water at levels close to human environmental exposure levels had increased osteoclast formation, as well as decreased trabecular number, decreased bone volume fraction, and increased trabecular separation, compared to controls [27]. Thus, the results from experimental studies show effects on both trabecular and cortical bone, consistent with the present study on elderly men.

A limitation of the study is the limited number of never-smokers, which makes it difficult to interpret the results in this group. In addition, less than half of the original cohort of 936 men could be analyzed. However, mean U-Cd and total body BMD were very similar in the two groups, so the 444 men in this study seem to be representative of the larger cohort (data not shown). Another limitation is that the study population consisted of only elderly men (older than 75 years). However, men are underrepresented in most previous studies of osteoporosis and related bone effects. Nevertheless, the results from this study must be confirmed by further studies on other groups, including women.

The longitudinal design of the study, which enabled us to investigate the association between cadmium in urine at baseline and bone microarchitecture parameters five to seven years later, could be considered as both a strength and a limitation. As U-Cd has a half-life of decades it reflects the body burden of cadmium due to intake over many years, and is not expected to change much in five years [33]. Furthermore, the effects of cadmium on bone are probably long-term effects. However, for the other covariates, it is a limitation that they were collected at baseline five years before the measurements of bone geometry. In addition, we have no baseline measurements for the bone microarchitecture variables, and they may have changed during the period due to other factors.

An additional strength is that the study population consisted of men from the general population with exposure to cadmium mainly from diet and smoking, and therefore the U-Cd levels were similar to those found in many countries such as Sweden and the U.S. The study is thus relevant to the general populations in many countries, especially as life expectancy is likely to continue to increase in the world, and osteoporosis and related fractures is a major problem in higher ages. Another strength is that the cohort is well-characterized. For example, we were able to use the variable pack-years (of cigarettes), which is a relatively good estimate of cumulative smoking during life. We were also able to analyze Cd in urine, which is considered the best measure of the accumulated lifetime exposure [10,33]. U-Cd is relatively stable as it mainly reflects the levels of Cd in the kidney. Cd in blood is more easily affected by sudden changes in exposure. The HR-pQCT measurements were made on tibia, i.e. weight bearing bone, which could be considered a strength as the most important fractures regarding morbidity and mortality, hip fractures, occur in weight bearing bone.

5. Conclusions

Urinary cadmium, even at low levels, was associated with reduced cortical thickness, cortical area and trabecular bone volume fraction. These results indicate that low-level cadmium exposure in the general population affects both cortical and trabecular bone, which may explain the increased risk of fracture.

CRedit authorship contribution statement

Maria Wallin: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Lars Barregard:** Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition. **Gerd Sallsten:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Thomas Lundh:** Validation, Formal analysis, Resources, Writing - review & editing. **Daniel Sundh:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Mattias Lorentzon:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Claes Ohlsson:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Dan Mellström:** Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bone.2020.115768>.

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