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Low-level cadmium exposure and cardiovascular outcomes in elderly Australian women: a cohort study

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Abstract

Background:

Cadmium has been associated with increased risk of cardiovascular disease (CVD) in observational studies, however there has been a limited focus on this relationship in women.

Objectives:

This study investigated the association of urinary cadmium (UCd) concentrations with CVD outcomes and all-cause mortality in elderly Western Australian (WA) women.

Methods:

UCd excretion was measured at baseline in 1359 women, mean age 75.2 ± 2.7 years and 14.5 years of atherosclerotic vascular disease (ASVD) hospitalisations and deaths, including both the principle cause of death and all associated causes of death. Health outcome data were retrieved from the Western Australian Data Linkage System. Cox regression analysis was used to estimate hazard ratios of ASVD and all-cause mortality. UCd was ln-transformed and models were adjusted for demographic and CVD risk factors.

Results:

Median (IQR) concentration of UCd was 0.18 (0.09-0.32) µg/L. In multivariable-adjusted analyses per ln unit (equivalent to ~2.7 fold) increase in UCd, there was a 36% increase in the risk of death from heart failure and 17% increase in the risk of a heart failure event, respectively (HR = 1.36, 95% CI 1.11-1.67; HR = 1.17, 95% CI 1.01- 1.35). When analyses were restricted to never smokers the relationship between UCd and death from heart failure remained (HR 1.29, 95% CI 1.01-1.63).

Conclusions:

This study suggests that even at low levels of exposure cadmium may be associated with heart failure hospitalisations and deaths in older women, however given the dilute nature of these urine samples, the results must be interpreted with caution.

Keywords: Cadmium; cardiovascular; heart failure; elderly women

Introduction

Cadmium is a widespread heavy metal released in industrial and agricultural processes and has been associated with many serious chronic diseases (Nawrot et al., 2010). Environmental exposure to cadmium is mainly due to exposure to tobacco smoke and diet, with diet being the main source of cadmium in non-smokers (Nawrot et al., 2010). Low environmental exposure to cadmium has been found to increase the risk of adverse health outcomes including decreased bone mineral density (Gallagher et al., 2008), impaired kidney function (Olsson et al., 2002) and various cancers (Åkesson et al., 2012; McElroy et al., 2006). An Australian study by Hinwood et al. (2013), examined exposure in 173 pregnant women and reported that one third had urinary cadmium concentrations ≥ 1 μg/g creatinine, which indicated an increased risk of health effects in this population. In older Australian women lower concentrations of urinary cadmium were reported, however, an association between urinary cadmium and decreased bone mineral density was still present at this low level of exposure (Callan et al., 2015).

A limited number of longitudinal studies of cadmium exposure, all-cause mortality and cardiovascular disease have been conducted, with most studied cohorts residing in the United States (Menke et al., 2009; Tellez-Plaza et al., 2013aa; Tellez-Plaza et al., 2013bb; Tellez-Plaza et al., 2012) or Japan (Nakagawa et al., 2006; Suwazono et al., 2014).

A recent meta-analysis calculated overall HRs for all-cause mortality (6 studies) and CVD mortality (5 studies) associated with increased urinary cadmium to be 1.44 (95% CI 1.25, 1.64) and 1.57 (95% CI 1.27, 1.95), respectively (Larsson and Wolk, 2015). When the analysis was restricted to the four studies that had undertaken subgroup analysis by sex, the HR for all-cause mortality for women in the highest vs lowest category of urinary cadmium

concentration was 1.50 (95% CI 1.08, 2.08) (Larsson and Wolk, 2015). A US study using the NHANES cohort concluded that cadmium exposure was associated with an increased risk of all-cause and cardiovascular disease mortality among men, but not women (Menke et al., 2009). An analysis of the 1999-2004 NHANES cohort found no differences in the association between cadmium exposure all-cause and CVD mortality by sex (Tellez-Plaza et al., 2012). A more recent systematic review of populations with low to moderate levels of cadmium exposure, however, found similar associations between cadmium exposure and cardiovascular disease between men and women, but the relationship was not significant for women (Tellez-Plaza et al., 2013cc). Conversely, a longitudinal study conducted in a cadmium contaminated area of Japan suggested that high levels of exposure to cadmium led to excess risk of mortality among women but not in men (Uetani et al., 2007). Whilst sex specific risk was suggested in these studies the results were inconsistent and the differential associations by sex may represent chance findings. Barregard et al., (2016) investigated the relationship between blood cadmium and incident cardiovascular disease (CVD) in men and women in Sweden and found increased hazard ratios for all CVD events for participants in the highest exposure quartile.

Mechanistically, cadmium exposure has been shown to elicit endothelial damage both in vitro and in murine models, with accelerated plaque formation observed (Knoflach et al., 2011; Messner et al., 2009). Cadmium exposure was found to be associated with the development of atherosclerotic plaques in 64 year old Swedish women after a 5 year follow up (Fagerberg et al., 2012), thus providing evidence of the pro-atherogenic potential effects of cadmium exposure.

Longitudinal studies of cadmium exposure and mortality are scarce, particularly in those with low levels of exposure. Given that women are known to have higher levels of cadmium exposure (Vahter et al., 2007) the contradictory results regarding the association between cadmium exposure and all-cause and CVD mortality in women, suggests that more studies focussed on women are warranted, especially older women. Furthermore, there have been limited studies on the relationship between cadmium exposure and non-fatal cardiovascular events.

CVD remains the number one cause of mortality in Australia and globally (Australian Bureau of Statistics [ABS], 2014; World Health Organization [WHO], 2013). Therefore, it is important to identify factors that increase the risk of developing CVD. The objective of this study was to examine the association between cadmium exposure and the incidence of mortality, cardiovascular mortality and (non-fatal) cardiovascular events in 14.5 year follow up of 1359 elderly Western Australian women. Coronary heart disease (CHD), stroke or cerebrovascular accident (CVA), heart failure (HF) and peripheral arterial disease (PAD), collectively known as atherosclerotic vascular disease (ASVD), were examined, as well as all-cause mortality.

Methods

Study population

Participants of this study (n = 1500) were women randomly recruited in the Perth metropolitan region from the Australian electoral roll in 1998. They were initially randomised into a 5-year, double-blinded, placebo-controlled calcium intervention trial of 1.2g of elemental calcium in the form of two tablets of calcium carbonate taken daily, or an identical placebo (Prince et al., 2006). Participants were aged ≥70 years, ambulant and

expected survival of at least 5-years. Participants were then followed for an additional 10-year observational extension study using the WA Data Linkage System (WADLS). At baseline written informed consent was obtained from all participants for the study and access to electronic health records. The Human Research Ethics Committee of the University of Western Australia approved the study protocol and consent form (approval number 05/06/004/H50). The Human Research Ethics Committee of the Western Australian Department of Health (DOHWA HREC) also approved the data linkage study (approval number #2009/24) and Edith Cowan University approved the current analysis (project number 12120).

Biochemistry

Baseline second morning void urine samples were collected in 1998 from 1359 participants, frozen and stored at -20 °C and available for analysis. These samples were collected when participants attended a clinic appointment. Participants had all been advised to be well hydrated prior to the appointment. In 2014, samples were thawed and prepared for metals analysis at Edith Cowan University, Joondalup, Western Australia. Metal free polypropylene tubes and pipette tips were used during sample preparation. Four hundred microlitres of urine was diluted 1 in 10 in 2% nitric Acid (Suprapur, Merck). Seven standards were prepared over the range of 0.02 to 500 mg/L in 2% nitric acid from stock standards containing elements of interest. An internal standard (containing Sc, Ge, Y, Rh, Te), was also prepared along with certified reference material (urine ClinChek level I and II, Recipe). The standards and controls were then diluted as per samples. Simultaneously, verification standards ICP-MS-E (High Purity Standards) of 2, 50, 100 mg/L were prepared in 2% nitric acid. The samples were analysed on a Thermo Fisher iCAP Q ICP-MS using an autosampler (Thermo Scientific Inc., New York, United States). New peristaltic pump tubing was used with each batch and

the rinse solution used was Merck Nitric acid 2% Suprapur with 50 mL/L of Triton X (Merck). Calibration checks were run every 20 samples; if check samples outside calibrations, new calibration was performed. New calibration curves were run routinely every 60 samples.

Urinary creatinine was measured by the Jaffe reaction using an Abbott Architect c16000 auto analyser (Abbott Laboratories, Illinois, USA). Urine specific gravity was measured using an Atago Master Refractometer (Atago Co., Tokyo, Japan), at the School of Medical and Health Sciences, Edith Cowan University.

A Hitachi 917 auto analyser (Roche Diagnostics, Mannheim, Germany) was used to measure total cholesterol, high-density lipoprotein cholesterol (HDLC) and serum triglyceride concentrations. Low-density lipoprotein cholesterol (LDLC) was calculated using Friedewald's method (Friedewald et al., 1972). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate estimated glomerular filtration rate (eGFR) using serum creatinine (Levey et al., 2009).

Baseline assessment

Smoking status was coded as non-smoker (i.e. never smoked) or former /current smoker (ever smoked) if they had consumed more than 1 cigarette per day for more than 3 months at any time in their life. Sixty women with a history of smoking provided incomplete smoking history records and were given a value of median pack years from the whole cohort. Body weight (kg) was measured using electronic scales (August Sauter GmbH D-7470 Albstadt 1 Ebingen) to the nearest 0.1 kg with participants wearing light clothes and no shoes. Height (m) was measured using a wall-mounted stadiometer (Holtain Limited, Crymych, Dyfed,

Britain) to the nearest 0.1 cm without socks or shoes. Body mass index (BMI) was calculated using the following equation = weight (kg) / [height (m)] ^{2}. Prevalent diabetes and hypertension, and the use of cardiovascular medications (anti-hypertensive agents, statins and low dose aspirin) were available from the demographic questionnaire. Systolic (SBP) and diastolic blood pressure (DBP) were measured using the right arm, after the subject had been reclined and rested for 5 minutes, using a mercury column manometer.

Prevalent atherosclerotic vascular disease at baseline was determined retrospectively from hospital discharge data 1980-1998 using the Western Australian Data Linkage System (WADLS), a population-based data linkage system, using diagnosis codes from the International Classification of Diseases, Injuries and Causes of Death Clinical Modification (ICD-9-CM) (World Health Organization., 1977). These codes included ischemic (coronary) heart disease (ICD-9-CM codes 410-414); heart failure (ICD-9-CM code 428); cerebrovascular disease excluding haemorrhage (ICD-9-CM codes 433-438); and peripheral arterial disease (ICD-9-CM codes 440-444).

Common Carotid Artery Intima-Media Thickness and Carotid Focal Plaques

Common carotid artery intimal medial thickness (CCA-IMT) and the presence of carotid focal plaques were determined at year 3 (2001) described elsewhere (Ivey et al., 2011). Briefly, six images taken from three different angles (anterolateral, lateral, and posterolateral) were examined and the CCA-IMT from each image was averaged to give an overall mean CCA-IMT and maximum CCA-IMT. A short-term precision study with repeat IMT measurements yielded a coefficient of variation of 5.98% as described previously (Bonnick et al., 2001). The complete carotid tree (common carotid artery, carotid bulb, internal and external carotid) was then examined for the presence of focal plaque, defined as an area of

focal increased thickness (≥1mm) of the intima-media layer. Severity of carotid plaque was further dichotomized by the degree of carotid stenosis as either none to minimal (<25%) or moderate to high (≥25%) (Wilson et al., 1997).

Outcome variables

The Western Australian Data Linkage System (WADLS) provided linked data for the study. WADLS combines seven core databases including inpatient hospital morbidity, inpatient and records and includes working links with special-purpose health service and research databases dating back to as early as 1966 **(Holman et al., 2008)**. The quality of WADLS linkage has been rigorously assessed by comparison with clerical investigation with estimates of invalid links (false-positives) and missed links (false-negatives) occurring in 0.11% of records **(Holman et al., 1999)**. Data for all hospitalisations from baseline (1998) until 14.5 years (2013) after their baseline visit was provided by the Hospital Morbidity Data Collection (HMDC) while deaths were provided by the Mortality Register, both of which are regularly audited **(Holman et al., 1999)**. . Therefore, if the elderly women remained in Western Australia we had complete follow-up for all hospitalizations and deaths over 14.5 years. Principal discharge diagnosis data from the Hospital Morbidity Data Collection was used to ascertain hospitalizations as the validity of these codes is higher than the additional discharge diagnoses fields **(Jamrozik et al., 2001).** International Classification of Diseases, Injuries and Causes of Death (ICD) multiple cause of death were determined from the coded death certificate using information in Parts 1 and 2 of the death certificate or all diagnosis text fields from the death certificate where ICD 10 coded death data were not yet available. ASVD deaths were defined using the primary diagnosis codes from the International Classification of Diseases, Injuries and Causes of Death: Clinical Modification (ICD-9-CM)

and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10-AM) between 1998 and 2013, inclusive. The following ICD9-CM and ICD10-AM codes were identified and utilised as part of the study: coronary heart disease (ICD-9-CM 410-414, ICD10-AM I20-I25), heart failure (ICD-9-CM 428, ICD10-AM I50), peripheral arterial disease excluding haemorrhagic (ICD9-CM 440-444, ICD10-AM I70-I74) and cerebrovascular disease excluding haemorrhagic (ICD-9-CM 433-438, ICD10-AM I63-I69). All-cause mortality was not grouped. Outcomes relating to the incidence of non-fatal CVD events were also used, in addition to CVD mortality. The event outcomes (ASVD event, coronary heart disease event, heart failure event, cerebrovascular disease event, and peripheral arterial disease event) included the fatal cases as well as hospitalisations linked to the respective disease codes. The validity of the diagnoses of myocardial infarction from HMDC, CHD death from the death registry and heart failure from the HMDC have been previously reported (Jamrozik et al., 2001; Teng et al., 2008).

Statistical methods

All statistical analyses were performed in IBM SPSS Statistics, Version 22.0 (2012, Armonk, NY: IBM Corp). Urinary cadmium was positively skewed and transformed using natural logarithm. Samples of urine that had concentrations below the limit of detection (LOD) (<0.05 μg/L) were assigned a value half of the LOD (Liu et al., 1997).

Urinary cadmium concentrations were grouped into tertiles and changes in demographic and health characteristics across tertiles were determined using Kruskal Wallis and Chi-squared analysis. Logistic regression was used to generate odds ratios (with 95% confidence intervals) for the presence of carotid focal plaques. Hazard ratios (with 95% confidence intervals) were estimated for all-cause mortality, CVD mortality and CVD event end points using Cox proportional hazards regression. Urinary cadmium concentrations were introduced

into regression models as tertiles or as continuous ln-transformed concentrations. The p value for the linear trend for the tertile models was calculated by running the models with the median urinary cadmium value for each tertile. Three statistical models were generated, with progressive adjustment for the following variables at baseline. Model 1 was adjusted for age and calcium treatment (yes/no). Model 2 was further adjusted for BMI, hypertension (yes/no), eGFR (ml/min/1.73m^2), prevalent ASVD (yes/no), diabetes (yes/no), low dose aspirin use (yes/no), statin use (yes/no). Model 3 was further adjusted for smoking status (ever/never) and cumulative dose (pack years). Participants were excluded if they had incomplete data for urinary cadmium or any of the confounders (for eGFR 132 participants had missing data, for BMI one participant had missing data and 7 had not responded regarding smoking status), leaving a sample size of 1219 in the final models. Models including urinary creatinine or specific gravity as a covariate were also run (supplementary data). As a sensitivity analysis Cox regression models were re-run excluding participants with urinary cadmium concentrations ≥10µg/L (n= 4), the findings remained unchanged (data not shown). Models were also re-run excluding participants with prevalent ASVD at baseline, to assess the relationship between urinary cadmium and incident ASVD.

Results

Urinary cadmium concentrations were low in this cohort with a mean concentration of 0.38 µg/L and median of 0.18 µg/L (Table 1). Cadmium concentrations were below the limit of detection in 13.8% of participants. Only 18 participants had urinary cadmium concentrations ≥2 µg/L. Urine samples were second morning void samples collected when the participants were well hydrated and were dilute, with 73% of samples having a urinary creatinine concentrations of <0.3 g/L. Urinary specific gravity was also very low in this population (median 1.005, range 1.001-1.029). To avoid potential artificial inflation of cadmium

exposure, the unadjusted cadmium concentrations are presented. However, models were also run including specific gravity and creatinine as covariates and these are presented in Supplementary tables 1 and 2.

The mean age for this sample of elderly women was 75.2 (± 2.7) years, with two thirds of participants being overweight or obese (BMI > 25.0 kg/m^2). One in five (23.2%) participants entered the study with prevalent CVD and one in eight had prevalent ASVD (13%). Thirty seven percent of participants reported being a current or former smoker, and significant increases in the incidence of smokers, and also the number of pack years smoked, were seen across the tertiles of urinary cadmium (Table 2). A difference in estimated glomerular filtration rate (eGFR) was also apparent across the tertiles. Participants in the highest two tertiles of urinary cadmium concentration had higher concentrations of low density lipoprotein (LDL) cholesterol than those with urinary cadmium concentrations ≤ 0.115 µg/L, however, this relationship was not significant (Table 2). Sixty two per cent of participants in the highest urinary cadmium tertile had hypertension at baseline, compared with 55% in the lowest tertile. Interestingly, significant differences in the presence of plaques, measured 3 years after baseline urine collection, were detected across the tertiles of urinary cadmium, with 45% of those in the highest tertile scoring positively for the presence of plaques, compared with 51% in the lowest tertile. Fifteen percent of participants had moderate to severe (≥25%) plaques identified at year 3 of the study. There were no differences in plaque severity across the tertiles of baseline urinary cadmium. Logistic regression revealed a decreased odds of having a plaque associated with increased urinary cadmium concentrations. In an adjusted model there was a 41% reduction in the odds of having a plaque for those in the highest tertile of urinary cadmium (Table 3).

Over a 14.5 year follow up period 479 participants (39%) died, with the number of deaths due to ASVD, CHD, heart failure, PAD and cerebrovascular accident 256, 152, 71, 16 and 99, respectively. The number of events (including both hospitalisations and deaths) for ASVD, CHD, heart failure, PAD and cerebrovascular accident were 529, 316, 144, 69 and 207, respectively. In fully adjusted models, urinary cadmium concentrations were associated with cardiovascular disease events, with a ln unit increase in urinary cadmium concentration (equivalent to a ~2.7 fold increase) associated with a 17% increase in risk of heart failure events (Table 4). Urinary cadmium concentrations were also positively associated with increased mortality. In models adjusted for BMI, hypertension (yes/no), eGFR (ml/min/1.73m^2), prevalent ASVD (yes/no), diabetes (yes/no), low dose aspirin use (yes/no) and statin use (yes/no), every unit increase in ln transformed urinary cadmium concentration was associated with a hazard ratio of 1.11 (95% CI 1.02-1.20) for all-cause mortality, 1.13 (95% CI 1.02-1.27) for ASVD mortality and 1.37 (95% CI 1.12-1.68) for heart failure mortality (Table 5). However, when these models were further adjusted for smoking history, only the relationship between urinary cadmium and heart failure death remained (HR 1.36 (95% CI 1.11-1.67), demonstrating the importance of smoking as a confounder. In analysis using tertiles of urinary cadmium concentrations and adjusted for cumulative smoking dose, those in the highest tertile had an increased risk of heart failure mortality with a hazard ratio of 2.30 (95% CI 1.25-4.25), indicating a more than 2 fold increase in risk, noting the wide confidence intervals (Table 5). No significant relationships were observed between urinary cadmium and mortality or events for coronary heart disease, stroke or peripheral artery disease (Tables 4 and 5). However the numbers of cases of PAD related mortality was small (n=16) which precluded a detailed analysis (data not shown) and is likely to have made hazard ratios for this outcome unstable.

Urinary specific gravity measurements and creatinine concentrations were low in this cohort. However, when either specific gravity or urinary creatinine were added as a covariate this attenuated the relationship between urinary cadmium and the outcomes total mortality, ASVD and heart failure mortality and heart failure event (HR 1.04 95% CI 0.95-1.14; 1.02 95% CI 0.90-1.17; 1.20 95% CI 0.94-1.54; 1.14 95% 0.96-1.35, respectively, including creatinine), (Supplementary Data Tables S1 and S2). Specific gravity was itself only a significant predictor in models for total mortality, ASVD mortality and heart failure mortality.

Sensitivity analysis was conducted by removing all participants with prevalent ASVD (n=152) at baseline from the models. Whilst no relationship was present between urinary cadmium concentrations and all-cause mortality or ASVD mortality, the relationship with heart failure mortality remained (multivariate adjusted hazard ratio comparing highest to lowest tertile of 1.99 (95% CI 1.02-3.89) and 1.96 (95% CI 1.00-3.85) for models 2 and 3, respectively). A second sensitivity analysis was conducted excluding participants with outlying urinary cadmium concentrations ≥10µg/L (n=4). The results and interpretation of the models was unchanged.

Amongst never smokers there was a 29% increased risk of heart failure mortality associated with a one ln unit increase in urinary cadmium, noting the wide confidence intervals HR 1.29 (95% CI 1.01-1.63). However, this relationship was also attenuated by adjustment for urinary creatinine (HR 1.19 (95% CI 0.72-1.97), indicating that the results must be interpreted with caution.

Discussion

In this cohort of elderly Australian women, urinary cadmium concentrations were associated with increased risk of heart failure hospitalisations and mortality, independent of age, smoking history and diabetes. The robustness of this association was confirmed in a sensitivity analysis excluding women with prevalent ASVD. However, attempts to adjust for urinary dilution weakened the associations between urinary cadmium and cardiovascular outcomes in this cohort.

The concentrations of urinary cadmium in this study were low, with a median concentration of 0.18 µg/L, indicating lower exposure than pregnant and older women in recent Australian studies (Callan et al., 2015; Hinwood et al., 2013). In contrast to the work of Fagerberg et al., (2012), no increase in the presence, or severity, of atherosclerotic plaques was seen with increasing urinary cadmium concentrations in this cohort. In fact, contrary to previous work, a decrease in the odds of having a carotid focal plaque was found to be associated with increased urinary cadmium. The reasons for this discrepancy are unclear, however, the Swedish study did report that the association between cadmium and atherosclerotic plaques was only present in multivariate models when blood cadmium, not urine, was used as the measure of exposure, which may indicate that recent cadmium exposure is more relevant to plaque prevalence than long term cadmium exposure. Similarly, no associations were observed between blood lipid profiles and urinary cadmium, which differs from the work of Kim et al., (2012) in a Korean population. Although there was a higher incidence of diagnosed hypertension at baseline in participants in the highest tertile of urine cadmium in this study, no association was observed between urinary cadmium and blood pressure similar to the results of Staessen et al., (2000). One explanation may be the relatively high frequency of anti-hypertensive medication use in our cohort (43%).

In fully adjusted multivariate models, urinary cadmium was not associated with a significant increase in all-cause mortality in this cohort. Most of the longitudinal studies of cadmium exposure and all-cause or cardiovascular mortality which have presented results for women in cohorts from the US (both NHANES and native American communities) or Japan, with limited studies on cohorts outside these areas. The two longitudinal studies that analyse the NHANES cohort report non-significant relationships between urinary cadmium concentrations and all-cause, cardiovascular and coronary heart disease mortality in women (Menke et al., 2009; Tellez-Plaza et al., 2012). In a cohort residing in a cadmium contaminated region of Japan, increased urinary cadmium was found to be associated with increased all-cause, cardiovascular disease and stroke mortality in women (Li et al., 2011; Nakagawa et al., 2006). Similarly, an increased risk of all-cause mortality was reported in women in non-polluted areas of Japan (Suwazono et al., 2014). In women from native American communities increased urinary cadmium was found to be associated with increased risk of incident cardiovascular disease, coronary heart disease, and peripheral artery disease, but not incident stroke or heart failure (Tellez-Plaza et al., 2013aa; Tellez-Plaza et al., 2013bb). The reasons for differences in the results presented in this study are unclear, however, the native American women (1750 participants) were younger than those in our cohort, with a mean age at baseline of 55.9 years. A Swedish study of post-menopausal women reported an increased risk of peripheral artery disease with increased urinary cadmium concentrations. Despite the relatively small sample size in the Swedish cohort (458 participants), the number of peripheral artery disease cases (55) was greater than in our study which may explain our failure to observe an increased risk of peripheral artery disease or related mortality (Fagerberg et al., 2013).

The mechanism by which cadmium exposure may increase the risk of heart failure is unclear. Although not redox active, cadmium has been found to elicit oxidative stress in numerous studies. Chronic cadmium exposure has been found to promote oxidative stress and endothelial damage in rat aortas (Almenara et al., 2013). Given that clinical and experimental studies have identified an increase in oxidative stress and reactive oxygen species in heart failure patients (reviewed by (Tsutsui et al., 2011), this may represent a potential mechanistic pathway, however the relationship between cadmium exposure and heart failure requires further investigation.

There were a number of strengths to this study including the long (14.5 year) follow up period and the ability to analyse both fatal and non-fatal cardiovascular events. Several of the previous studies investigating cadmium exposure and cardiovascular outcomes have been restricted to cardiovascular mortality, which represents a distinct subset of cardiovascular disease events. Also by excluding participants with prevalent ASVD at baseline we were able to examine incident cardiovascular disease, although the relationship between urinary cadmium and incident cardiovascular disease was only significant for heart failure. There have been a limited number of previous studies which have focused specifically on the effects of low cadmium exposure in older women. Furthermore the ability to adjust the analyses for a number of baseline characteristics and known cardiovascular risk factors is a strength of the study. However it is possible that the lifetime cumulative effects of smoking may not have been adequately captured in the adjustment variables. Similarly, cotinine determinations were not available in the present study, thus residual confounding by second hand smoke cannot be discarded as a contributor to cadmium concentrations in these women.

In terms of limitations, as this is an observational study causality cannot be determined. Secondly, in this study urine samples were morning second void samples from well hydrated participants. Thus a large proportion of samples were dilute with urinary creatinine concentrations <0.3 g/L, indicating that creatinine adjustment was not advisable in this cohort (WHO, 1996). This lack of adjustment may have led to exposure misclassification and a failure to adequately adjust for kidney function, which is itself impacted by cadmium exposure. Regression models were adjusted for estimated glomerular filtration rate to try to adjust for (chronic) kidney function, however, given that the inclusion of either specific gravity or creatinine in the models as covariates attenuated the relationships between cadmium and cardiovascular outcomes (Supplementary Data Tables 1 and 2) this suggests that unresolved confounding from kidney function was present in this study. However given the fact that the women were elderly and likely to have reduced creatinine excretion and specific gravity (Davies et al., 2002; Moriguchi et al., 2005), adjustment of urinary cadmium concentrations in this cohort may have led to overestimation of exposure, which is why a conservative approach without adjustment was adopted for the initial models, with further models presented in Supplementary data for completeness. Nonetheless, the fact that creatinine attenuated the associations between urinary cadmium and cardiovascular outcomes in this cohort indicates that the results are only suggestive and require confirmation in a cohort in which first morning void samples are available for analysis. Thirdly, most of the observed associations were became non-significant after adjusting for self-reported smoking history suggesting the association may not be independent of smoking history. A further limitation is that there was missing data in a number of the covariates used to adjust the models which led to a reduction in sample size and power, and may have introduced bias. However the covariates included in the model were known predictors of mortality and cardiovascular disease and therefore allowed for a comprehensive analysis of the relationship

of low cadmium exposure with all-cause mortality and cardiovascular disease, albeit in a reduced study cohort. Finally, for heart failure events and in particular heart failure deaths, there were few events. As such the findings should be interpreted with caution until the relationship between urinary cadmium and heart failure events can be confirmed in other prospective studies.

Conclusions

In this cohort of elderly women with low cadmium exposure, increased urinary cadmium was associated with increased heart failure mortality and non-fatal heart failure events over a 14.5 year follow up period, although adjustment for urinary specific gravity or creatinine attenuated these relationships, therefore the results must be interpreted with caution. The relationship with heart failure mortality was observed in never smokers, although residual confounding by second hand smoke exposure cannot be ruled out, these results suggest that low level (dietary) cadmium exposure may be associated with increased heart failure mortality in women, however given the dilution of the urine samples in this study these findings require confirmation in other cohorts. Given the low urinary cadmium concentrations in this study the results appear to support the concept that cadmium exposure may be a risk factor for cardiovascular outcomes, however the importance of confounding from kidney function was evident.

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Table 1. Urinary cadmium concentrations in elderly women (n=1219).

		Percentiles				
Urinary measurement	**Mean**	**5th**	**25th**	**50th**	**75th**	**95th**
Cadmium unadjusted (μg/L)	0.37	<0.05	0.09	0.18	0.32	0.95
Cadmium Adjusted (μg/g creatinine)*	0.94	0.12	0.44	0.65	1.06	2.30

IQR = interquartile range

*Creatinine adjusted results presented only for women with urinary creatinine >0.3g/L (n=327)

Table 2. Baseline characteristics of the study participants overall and expressed by tertiles of urinary cadmium (n = 1219, unless otherwise specified).

			Cadmium Tertiles (µg/L)						
	Overall		**≤ 0.115**		**> 0.115 – 0.255**		**> 0.255**		***p* value**
Age, (years)[a]	75.2	± 2.7	75.0	± 2.7	75.2	± 2.7	75.4	± 2.7	0.084
BMI, (Kg/m^2)[a]	27.1	± 4.7	26.9	± 4.9	27.1	± 4.3	27.4	± 4.8	0.232
Smoking									
Ever smoker[b]	451	(37)	122	(30)	149	(37)	180	(44)	**<0.001***
Pack years[a]	7.2	± 16.6	4.00	± 10.2	6.3	±14.2	11.4	±22.2	**<0.001***
Cholesterol mg/dL[a,e]	226.8	± 42.9	223.1	± 43.8	228.7	± 39.9	228.5	± 44.7	0.185
HDL cholesterol mg/dL[a,e]	56.2	± 14.7	56.4	± 15.6	56.4	± 14.2	55.9	± 14.2	0.954
Triglycerides mg/dL[a,e]	138.4	± 64.0	139.4	± 68.7	138.8	± 63.6	137.1	± 59.7	0.932
LDL cholesterol mg/dL[a,e]	142.5	± 39.3	138.7	± 38.8	144.2	± 36.4	144.6	± 42.1	0.151
Mean systolic blood pressure (mmHg)[a,e]	137.7	± 17.9	138.5	± 19.0	137.8	± 16.6	137.0	± 18.0	0.832
Mean diastolic blood pressure (mmHg)[a,e]	73.1	± 11.0	72.6	± 10.9	73.0	± 10.4	73.7	± 11.7	0.334
Mean arterial pressure (mmHg)[a,e]	94.6	± 11.7	94.5	± 12.0	94.6	± 10.9	94.8	± 12.3	0.825
Mean of mean intima-media thickness (mm)[a,e]	0.78	± 0.13	0.78	± 0.13	0.78	± 0.12	0.77	± 0.12	0.594
Mean of maximum intima-media thickness (mm)[a,e]	0.92	± 0.15	0.93	± 0.16	0.92	± 0.15	0.91	± 0.15	0.524
Presence of atherosclerotic plaque[b,c,d,e]	472	(50)	158	(51)	172	(55)	142	(45)	**0.032***
Moderate to severe plaque (≥25%)[d,e]	138	(15)	45	(15)	48	(15)	45	(14)	0.919
eGFR (ml/min/1.73 m^2)[a]	66.5	± 13.2	68.0	± 13.0	65.5	± 13.3	66.0	± 13.5	**0.019***
Hypertension[b]	688	(56)	223	(55)	214	(53)	251	(62)	**0.024***
Diabetes[b]	81	(6.6)	31	(7.6)	20	(4.9)	30	(7.4)	0.239
Low dose aspirin use[b]	263	(22)	86	(21)	85	(21)	92	(23)	0.809
Statin use	226	(19)	65	(16)	81	(20)	80	(20)	0.250
Prevalent ASVD[b]	152	(13)	44	(11)	50	(12)	58	(14)	0.318

[a]mean ± standard deviation; [b]number (percentage); [c](n=929); [d]measured at year 3, not baseline; [e]Missing data – n for cholesterol measures n=908, for blood pressure n=1180, intima-media thickness n=922 and presence of plaque n=937.

p values were calculated using Kruskal-Wallis or Chi-squared tests. *Relationship is significant at the 0.05 level (2-tailed).

Table 3: Presence of carotid focal plaque (measured at year 3) by urinary cadmium concentration (n=937)*

	Cadmium Tertiles (µg/L)						**Continuous**		
	≤ 0.115	**> 0.115 – 0.255**			**> 0.255**				
Model 1	1.00	1.14	(0.83	1.57)	**0.73**	**(0.53**	**1.00)**	0.90	(0.80	1.02)
Model 2	1.00	1.16	(0.84	1.60)	**0.68**	**(0.49**	**0.94)**	0.89	(0.79	1.00)
Model 3	1.00	1.11	(0.80	1.54)	**0.59**	**(0.42**	**0.83)**	**0.85**	**(0.75**	**0.96)**

* Presented as Odds ratios (95% CI)

Model 1 adjusted for age at baseline and calcium treatment (yes/no). Model 2 further adjusted for BMI, hypertension (yes/no), eGFR (ml/min/1.73m^2), Prevalent ASVD (yes/no), diabetes (yes/no), low dose aspirin use (yes/no) and statin use (yes/no). Model 3 further adjusted for smoking status (ever/never) and cumulative dose (pack years). Bold values indicate significance at p <0.05. Continuous cadmium concentration per ln unit (equivalent to ~2.7 fold) increase in urinary cadmium. Further adjustment for urinary specific gravity had no impact on the interpretation of these models.

Table 4: Atherosclerotic vascular disease events (death or hospitalisation) Endpoints by Urine Cadmium Concentrations (n=1219)*

	Cadmium Tertiles (µg/L)					
	≤ 0.115	**> 0.115 – 0.255**	**> 0.255**	***p* for trend**	**Continuous**	***p* value**
	Atherosclerotic vascular disease event					
No. cases/noncases	165/408	184/405	180/406		529/1219	
Model 1	1.00	1.07 (0.84, 1.35)	1.13 (0.89, 1.43)	0.326	1.05 (0.97, 1.14)	0.245
Model 2	1.00	1.06 (0.84, 1.34)	1.03 (0.82, 1.30)	0.878	1.03 (0.95, 1.13)	0.441
Model 3	1.00	1.04 (0.82, 1.31)	0.99 (0.78, 1.25)	0.835	1.02 (0.93, 1.11)	0.673
	Coronary heart disease event					
No. cases/noncases	99/408	111/405	106/406		316/1219	
Model 1	1.00	1.15 (0.88, 1.51)	1.09 (0.83, 1.43)	0.680	1.07 (0.97, 1.18)	0.203
Model 2	1.00	1.14 (0.87, 1.51)	0.97 (0.74, 1.28)	0.622	1.04 (0.94, 1.15)	0.447
Model 3	1.00	1.12 (0.85, 1.47)	0.92 (0.69, 1.21)	0.365	1.02 (0.92, 1.13)	0.697
	Heart failure event					

No. cases/noncases	39/408	46/405			59/406				144/1219		
Model 1	1.00	1.20	(0.78,	1.84)	**1.57**	**(1.04,**	**2.35)**	**0.027**	**1.19**	**(1.03,**	**1.37)**	**0.019**
Model 2	1.00	1.24	(0.80,	1.91)	1.47	(0.98,	2.21)	0.071	**1.17**	**(1.01,**	**1.36)**	**0.032**
Model 3	1.00	1.24	(0.80,	1.91)	1.46	(0.97,	2.21)	0.080	**1.17**	**(1.01,**	**1.35)**	**0.039**
	Peripheral artery disease event											
No. cases/noncases	15/405	23/401			29/403				69/1215		
Model 1	1.00	1.47	(0.78,	2.77)	1.79	(0.97,	3.30)	0.079	1.18	(0.96,	1.45)	0.110
Model 2	1.00	1.53	(0.81,	2.91)	1.70	(0.92,	3.15)	0.127	1.17	(0.95,	1.44)	0.133
Model 3	1.00	1.45	(0.76,	2.75)	1.55	(0.83,	2.90)	0.231	1.15	(0.92,	1.43)	0.222
	Cerebrovascular accident event											
No. cases/noncases	70/408	70/405			67/406				207/1219		
Model 1	1.00	1.00	(0.72,	1.39)	0.96	(0.69,	1.35)	0.807	1.01	(0.89,	1.14)	0.916
Model 2	1.00	1.00	(0.72,	1.40)	0.90	(0.65,	1.27)	0.517	0.99	(0.88,	1.12)	0.894
Model 3	1.00	1.00	(0.71,	1.40)	0.90	(0.64,	1.27)	0.528	1.00	(0.88,	1.13)	0.937

*HRs (95% CI)

Model 1 adjusted for age at baseline and calcium treatment (yes/no). Model 2 further adjusted for BMI, hypertension (yes/no), eGFR (ml/min/1.73m2), Prevalent ASVD (yes/no), diabetes (yes/no), low dose aspirin use (yes/no) and statin use (yes/no). Model 3 further adjusted for smoking status (ever/never) and cumulative dose (pack years). *p value for linear trend calculated by running model with median value for each tertile. Bold values indicate significance at p <0.05. Continuous cadmium concentrations per ln unit (equivalent to ~2.7 fold) increase in urinary cadmium.

Table 5: Mortality Endpoints by Urine Cadmium Concentrations (n=1219)*

	Cadmium Tertiles (µg/L)					
	≤ 0.115	**> 0.115 – 0.255**	**> 0.255**	***p* for trend***	**Continuous**	***p* value**
	Total mortality					
No. cases/noncases	145/408	158/405	176/406		479/1219	
Model 1	1.00	1.10 (0.88, 1.38)	1.24 (0.99, 1.54)	0.058	**1.12 (1.03, 1.21)**	**0.007**
Model 2	1.00	1.11 (0.88, 1.39)	1.19 (0.95, 1.48)	0.148	**1.11 (1.02, 1.20)**	**0.014**
Model 3	1.00	1.06 (0.85, 1.34)	1.10 (0.87, 1.37)	0.463	1.08 (0.99, 1.17)	0.081
	Atherosclerotic vascular disease mortality					
No. cases/noncases	70/408	90/405	96/406		256/1219	
Model 1	1.00	1.29 (0.94, 1.76)	**1.38 (1.02, 1.88)**	0.064	**1.15 (1.03, 1.28)**	**0.011**
Model 2	1.00	1.29 (0.94, 1.77)	1.28 (0.94, 1.74)	0.207	**1.13 (1.02, 1.27)**	**0.025**
Model 3	1.00	1.24 (0.90, 1.71)	1.21 (0.88, 1.66)	0.359	1.12 (1.00, 1.25)	0.055
	Coronary heart disease mortality					
No. cases/noncases	42/408	57/405	53/406		152/1217	

Model 1	1.00		1.36	(0.91,	2.03)	1.27	(0.85,	1.91)	0.379	1.12	(0.97,	1.28)	0.122
Model 2	1.00		1.34	(0.89,	2.00)	1.15	(0.76,	1.73)	0.761	1.10	(0.95,	1.26)	0.214
Model 3	1.00		1.31	(0.88,	1.97)	1.11	(0.74,	1.69)	0.880	1.09	(0.94,	1.26)	0.258
	Heart failure mortality												
No. cases/noncases	15/408		19/405			37/406				71/1219			
Model 1	1.00		1.28	(0.65,	2.51)	**2.49**	**(1.36,**	**4.54)**	**0.001**	**1.37**	**(1.13,**	**1.65)**	**0.001**
Model 2	1.00		1.28	(0.65,	2.54)	**2.36**	**(1.29,**	**4.32)**	**0.002**	**1.37**	**(1.12,**	**1.68)**	**0.002**
Model 3	1.00		1.26	(0.63,	2.51)	**2.30**	**(1.25,**	**4.25)**	**0.003**	**1.36**	**(1.11,**	**1.67)**	**0.003**
	Cerebrovascular accident mortality												
No. cases/noncases	30/408		36/405			33/406				99/1216			
Model 1	1.00		1.20	(0.74,	1.95)	1.11	(0.67,	1.82)	0.806	1.04	(0.87,	1.23)	0.703
Model 2	1.00		1.18	(0.72,	1.92)	1.03	(0.62,	1.69)	0.939	1.02	(0.85,	1.22)	0.824
Model 3	1.00		1.13	(0.69,	1.85)	0.96	(0.58,	1.59)	0.750	1.00	(0.83,	1.20)	0.975

*HR (95% CI)

Model 1 adjusted for age at baseline and calcium treatment (yes/no). Model 2 further adjusted for BMI, hypertension (yes/no), eGFR (ml/min/1.73m^2), Prevalent ASVD (yes/no), diabetes (yes/no), low dose aspirin use (yes/no) and statin use (yes/no). Model 3 further adjusted for

smoking status (ever/never) and cumulative dose (pack years). *p value for linear trend calculated by running model with median value for each tertile. Bold values indicate significance at p <0.05. Continuous cadmium concentration per ln unit (equivalent to ~2.7 fold) increase in urinary cadmium.