Urine cadmium and acute myocardial infarction among never smokers in the Danish Diet, Cancer and Health cohort

Sears, Clara G.; Poulsen, Aslak Harbo; Eliot, Melissa; Howe, Chanelle J.; James, Katherine A.; Harrington, James M.; Roswall, Nina; Overvad, Kim; Tjonneland, Anne; Raaschou-Nielsen, Ole; Wellenius, Gregory A.; Meliker, Jaymie

Published in:
Environment International

DOI:
10.1016/j.envint.2021.106428

Publication date:
2021

Document version
Publisher's PDF, also known as Version of record

Environment International 150 (2021) 106428

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Urine cadmium and acute myocardial infarction among never smokers in the Danish Diet, Cancer and Health cohort

Clara G. Sears [a,*], Aslak Harbo Poulsen [b], Melissa Eliot [a], Chanelle J. Howe [a], Katherine A. James [c], James M. Harrington [d], Nina Roswall [b], Kim Overvad [e,f], Anne Tjønneland [b,g], Ole Raaschou-Nielsen [b,h], Gregory A. Wellenius [a,i], Jaymie Meliker [j]

[a] Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA
[b] Danish Cancer Society Research Center, Copenhagen, Denmark
[c] Department of Environmental and Occupational Health, Colorado School of Public Health, University of Colorado-Anschutz Medical Campus, CO, USA
[d] Center for Analytical Science, Research Triangle Institute, Research Triangle Park, NC, USA
[e] Department of Public Health, Aarhus University, Aarhus, Denmark
[f] Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark
[g] Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[h] Department of Environmental Science, Aarhus University, Roskilde, Denmark
[i] Department of Environmental Health, Boston University, Boston, MA, USA
[j] Program in Public Health, Department of Family, Population, & Preventive Medicine, Stony Brook University, Stony Brook, NY, USA

ARTICLE INFO

Handling Editor: Shoji Nakayama

Keywords:
Cadmium
Acute myocardial infarction
Cardiovascular disease
Case-cohort study

ABSTRACT

Cadmium exposure has been associated with cardiovascular disease. Cigarette smoking is a key source of cadmium exposure and thus a potential confounder in observational studies of environmental cadmium and cardiovascular disease that include tobacco smokers. We leveraged up to 20 years of follow-up in the Danish Diet, Cancer and Health cohort to test the hypothesis that cadmium exposure is associated with acute myocardial infarction (AMI) among people who never smoked. Between 1993 and 1997, 19,394 never-smoking participants (ages 50–64 years) were enrolled and provided a urine sample. From this sample, we randomly selected a subcohort of 600 males and 600 females. We identified 809 AMI cases occurring between baseline and the end of 2015 using the Danish National Patient Registry. We quantified cadmium, creatinine, and osmolality in baseline urine samples. Using an unweighted case-cohort approach, we estimated adjusted hazard ratios (aHR) for AMI in Cox proportional hazards models with age as the time axis. Participants had relatively low concentrations of urinary cadmium, as expected for never smokers (median = 0.20; 25th, 75th = 0.13, 0.32 μg cadmium/g creatinine). We did not find strong evidence to support an association between higher urinary cadmium and AMI when comparing the highest versus lowest quartile (aHR = 1.16; 95% CI: 0.86 – 1.56) and per IQR increment in cadmium concentration (aHR = 1.02; 95% CI: 0.93 – 1.12). Results were not materially different across strata defined by sex. Results were generally similar using creatinine or osmolality to account for differences in urine dilution. While cadmium exposure has been identified as a risk factor for cardiovascular disease, we did not find strong evidence that urinary cadmium at relatively low-levels is associated with AMI among people who have never smoked.

1. Introduction

Cadmium is a highly persistent, naturally-occurring heavy metal. In addition to background levels found in soil, application of phosphate fertilizers and sewage sludge to cropland increases the amount of cadmium absorbed by foodstuffs and tobacco (Järup and Åkesson, 2009). As a result, cadmium is present in virtually all foods, with more than 80% of food-derived cadmium coming from cereals, vegetables, and potatoes (Olsson et al., 2005). Average cadmium intake in food varies from 8 to 25 μg/day, and an individual absorbs approximately 1

* Corresponding author at: Postdoctoral Research Associate, Department of Epidemiology, Brown University School of Public Health, Box G-S121-2, Providence, RI 02912, USA.
E-mail address: clara_sears@brown.edu (C.G. Sears).

https://doi.org/10.1016/j.envint.2021.106428
Received 5 October 2020; Received in revised form 25 January 2021; Accepted 27 January 2021
Available online 8 February 2021

μg/day of cadmium from smoking 20 cigarettes per day (Järup and Åkesson, 2009; Sahmoun et al., 2005).

Cadmium has been recognized as an occupational health hazard for decades, most notably as a risk factor for lung cancer, impaired renal function, and bone disease (Amzal et al., 2009; Järup and Åkesson, 2009; Murata et al., 1970; IARC, 1993). At lower levels of exposure more relevant for the general population, there is growing evidence of cadmium as a cardiovascular risk factor (Chowdhury et al., 2018; Domingo-Relloso et al., 2019). Experimental studies have demonstrated cadmium's ability to damage vascular tissues (Abraham et al., 2000; Fujiwara et al., 1998; Jeong et al., et al., 2004), induce endothelial dysfunction (Dong et al., 2014; Pearson et al., 2003; Prozialeck, 2000; Prozialeck et al., 2006; Wang et al., 2018; Woods et al., 2008), and promote atherosclerosis (Hernández and Macia, 1996; Kaji et al., 1994; Knoflach et al., 2011; Messner et al., 2009; Oliveira et al., 2019; Yamamoto et al., 1993).

The first case report of cadmium-associated acute myocardial infarction (AMI) is from a worker exposed to cadmium fumes in 1970 (Zavon and Meadows 1970). Prospective epidemiologic studies initially investigated the relationship of cadmium biomarkers with coronary heart disease (CHD) and most (Barregard et al., 2016; Menke et al., 2009; Tellez-Plaza et al., 2012; 2013a, systematic review: 2013b) – but not all (Nawrot et al., 2008) – show positive associations, with Menke et al reporting these positive associations among men but not women (Menke et al., 2009). One of these prospective studies suggests a positive association between blood cadmium and AMI specifically, but these results are not consistent with studies using dietary estimates of cadmium exposure (Julin et al., 2011; 2013a; 2013b).

Cadmium is found in cigarettes and smoking is an established risk factor for AMI. Therefore, smoking stands to be an important source of residual confounding in existing observational studies of relations between cadmium and AMI. Two prior prospective studies of cadmium biomarkers and AMI or CHD among never smokers suggest cadmium is associated with AMI, but these studies were underpowered (Barregard et al., 2016; Tellez-Plaza et al., 2013a). Quantifying the risk of AMI associated with cadmium from sources other than smoking is critical for policy-makers considering regulating key dietary or industrial sources of cadmium exposure. Accordingly, to investigate whether cadmium is associated with AMI independent of smoking, we conducted a case-cohort study among people who never smoked tobacco in the Danish Diet, Cancer and Health (DCH) cohort. We chose a case-cohort design to efficiently leverage stored urine samples collected at baseline, along with existing data from up to 20 years of follow-up.

2. Methods

We used existing data and urine samples from the prospective DCH cohort to evaluate the association between urinary cadmium concentrations and AMI among men and women who never smoked. Details of sampling, recruitment, and follow-up procedures for the DCH cohort are provided elsewhere (Tjønneland et al., 2007). Briefly, between December 1993 and May 1997, 160,725 individuals (ages 50–64 years) living in the area surrounding Copenhagen or Aarhus were invited to participate in the study, 57,053 (35.5%) of which consented to participate. At enrollment, participants completed questionnaires to assess smoking habits, socioeconomic characteristics, diet, and physical activity. Trained research personnel also collected anthropometric measurements and urine samples. The DCH cohort was established in accordance with the Helsinki Declaration and approved by the local Ethics Committees. Written informed consent was obtained from all participants.

For this study, we excluded from analyses participants with prevalent cancer at baseline (n = 581), missing a urine sample because it was not provided (n = 390) or not available in the biobank (n = 772), or missing information on self-reported smoking status (n = 69) (Fig. 1). Among the remaining participants, only participants who self-identified

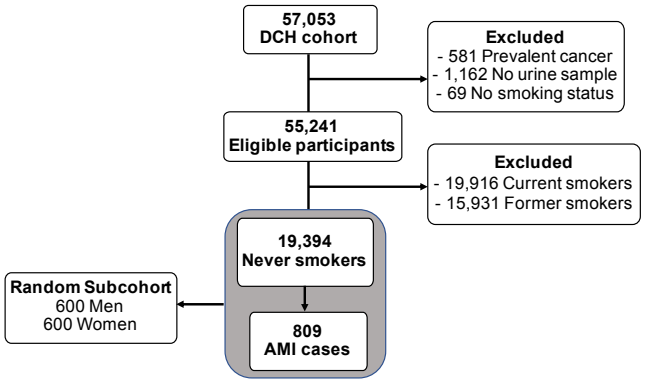

Fig. 1. Flow diagram of case-cohort study design with participants from the Diet, Cancer and Health cohort (Copenhagen and Aarhus, Denmark; 1993–1997).

at baseline as never smokers (n = 19,394 total; n = 6,821 men and n = 12,573 women) were eligible for inclusion.

Among all eligible participants who never smoked, we identified AMI cases that occurred between individual enrollment dates and December 31st, 2015 using the Danish National Patient Registry (NPR) (Schmidt et al., 2015; Lynge et al., 2011). The Danish National Health Service provides free universal tax-supported health and hospital care for all residents. Since 1977, all patient diagnoses in somatic hospitals have been recorded in the NPR and coded using ICD 8 (prior to 1994) or ICD 10 (implemented in 1994). Beginning in 1995, diagnoses from emergency rooms and outpatient visits were also included in the NPR (Schmidt et al., 2015; Lynge et al., 2011). Using the unique civil registration number assigned to all Danes at birth, we identified DCH cohort participants registered with a diagnosis of AMI in the NPR (primary or secondary diagnosis of ICD-8 410–410.99, 427.27 and ICD-10: I21.0-I21.9 and I46.0-I46.9) (Pedersen, 2011; Schmidt et al., 2014). From study baseline through 2003, a physician with experience in cardiology reviewed the medical records of potential AMI cases and confirmed diagnoses according to AMI criteria set by the American Heart Association and the European Society of Cardiology for use in epidemiology. The positive predictive value of this approach was greater than 92% (Joensen et al., 2009). Therefore, beginning in 2004, participants identified through the NPR as having an AMI were accepted as cases without further adjudication. Cases of cardiac arrest (ICD-8: 427.27 or ICD-10: I46.0-I46.9) were an exception and were only included if deemed to be of cardiac origin by a skilled physician through 2013 (Monrad et al., 2017). Through 2013 we used the Danish Cause of Death Registry to identify fatal AMI cases that were not included in the NPR. Between 2013 and 2015, we identified additional DCH participants with AMI using ICD codes registered in the NPR.

We identified a total of 809 cases of incident AMI among all eligible participants who never smoked. We selected a referent subcohort by randomly sampling 600 men and 600 women (total n = 1,200) who never smoked, and 61 of these subcohort members were AMI cases. We censored participants at the emigration date recorded in the Danish Central Person Registry, the date of death documented in the Danish Cause of Death Registry, or December 31st, 2015, whichever occurred first (Helweg-Larsen, 2011; Pedersen, 2011; Schmidt et al., 2014).

2.1. Urinary Cadmium, Creatinine, and osmolality

Spot urine samples were collected in a transparent polypropylene cup at baseline using trace metal-free techniques (Tjønneland et al., 2007). Within 2 hours of collection, a laboratory technician aliquoted urine samples and stored them in the biobank at −150 °C. The urine was never in contact with any metal equipment nor any colored plastic (dyes used to color plastic can contain cadmium). Urine samples from cases

and subcohort members were mixed and anonymized before shipment on dry ice to RTI International's Trace Metals Laboratory (Research Triangle Park, NC) to ensure completely blinded analysis and to account for potential batch to batch variation in urine analysis.

High purity HNO_3 was prepared from trace metals grade concentrated nitric acid (JT Baker, Phillipsburg, NJ) by acid distillation (Milestone DuoPur, Shelton, CT) and screened for impurities before use. National Institute of Standards and Technology (NIST, Gaithersburg, MD) traceable stock standards of analytes and internal standards (bismuth, praseodymium, holmium, scandium, and yttrium) were purchased commercially (High Purity Standards, Charleston, SC) and used to prepare calibration standards. High-purity deionized water (16MΩ or better, Pure Water Solutions) was used for preparation of all samples and standards.

Urine samples were thawed to room temperature and vortexed to mix thoroughly. A 0.50 mL aliquot of each sample was transferred to 15-mL polypropylene tubes. We added 0.50 mL HNO_3 and 0.050 mL of concentrated HCl to each digestion vessel and heated in a graphite heating block (SCP Science, Baie d'Urfe, Quebec) at 95 °C for 30 min. Samples were allowed to cool to room temperature and we added 0.25 mL of 30% hydrogen peroxide solution (EMD Millipore, Burlington, MA). The resulting mixture was digested at 95 °C for an additional 30 min. Tubes were cooled to room temperature, spiked with an internal standard mixture to a final concentration of 5 μg/L, and diluted to a final volume of 5 mL with deionized water with mixing.

An iCAP Q ICP-MS system (Thermo Scientific, Waltham, MA) equipped with a helium gas collision cell was used for the determination of a suite of 19 elements including cadmium. The instrument was tuned daily to maximize sensitivity and signal stability across the mass range and to mitigate polyatomic interferences. We monitored multiple isotopes for most elements to account for analytical interferences (polyatomic and isobaric), such as molybdenum for cadmium (MoO).

NIST standard reference material (SRM) 2668 – Toxic Elements in Frozen Human Urine was used as a quality control sample to verify method accuracy and reproducibility. Mean recovery for cadmium in NIST SRM 2668 was 99.1% with a coefficient of variation (CV) of 4.2% over 47 sample batches and 9 months. Approximately 10% of samples were reanalyzed to provide an additional measure of method accuracy and reproducibility. Samples were selected at random within 3 concentration ranges: low (<0.25 μg Cd/mL), medium (0.25–0.50 μg Cd/mL) and high (>0.50 μg Cd/mL) to provide a measure of reproducibility across the range of observed sample concentration ranges. These samples demonstrated good reproducibility of 6.6% CV for samples above the LOD and a Pearson's correlation coefficient of 0.93.

We quantified urinary creatinine colorimetrically by the Jaffe reaction with a Cayman Chemicals (Ann Arbor, MI) Creatinine Assay Kit following the manufacturer's instructions. NIST SRM 2668 has an informational value for creatinine (626 mg/L in Level 1), so it was measured as a quality control sample. Recovery of the SRM generally fell within 90–110% of the informational value. We reanalyzed ~ 10% of samples as incurred samples, and the incurred values exhibited a CV of 3.9% for samples above the limit of detection.

We measured urinary osmolality by the freezing point depression method using a Model 3320 Micro-Osmometer by Advanced Instruments, Inc. (Norwood, MA). We measured Clinitrol Reference Solution (Advanced Instruments, certified value 290 mOsm) and 800 mOsm Renol Urine Osmolality Control solution as daily QC samples and report CV = 1.3%, 0.7% for the two reference solutions.

For the main analyses, we used the quantified cadmium concentrations for values below the LOD. Among AMI cases and the subcohort members, 17 individuals (2.1%) and 22 individuals (1.8%) had urinary cadmium concentrations below the LOD (median LOD [25th, 75th] = 0.003 [0.001, 0.003] μg/L). We calculated creatinine-standardized cadmium concentrations by dividing urinary cadmium concentrations (μg) by creatinine (g).

2.2. Urinary cotinine and Self-reported Second-hand tobacco exposure

We measured urinary cotinine, an indicator of tobacco smoke exposure, using a cotinine ELISA bioassay kit by Abnova Corporation (Taipei, Taiwan). We followed the manufacturer's protocol for analysis, calibrating the analysis over the range of 0–100 μg cotinine/L urine. Incurred sample reanalysis results exhibited a CV of 2.9%. Over-range samples were diluted into the linear range with deionized water and remeasured.

At baseline, participants also self-reported secondhand smoke exposure at home and at work during each decade of their life prior to and at DCH cohort enrollment (ages 0–9, 10–19, 20–29, 30–39, 40–49, ≥50 years). Using responses to these questions we grouped participants based on secondhand smoke exposure level into: 1) none; 2) exposure only before age 50 years (i.e. earliest possible age of DCH cohort enrollment); and 3) exposure at age 50 years and older (i.e. at enrollment).

2.3. Covariates

Participants self-reported age, sex, education, and physical activity on questionnaires administered at baseline, as previously described (Tjønneland et al., 2007). Women self-reported menopausal status and parity. At baseline, study personnel measured height and weight, and calculated body mass index (BMI). We identified potential confounders, sources of selection bias, and covariates to include in statistical models *a priori* based on knowledge about the risk factors for cadmium exposure and AMI (Hernán, 2002).

2.4. Statistical analyses

We used the Prentice case-cohort approach to estimate associations between creatinine-standardized urinary cadmium concentrations and AMI (Barlow et al., 1999). In this time-to-event analysis, follow-up for each participant in the subcohort started on the age at their specific DCH cohort enrollment date and ran until the age at the earlier of date of AMI diagnosis or date of censoring due to emigration, death, or the end of the follow-up period. AMI cases among DCH participants who were not included in the subcohort were not considered to be *at risk* for AMI until immediately preceding the date of AMI diagnosis. Based on the Prentice method, we estimated hazard ratios and robust 95% confidence intervals for AMI using Cox proportional hazards models (Barlow et al., 1999). We used age as the time axis because it is related to the outcome and using time-on-study as the axis may bias results, even when adjusting for age as a covariate (Thiébaut and Bénichou, 2004). We estimated the association between creatinine-standardized cadmium and AMI in minimally-adjusted models including sex as a covariate and in fully-adjusted models including sex, BMI (continuous-linear term), education (categorical), and urinary cotinine concentrations (continuous-linear term). We evaluated the association between quartiles of creatinine-standardized cadmium and AMI and conducted a linear test for trend by assigning observations in each quartile of creatinine-standardized cadmium the median concentration of the quartile, treating the variable as continuous, and assessing the p-value for this term (Rothman et al., 2008). We further assessed the linearity of the relationship between creatinine-standardized cadmium and AMI by modeling cadmium using a natural cubic spline with three degrees of freedom (knots at 33rd and 67th percentiles). We visually assessed the linearity and ran an ANOVA to compare this spline model to the model with a linear term. We assessed the p-value from the ANOVA as the non-linearity p-value.

In secondary analyses, we also included a sex by cadmium product term in adjusted models, as well as estimated the association between creatinine-standardized urinary cadmium concentrations and AMI in sex-stratified analyses. For the strata of women, we included menopausal status as a covariate in adjusted models. In the stratified analysis,

we visually evaluated linearity and compared the fit of the linear models to spline models with three degrees of freedom, as described for the main analysis.

2.5. Sensitivity analyses

We conducted a series of sensitivity analyses to assess the robustness of our findings to outliers and cadmium concentrations below the LOD. First, we repeated the main analyses with all cadmium concentrations below the LOD assigned the LOD/sqrt 2 (Hornung and Reed, 1990). Separately, we conducted additional analyses restricting the study sample to participants with creatinine-standardized cadmium concentrations between the 5th and 95th percentiles.

We conducted a series of sensitivity analyses to evaluate the impact of using creatinine standardization to account for variation in urine dilution. We compared our results to hazard ratios from adjusted models that included creatinine as a covariate. Based on methods described by O'Brien et al (2016), we also calculated a covariate adjusted creatinine-standardized cadmium ratio (O'Brien et al., 2016). First, we fit a linear regression model predicting log creatinine concentrations based on age, sex, physical activity level, and BMI. Next, we divided urinary cadmium concentrations by the ratio of quantified urinary creatinine concentrations to predicted creatinine concentrations. We report continuous results per interquartile range difference in the covariate-adjusted creatinine-standardized cadmium ratio to facilitate comparison across different methods. We also assessed how using osmolality to account for variation in urine dilution alters our results. We conducted osmolality-adjusted analyses which included osmolality, instead of creatinine, as a covariate in adjusted models. We also assessed the impact of using osmolality-standardized cadmium concentrations in adjusted models. Moreover, we evaluated how using these different methods to account for variation in urine dilution altered results from the secondary sex-stratified analyses. In models adjusted for creatinine, we evaluated the association between sex-specific quartiles of cadmium and AMI. We also assessed linearity of the relationship of cadmium with AMI when adjusting for creatinine by comparing linear models to natural cubic splines with three degrees of freedom (knots at 33rd and 67th percentiles), as described for the main analyses.

Finally, we excluded participants with prevalent diabetes prior to baseline (n = 16 subcohort members and n = 33 non-subcohort AMI cases) and additionally adjusted for incident diabetes (n = 187) during the follow-up period in the fully-adjusted model. In a separate sensitivity analysis, we excluded all participants with diabetes prior to or during follow-up. Diabetes could potentially confound the relationship of urinary cadmium concentrations with AMI. However, diabetes and kidney function could also be plausible causal intermediaries on a pathway between cadmium exposure and cardiovascular disease, and therefore, including diabetes as a covariate may result in over-adjustment (VanderWeele, 2019). We conducted additional analysis in the fully adjusted model: (1) adjusting for hypertension, hypercholesteremia, and diabetes; (2) excluding participants with cotinine $\geq$ 200 μg/L (n = 40 who may be current smokers (Kim, 2016); (3) excluding participants with creatinine < 0.03 g/L and greater than 3.0 g/L ; and 4) excluding participants with heart failure or stroke.

All analyses were conducted in R version 3.6.2 using the survival package and functions to account for the case-cohort study design.

3. Results

3.1. Participant characteristics

We identified 809 cases of AMI, predominantly among males (60.3%). Among subcohort members, we censored 3 participants due to emigration and 138 due to death (median follow-up time [25th, 75th percentile] = 14.3 [8.7, 16.6] years). Participants who subsequently developed AMI tended to have a lower level of education and be more obese compared with subcohort members (Table 1).

Overall, the median (25th, 75th) cotinine concentration was 16.8 (5.6, 34.4) μg/L. Participants who reported being exposed to secondhand smoke at age $\geq$ 50 years had a higher median cotinine concentration (21.0 [7.5, 39.8] μg/L) compared with participants who reported secondhand smoke exposure only prior to age 50 years (8.8 [3.5, 20.8]

Table 1
Baseline characteristics of AMI cases and subcohort members.

Baseline characteristics	Subcohort (n = 1200)		AMI cases (n = 809)	
	No (%)	Median (25th, 75th)	No (%)	Median (25th, 75th)
Age at enrollment (years)	1200	55.8 (52.5, 59.9)	809	58.0 (54.1, 61.3)
Sex				
Male	600 (50)		488 (60)	
Female	600 (50)		321 (40)	
Education				
Low (<8 years)	344 (28)		272 (34)	
Medium (8–10 years)	549 (46)		380 (47)	
High (>10 years)	307 (26)		157 (19)	
Parity (women only)				
0	77 (13)		34 (11)	
1 to 2	360 (60)		190 (59)	
3 to 8	163 (27)		97 (30)	
Menopausal status (women only)				
Post	349 (58)		221 (69)	
Pre	103 (17)		33 (10)	
Unknown	148 (25)		67 (21)	
Cotinine concentration				
<= 20 μg/L	690 (58)		439 (54)	
>20 μg/L to <50 μg/L	354 (29)		248 (31)	
>=50 μg/L to <200 μg/L	134 (11)		104 (13)	
>=200 μg/L	22 (2)		18 (2)	
Self-reported secondhand smoke exposure at home or work				
None	26 (2)		15 (2)	
Before age 50 (but not after)	334 (28)		212 (26)	
After age 50	840 (70)		582 (72)	
BMI				
< 25	479 (40)		228 (28)	
25 to < 30	523 (44)		366 (45)	
30 +	198 (16)		215 (27)	
Leisure-time physical activity				
No	465 (39)		370 (46)	
Yes	735 (61)		439 (54)	
Urine Cd concentration (μg/L)		0.19 (0.09, 0.34)		0.22 (0.11, 0.37)
Urine creatinine (g/L)		0.99 (0.47, 1.64)		1.08 (0.53, 1.66)
Osmolality (mOsM)		583 (312, 784)		602 (334, 795)

μg/L) or no secondhand smoke exposure (10.5 [2.7, 16.9] μg/L). Urinary cadmium concentrations at study baseline were similar between males and females among both the subcohort and AMI cases (Table 2).

The median urinary cadmium concentration among females who developed AMI was higher compared with the average for females in the subcohort, while the median cadmium concentrations among male AMI cases was similar to the median for males in the subcohort. Overall, males had higher concentrations of urinary creatinine and osmolality, as well as lower creatinine and osmolality standardized cadmium concentrations compared with females (Supplemental Table 1).

3.2. Urinary cadmium and AMI

We observed that the hazard ratio for AMI increased monotonically across quartiles of cadmium concentrations, reaching a fully-adjusted hazard ratio of 1.16 (95% CI: 0.86 – 1.56) in the highest versus lowest quartile (p-trend: 0.19) (Table 3). When considering urinary cadmium concentrations as a linear continuous variable, an interquartile range difference (0.19 μg/g) in creatinine-standardized cadmium concentrations was associated with a fully-adjusted hazard ratio of 1.02 (0.93, 1.12). Results were not materially different in minimally and fully-adjusted models. Models using spline functions of cadmium did not suggest a monotonic exposure–response function (Supplemental Fig. 1).

3.3. Sex-Specific relationship between cadmium and AMI

We did not find strong evidence to support an association between cadmium and AMI in either sex strata (Table 4; e.g., fully-adjusted hazard ratio (aHR) = 1.29; 95% CI: 0.80 – 2.08 for males and aHR = 1.00; 95% CI: 0.63 – 1.60 for females comparing the highest and lowest quartiles). Analyses considering cadmium as a linear continuous variable (males: aHR = 1.01; 95% CI: 0.82 – 1.26; females: aHR = 1.02; 95% CI: 0.92 – 1.12) or more flexibly using splines (Supplemental Fig. 1) did not provide evidence of an association between urinary cadmium and AMI for either of the sexes.

3.4. Sensitivity analyses

We performed multiple sensitivity analyses to evaluate the robustness of these results to different analytic choices. Results were not materially different in sensitivity analyses modeling values of cadmium below the LOD with LOD/sqrt 2 rather than with the quantified cadmium concentrations or in analyses trimming cadmium values within the 5th and 95th percentiles (Supplemental Table 2). However, results did differ somewhat depending on the approach used to adjust for variation in urine dilution. We observed stronger evidence for an association for creatinine-adjusted and osmolality-adjusted analyses (Supplemental Table 3). For example, we observed fully-adjusted hazard ratios of 1.51 (95% CI: 1.03 – 2.20) and 1.33 (95% CI: 0.93 – 1.90) comparing the highest versus lowest quartiles in creatinine adjusted and osmolality adjusted models, respectively, but fully-adjusted hazard ratios of 1.15 (95% CI: 0.88 – 1.51) and 1.00 (95% CI: 0.76 – 1.32) in models using covariate-adjusted creatinine standardization or osmolality standardization, respectively. Models with spline representations of creatinine adjusted cadmium concentrations suggested an association among both sexes combined and perhaps among women (Supplemental Figure 2). Nonetheless, in analyses considering cadmium as a linear continuous variable, there was not strong evidence to support an association regardless of the modeling approach, with adjusted hazard ratios per IQR ranging from 0.99 to 1.02.

Regardless of the approach to adjust for variation in urine dilution, we did not observe strong evidence of effect modification by sex (Supplemental Tables 4 and 5). The exclusion of prevalent diabetes cases at baseline and adjustment for incident diabetes or other CVD conditions did not materially change the results (Supplemental Table 6 and 7). The exclusion of participants with cotinine concentrations ≥ 200 μg/L, extreme creatinine concentrations, or cases of stroke or heart failure, also did not meaningfully change our results.

Table 2
Urinary cadmium, creatinine and osmolality concentrations by participant sex.

	Subcohort		AMI Cases	
	Female	Male	Female	Male
Total n (%)	600 (50)	600 (50)	321 (40)	488 (60)
	Median (25th, 75th)	Median (25th, 75th)	Median (25th, 75th)	Median (25th, 75th)
Cadmium (μg/L)	0.18 (0.08, 0.36)	0.20 (0.10, 0.33)	0.24 (0.10, 0.42)	0.21 (0.12, 0.34)
Creatinine (g/L)	0.65 (0.31, 1.26)	1.35 (0.80, 1.89)	0.75 (0.37, 1.30)	1.31 (0.75, 1.82)
Creatinine standardized Cadmium (μg/g creatinine)	0.28 (0.19, 0.40)	0.16 (0.11, 0.21)	0.32 (0.21, 0.43)	0.16 (0.11, 0.23)
Osmolality (mOsM)	447 (235, 704)	697 (460, 855)	470 (289, 716)	692 (437, 854)
Osmolality standardized Cadmium (μg/mOSM)	0.44 (0.26, 0.72)	0.30 (0.19, 0.46)	0.47 (0.30, 0.74)	0.33 (0.20, 0.50)

4. Discussion

In this case-cohort study of never smokers with up to 20 years of follow-up, we did not find strong evidence supporting an association between urinary cadmium and AMI. Recent reviews find growing evidence of cadmium exposure as a risk factor for different dimensions of CVD, including AMI (Tellez-Plaza et al., 2013b; Tinkov et al., 2018). Although prior studies have adjusted for smoking, few have been designed to evaluate whether cadmium is associated with AMI at levels consistent with populations who have never smoked tobacco. Quantifying this relationship is critical information for policy-makers considering the value of regulating environmental sources of cadmium exposure. Our findings suggest that cadmium is not a substantial risk factor for AMI among never-smokers at exposures corresponding to urinary levels generally below 0.5 μg/g creatinine. However, we cannot exclude the possibility of potentially important but relatively small effect sizes among this non-smoking population.

Cadmium's effects on the vascular endothelium have been known for decades, and a number of animal (murine and bovine) and cellular studies have suggested a link between cadmium and atherosclerosis and hypertension (Prozialeck et al., 2006). Cadmium can induce release of a variety of inflammatory markers (Knoflach et al., 2011), stimulate the release of antithrombolytic agents and facilitate the adhesion of leukocytes and platelets to the endothelium (Hernández and Macia, 1996; Kaji et al., 1994; Yamamoto et al., 1993), and enhance production of extracellular matrix components that increase blood vessel stiffness (Abraham et al., 2000; Fujiwara et al., 1998; Jeong et al., 2004). However, the internal dose of cadmium that will elicit these effects in humans has not yet been characterized.

Prior studies have mostly (Barregard et al., 2016; Menke et al., 2009; Tellez-Plaza et al., 2012; 2013a) but not always (Nawrot et al., 2008; Jeong et al., 2020) reported positive associations between cadmium biomarkers and CHD or ischemic heart disease. Two prospective studies report consistent positive association between cadmium biomarkers and incident AMI or CHD when adjusting for tobacco use, and among never smokers in stratified analyses (Barregard et al., 2016; Tellez-Plaza et al., 2013a). For example, in the Malmö Diet and Cancer Study, Barregard et al. (2016) reported a hazard ratio of 1.7 (95% CI: 1.1 – 2.7) for AMI when comparing participants in the highest quartile of blood cadmium with participants in the lowest quartile, while adjusting models for

Table 3
Adjusted hazard ratio (HR) for risk of AMI per interquartile range difference and quartile of urinary cadmium concentration (creatinine standardized).

U-Cd (μg/g creatinine)	Total	Cases (n)	Urinary Cd Median (μg/g creatinine)	Minimally-adjusted HR [a] (95% CI)	Fully-adjusted HR [b] (95% CI)	Trend Test P-value
Continuous [c]	1948	809		1.02 (0.94–1.12)	1.02 (0.93–1.12)	
Quartile 1: less than or equal to 0.13	489	207	0.097	Ref	Ref	0.19
Quartile 2: greater than 0.13 to 0.20	459	183	0.17	0.90 (0.69–1.17)	0.91 (0.69–1.19)	
Quartile 3: greater than 0.20 to 0.32	502	213	0.25	1.10 (0.85–1.44)	1.11 (0.84–1.45)	
Quartile 4: greater than 0.32	498	206	0.43	1.16 (0.86–1.55)	1.16 (0.86 –1.56)	

[a] Minimally-adjusted model includes sex.
[b] Fully-adjusted model includes: sex (categorical), BMI (continuous), education (categorical), and cotinine (continuous).
[c] Continuous rate ratio for an interquartile range (IQR) = 0.19 μg/g difference in creatinine standardized urinary cadmium concentration. Interquartile range and creatinine standardized cadmium quartiles are based on concentrations among subcohort members.

Table 4
Fully-adjusted hazard ratio (HR) for risk of AMI per interquartile range difference and quartile of urinary cadmium concentration (creatinine standardized) in sex-stratified analyses.

	Male			Female		
U-Cd (μg/g creatinine)	Total N	Cases n	Fully-adjusted HR (95% CI)	Total N	Cases n	Fully-adjusted HR (95% CI)
Continuous [a]	1040	488	1.01 (0.82–1.26)	908	321	1.02 (0.92–1.12)
Quartile 1: less than or equal to 0.13	384	172	Ref	105	35	Ref
Quartile 2: greater than 0.13 to 0.20	316	143	0.97 (0.71–1.32)	143	40	0.72 (0.41–1.27)
Quartile 3: greater than 0.20 to 0.32	252	125	1.15 (0.83–1.60)	250	88	1.04 (0.63–1.71)
Quartile 4: greater than 0.32	88	48	1.29 (0.80–2.08)	410	158	1.00 (0.63–1.60)

Fully-adjusted model includes: BMI (continuous), education (categorical), and cotinine (continuous). Female model also adjusted for menopause status.
P-value for sex by cadmium product term in fully-adjusted model: p = 0.30.
[a] Continuous HR for an interquartile range (IQR) = 0.19 μg/g creatinine difference. Interquartile range and creatinine standardized cadmium quartiles are based on concentrations among subcohort members.

tobacco smoking. In stratified analyses, among participants who never smoked, the authors reported a hazard ratio of 2.4 (95% CI: 1.1 – 5.4) for AMI when comparing those in the highest versus lowest quartile of blood cadmium, but there were only seven cases in the highest quartile. Similarly, in the Strong Heart Study, Tellez-Plaza et al. (2013a) reported that higher concentrations of urinary cadmium were associated with incident CHD when adjusting for demographics, smoking status, and cumulative smoking dose (aHR = 1.22 comparing 80th [1.62 μg/g] with 20th [0.55 μg/g] percentile of log-transformed creatinine-standardized cadmium concentrations; 95% CI: 1.08 – 1.38). These results were consistent with those reported among the Strong Heart Study group of never smokers with 243 CHD cases (HR = 1.16 comparing 80th with 20th percentile; 95% CI: 0.96, 1.39). While the mean participant age and follow-up duration in these studies were similar, neither are directly comparable to our analysis. Specifically, Barregard et al. used blood-cadmium while Tellez-Plaza et al. used the broader CHD outcome and, on average, participants had higher urinary cadmium concentrations. These two studies, along with a study of cardiovascular mortality using NHANES data (Tellez-Plaza et al., 2012), were included in a recent *meta*-analysis by Chowdhury et al., 2018 that reported a relative risk for CHD of 1.43 (95% CI: 1.12, 1.84) among never-smoking participants in the highest compared to lowest third of cadmium concentrations (Chowdhury et al., 2018). Our results, based on 809 cases of AMI, add to this existing literature and provide little evidence that at lower concentrations of cadmium, commonly observed among never smokers, higher urinary cadmium is associated with AMI.

In our analysis we assessed urinary biomarkers of cadmium exposure. Epidemiologic studies of cadmium benefit from the availability of well-established and validated biomarkers of exposure in urine or blood, but not in plasma because cadmium is concentrated in red blood cells (Yuan et al., 2017). Following exposure, cadmium accumulates in the kidneys where it remains for many years (half-life: 10–30 years) (Amzal et al., 2009; Fowler et al., 2015). A small portion of cadmium is continuously but slowly excreted in urine (Fowler et al., 2015). Therefore, urinary concentrations of cadmium provide estimates of long-term exposure while blood cadmium reflects a combination of both long-term and more recent exposures (Nordberg, 2009). As recently reviewed (Vacchi-Suzzi et al., 2016), most studies examining temporal variability of urinary cadmium suggest that a single spot urine sample is a stable estimate (e.g., see Akerstrom et al., 2014; Arisawa et al., 1997; Meliker et al., 2019; Smolders et al., 2014; Vacchi-Suzzi et al., 2017). At low levels of urine cadmium, we might expect urinary excretion of proteins and urinary flow rate to contribute to the variability of creatinine-adjusted urine cadmium in spot urine samples making one-time measures less representative; however, we previously reported an ICC = 0.78 for a small cohort with low levels of urine cadmium (median = 0.21 μg/g) (Vacchi-Suzzi et al., 2017). It is also possible that blood cadmium may be a more relevant measure of exposure if cadmium is linked with atherosclerosis although this remains to be determined.

When using urine biomarkers, it is essential to control for differences in urine dilution across participant samples, with many options available but little consensus on the optimal approach. The most widely used approaches account for variation in urine dilution by considering urinary creatinine concentrations, even though creatinine concentrations can be influenced by muscle mass (Suwazono et al., 2005). In our analysis we also measured osmolality – an index of the concentration of osmotically active particles, particularly chloride, sodium, urea, and potassium – which is strongly correlated (ρ = 0.75) with specific gravity (Imran et al., 2010; Voinescu et al., 2002). Various statistical approaches have also been applied to adjust for variations in the urine dilution measure. Traditionally investigators divide by the measure (e.g., cadmium/creatinine), but some have argued for statistical adjustment in a regression model (Barr et al., 2005) or adjustment for a covariate-adjusted density measure (O'Brien et al., 2016). We chose cadmium/creatinine for our primary analysis, both because it is the most commonly used method in prior studies and because of the high degree of temporal stability in the measure (intraclass correlation coefficient greater than 0.80) in spot samples collected years apart (Meliker et al., 2019; Vacchi-Suzzi et al., 2016). However, we also report results from a series of sensitivity analyses in order to assess the robustness of our results across different methods of adjusting for variation in urine dilution and found that results were generally similar. When cadmium was

treated as a linear continuous variable, a range of point estimates in fully-adjusted models (aHR = 0.97–1.02) was observed for the IQR increment across the analyses. Results were somewhat more variable when considering quartiles of cadmium, and the upper quartile was generally elevated more so in the creatinine-adjusted model (Supplemental Table 3). Resolving the best strategy for urine dilution adjustment is an important and active area of inquiry for epidemiologic studies of urine biomarkers.

CHD tends to develop later in women compared with men (Maas and Appelman, 2010), and presentation of risk factors can also vary by sex (Grundtvig et al., 2009; Keteepe-Arachi and Sharma, 2017). Most studies have not shown sex differences in the relationship between cadmium and CVD outcomes (as reviewed, Tellez-Plaza et al., 2013b; Tinkov et al., 2018), however, one study reported associations with CVD and CHD mortality in men but not in women (Menke et al., 2009), and ours is the first study to soundly investigate sex differences among never smokers. More research is needed to better understand potential sex differences in relationships between cadmium and CVD health outcomes, especially in light of higher levels of cadmium in women. Sex differences in cadmium levels are thought to reflect lower iron levels, which may increase cadmium absorption in the digestive tract (Nishijo et al., 2004; Vahter et al., 2007). Future studies may also consider using multiple methods to account for variations in urine dilution due to the sex differences in creatinine concentrations and osmolality.

Our study has several limitations. We adjusted statistical models for covariates that we selected *a priori,* but residual confounding due to other characteristics that we did not control for is always possible. It is also possible that our results are impacted by selection bias due to non-participation in the DCH cohort. Like many studies, participants tended to be better educated, wealthier, more often in white-collar jobs, and more likely to be married than their non-participating counterparts (Tjønneland et al., 2007). Furthermore, we identified incident AMI using national registries and based on diagnosis codes, which could result in some misclassification of the outcome. We were not able to examine the pathophysiologic etiology (atherosclerosis vs other) of the AMI diagnoses in detail due to the lack of information extracted from the NPR. Given the documented high specificity of our approach to outcome assessment, we expect this misclassification to increase the variance in the estimated relationship of urinary cadmium and AMI but not considerably bias the results. We also did not identify prevalent AMI among subcohort members who did not have an additional diagnosis of AMI in the NPR during the follow-up period. However, the prevalence of AMI at baseline in the DCH cohort at baseline was<2%; therefore, we expect this misclassification to be small. We were powered to detect a hazard ratio of 1.3 in the main analysis by quartiles, however we observed a hazard ratio of 1.16 with wide confidence intervals. Therefore, we cannot exclude the possibility that with a larger sample size our inferences may have been different. Lastly, it is possible that for some participants in the subcohort and larger DCH cohort death may have precluded AMI, and therefore could be considered a competing risk that would bias our results. However, we do not expect this to substantially impact our results since few subcohort members were censored due to death and the median follow-up time for subcohort members who died was slightly longer than the follow-up duration for participants who were AMI cases (median [25th, 75th] = 12.2 [7.1, 16.6] years).

Our study sample is predominantly non-Hispanic white, and therefore the results may not be generalizable to other racial and ethnic groups. We expect our results to be generalizable to other Scandinavian and non-Hispanic white populations of never smokers. Cadmium exposure among this sample of never smokers is likely to be similar to, or lower than, levels reported for never smokers in the United States (Riederer et al., 2013).

Our study also has several strengths. We used the urine cadmium biomarker as a proxy for exposure. This biomarker is an indicator of body burden in the kidney, is temporally stable, and reflects key sources of exposure such as those from diet or cigarettes (Amzal et al., 2009; Fowler et al., 2015; Vacchi-Suzzi et al., 2016). Estimating exposure to key dietary sources of cadmium via a food frequency questionnaire has proved challenging because of the diverse foodstuffs that accumulate cadmium from application of phosphate fertilizers and sewage sludge (Singh, 1994; Vacchi-Suzzi et al., 2015), further pointing to the utility of the urinary cadmium biomarker as a proxy for exposure. Another strength of our study is that we used multiple approaches to account for urine dilution. Additionally, we used data from a prospective, population-based cohort with a long follow-up period. This prospective design and use of national registry for case identification can reduce the likelihood that disease status would influence participation or that differences in participant characteristics would impact the reporting of disease status. Furthermore, urine was collected before diagnosis. We also assessed tobacco smoking behaviors and secondhand tobacco smoke exposure history at baseline, which are potential confounders.

4.1. Conclusions

The results of this prospective study among people who never smoked did not provide strong evidence to support the presence of an association between higher cadmium exposure and AMI at levels of urine cadmium < 0.5 μg/g creatinine; however, we also cannot rule out the possibility that there is a small association that we were underpowered to detect. Future studies considering sex differences in the relationship between cadmium and CHD or AMI may want to use multiple methods to account for variations in urine dilution given the observed sex-differences in cadmium, creatinine, and osmolality.

CRediT authorship contribution statement

Clara G. Sears: Methodology, Investigation, Writing - original draft, Project administration. **Aslak Harbo Poulsen:** Methodology, Investigation, Writing - review & editing. **Melissa Eliot:** Software, Formal analysis, Visualization. **Chanelle J. Howe:** Methodology, Writing - review & editing, Funding acquisition. **Katherine A. James:** Methodology, Writing - review & editing. **James M. Harrington:** Methodology, Validation, Investigation, Writing - original draft, Funding acquisition. **Nina Roswall:** Data curation, Writing - review & editing. **Kim Overvad:** Writing - review & editing. **Anne Tjønneland:** Writing - review & editing. **Ole Raaschou-Nielsen:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Gregory A. Wellenius:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Funding acquisition. **Jaymie Meliker:** Conceptualization, Methodology, Investigation, Writing - original draft, Project administration, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Dr. Wellenius has received consulting fees from the Health Effects institute (Boston, MA) and serves as a paid visiting scientist at Google Research. The other co-authors declare no known financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Funding

Funding for this work came from NIEHS R01ES026614. The establishment and running of The Diet, Cancer and Health cohort was funded by the Danish Cancer Society.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.envint.2021.106428.

References

Abraham, D., Hofbauer, R., Schäfer, R., Blumer, R., Paulus, P., Miksovsky, A., et al., 2000. Selective downregulation of VEGF-A(165), VEGF-R(1), and decreased capillary density in patients with dilative but not ischemic cardiomyopathy. Circ Res 87 (8), 644–647. https://doi.org/10.1161/01.res.87.8.644.

Akerstrom, M., Barregard, L., Lundh, T., Sallsten, G., 2014. Variability of urinary cadmium excretion in spot urine samples, first morning voids, and 24 h urine in a healthy non-smoking population: implications for study design. J Expo Sci Environ Epidemiol 24 (2), 171–179. https://doi.org/10.1038/jes.2013.58.

Amzal, B., Julin, B., Vahter, M., Wolk, A., Johanson, G., Akesson, A., 2009. Population toxicokinetic modeling of cadmium for health risk assessment. Environ Health Perspect 117 (8), 1293–1301. https://doi.org/10.1289/ehp.0800317.

Arisawa, K., Nakano, A., Honda, S., Saito, H., 1997. Reproducibility of urinary beta 2-microglobulin and cadmium excretion among residents in a cadmium-polluted area during a 3-year period. Toxicol Lett 91 (2), 147–152. https://doi.org/10.1016/s0378-4274(97)03884-8.

Barlow, W.E., Ichikawa, L., Rosner, D., Izumi, S., 1999. Analysis of case-cohort designs. J Clin Epidemiol 52 (12), 1165–1172.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113 (2), 192–200. https://doi.org/10.1289/ehp.7337.

Barregard, L., Sallsten, G., Fagerberg, B., Borné, Y., Persson, M., Hedblad, B., et al., 2016. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish adults: The Malmö Diet and Cancer Study. Environ Health Perspect 124 (5), 594–600. https://doi.org/10.1289/ehp.1509735.

Chowdhury, R., Ramond, A., O'Keeffe, L.M., Shahzad, S., Kunutsor, S.K., Muka, T., et al., 2018. Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis. BMJ 362, k3310. https://doi.org/10.1136/bmj.k3310.

Domingo-Relloso, A., Grau-Perez, M., Briongos-Figuero, L., Gomez-Ariza, J.L., Garcia-Barrera, T., Dueñas-Laita, A., et al., 2019. The association of urine metals and metal mixtures with cardiovascular incidence in an adult population from Spain: the Hortega Follow-Up Study. International Journal of Epidemiology 48 (6), 1839–1849. https://doi.org/10.1093/ije/dyz061.

Dong, F., Guo, F., Li, L., Guo, L., Hou, Y., Hao, E., et al., 2014. Cadmium induces vascular permeability via activation of the p38 MAPK pathway. Biochem Biophys Res Commun 450 (1), 447–452. https://doi.org/10.1016/j.bbrc.2014.05.140.

Fujiwara, Y., Watanabe, S., Kaji, T., 1998. Promotion of cultured vascular smooth muscle cell proliferation by low levels of cadmium. Toxicol Lett 94 (3), 175–180. https://doi.org/10.1016/S0378-4274(98)00005-8.

Fowler, B.A., Alexander, J., Oskarsson, A., 2015. Toxic Metals in Food; Cadmium. In: Nordberg, G.F., Fowler, G.A., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals. Academic Press/Elsevier, Amsterdam and Boston.

Grundtvig, M., Hagen, T.P., German, M., Reikvam, A., 2009. Sex-based differences in premature first myocardial infarction caused by smoking: twice as many years lost by women as by men. Eur J Cardiovasc Prev Rehabil Off J Eur Soc Cardiol Work Groups Epidemiol Prev Card Rehabil Exerc Physiol 16 (2), 174–179. https://doi.org/10.1097/HJR.0b013e328325d7f0.

Helweg-Larsen, K., 2011. The Danish Register of Causes of Death. Scand J Public Health 39 (Suppl 7), 26–29. https://doi.org/10.1177/1403494811399958.

Hernán, M.A., 2002. Causal knowledge as a prerequisite for confounding evaluation: An application to birth defects epidemiology. Am J Epidemiol 155 (2), 176–184. https://doi.org/10.1093/aje/155.2.176.

Hernández, M., Macia, M., 1996. Free peripheral sulfhydryl groups, CD11/CD18 integrins, and calcium are required in the cadmium and nickel enhancement of human-polymorphonuclear leukocyte adherence. Arch Environ Contam Toxicol 30, 437–443. https://doi.org/10.1007/BF00213393.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of Nnndetectable values. Appl Occup Environ Hyg 5 (1), 46–51. https://doi.org/10.1080/1047322X.1990.10389587.

Imran, S., Eva, G., Christopher, S., Flynn, E., Henner, D., 2010. Is specific gravity a good estimate of urine osmolality? J Clin Lab Anal 24, 426–430. https://doi.org/10.1002/jcla.20424.

International Agency for Research on Cancer (IARC). 1993. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. IARC Monographs on the Evaluation of Carcinogenic risk to Humans, vol. 58. IARC, Lyon, Fr. 444 pp.

Järup, L., Åkesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238 (3), 201–208. https://doi.org/10.1016/j.taap.2009.04.020.

Jeong, E.-M., Moon, C.-H., Kim, C.-S., Lee, S.H., Baik, E.J., Moon, C.K., et al., 2004. Cadmium stimulates the expression of ICAM-1 via NF-kappaB activation in cerebrovascular endothelial cells. Biochem Biophys Res Commun 320 (3), 887–892. https://doi.org/10.1016/j.bbrc.2004.05.218.

Jeong, J., Yun, S., Kim, M., Koh, Y.H., 2020. Association of blood cadmium with cardiovascular disease in Korea: from the Korea National Health and Nutrition Examination Survey 2008–2013 and 2016. Int J Environ Res Public Health 17 (6288). https://doi.org/10.3390/ijerph17176288.

Joensen, A.M., Jensen, M.K., Overvad, K., Dethlefsen, C., Schmidt, E., Rasmussen, L., et al., 2009. Predictive values of acute coronary syndrome discharge diagnoses differed in the Danish National Patient Registry. J Clin Epidemiol 62 (2), 188–194. https://doi.org/10.1016/j.jclinepi.2008.03.005.

Julin, B., Bergkvist, C., Wolk, A., Åkesson, A., 2013a. Cadmium in diet and risk of cardiovascular disease in women. Epidemiol Camb Mass 24, 880–885. https://doi.org/10.1097/EDE.0b013e3182a777c9.

Julin, B., Vahter, M., Amzal, B., Wolk, A., Berglund, M., Åkesson, A., 2011. Relation between dietary cadmium intake and biomarkers of cadmium exposure in premenopausal women accounting for body iron stores. Environ Health 10 (105). https://doi.org/10.1186/1476-069X-10-105.

Julin, B., Wolk, A., Thomas, L.D., Akesson, A., 2013b. Exposure to cadmium from food and risk of cardiovascular disease in men: a population-based prospective cohort study. Eur J Epidemiol 28, 837–840. https://doi.org/10.1007/s10654-013-9841-8.

Kaji, T., Ohkawara, S., Inada, M., Yamamoto, C., Sakamoto, M., Kozuka, H., 1994. Cadmium stimulation of glycosaminoglycan synthesis by cultured vascular endothelial cells: Comparison of various cell types. Biol Pharm Bull 17 (3), 454–457. https://doi.org/10.1248/bpb.17.454.

Keteepe-Arachi, T., Sharma, S., 2017. Cardiovascular disease in women: Understanding symptoms and risk factors. Eur Cardiol 12 (1), 10–13. https://doi.org/10.15420/ecr.2016:32:1.

Kim, S., 2016. Overview of cotinine cutoff values for smoking status classification. Int J Environ Res Public Health 13 (1236). https://doi.org/10.3390/ijerph13121236.

Knoflach, M., Messner, B., Shen, Y.H., Frotschnig, S., Liu, G., Pfaller, K., et al., 2011. Non-toxic cadmium concentrations induce vascular inflammation and promote atherosclerosis. Circ J 75, 2491–2495. https://doi.org/10.1253/circj.CJ-11-0196.

Lynge, E., Sandegaard, J.L., Rebolj, M., 2011. The Danish National Patient Register. Scand J Public Health 39 (Suppl 7), 30–33. https://doi.org/10.1177/1403494811401482.

Maas, A.H.E.M., Appelman, Y.E.A., 2010. Gender differences in coronary heart disease. Neth Heart J 18 (12), 598–602. https://doi.org/10.1007/s12471-010-0841-y.

Meliker, J.R., Vacchi-Suzzi, C., Harrington, J., Levine, K., Lui, L.-Y., Bauer, D.C., et al., 2019. Temporal stability of urinary cadmium in samples collected several years apart in a population of older persons. Int J Hyg Environ Health 222 (2), 230–234. https://doi.org/10.1016/j.ijheh.2018.10.005.

Menke, A., Muntner, P., Silbergeld, E.K., Platz, E.A., Guallar, E., 2009. Cadmium levels in urine and mortality among U.S. adults. Environ Health Perspect 117 (2), 190–196. https://doi.org/10.1289/ehp.11236.

Messner, B., Knoflach, M., Seubert, A., Ritsch, A., Pfaller, K., Henderson, B., et al., 2009. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. Arterioscler Thromb Vasc Biol 29, 1392–1398. https://doi.org/10.1161/ATVBAHA.109.190082.

Monrad, M., Ersbøll, A.K., Sørensen, M., Baastrup, R., Hansen, B., Gammelmark, A., et al., 2017. Low-level arsenic in drinking water and risk of incident myocardial infarction: A cohort study. Environ Res 154, 318–324. https://doi.org/10.1016/j.envres.2017.01.028.

Murata, I., Hirono, T., Saeki, Y., Nakagawa, S., 1970. Cadmium enteropathy, renal osteomalacia ("Itai Itai" disease in Japan). Bull Soc Int Chir 29 (1), 34–42.

Nawrot, T.S., Van Hecke, E., Thijs, L., Richart, T., Kuznetsova, T., Jin, Y., et al., 2008. Cadmium-related mortality and long-term secular trends in the cadmium body burden of an environmentally exposed population. Environ Health Perspect 116 (12), 1620–1628. https://doi.org/10.1289/ehp.11667.

Nishijo, M., Satarug, S., Honda, R., Tsuritani, I., Aoshima, K., 2004. The gender differences in health effects of environmental cadmium exposure and potential mechanisms. Mol Cell Biochem 255, 87–92. https://doi.org/10.1023/b:mcbi.0000007264.37170.39.

Nordberg, G.F., 2009. Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol 238 (3), 192–200. https://doi.org/10.1016/j.taap.2009.03.015.

O'Brien, K.M., Upson, K., Cook, N.R., Weinberg, C.R., 2016. Environmental chemicals in urine and blood: Improving methods for creatinine and lipid adjustment. Environ Health Perspect 124 (2), 220–227. https://doi.org/10.1289/ehp.1509693.

Oliveira, T.F., Batista, P.R., Leal, M.A., Campagnaro, B.P., Nogueira, B.V., Vassallo, D.V., et al., 2019. Chronic cadmium exposure accelerates the development of atherosclerosis and induces vascular dysfunction in the aorta of ApoE-/- Mice. Biol Trace Elem Res 187 (1), 163–171. https://doi.org/10.1007/s12011-018-1359-1.

Olsson, I.-M., Eriksson, J., Öborn, I., Skerfving, S., Oskarsson, A., 2005. Cadmium in food production systems: A health risk for sensitive population groups. Ambio 34 (4), 344–351. https://doi.org/10.1579/0044-7447-34.4.344.

Pearson, C.A., Lamar, P.C., Prozialeck, W.C., 2003. Effects of cadmium on E-cadherin and VE-cadherin in mouse lung. Life Sci 72 (11), 1303–1320. https://doi.org/10.1016/s0024-3205(02)02379-2.

Pedersen, C.B., 2011. The Danish Civil Registration System. Scand J Public Health 39 (7 Suppl), 22–25. https://doi.org/10.1177/1403494810387965.

Prozialeck, W.C., 2000. Evidence that E-cadherin may be a target for cadmium toxicity in epithelial cells. Toxicol Appl Pharmacol 164 (3), 231–249. https://doi.org/10.1006/taap.2000.8905.

Prozialeck, W.C., Edwards, J.R., Woods, J.M., 2006. The vascular endothelium as a target of cadmium toxicity. Life Sci 79 (16), 1493–1506. https://doi.org/10.1016/j.lfs.2006.05.007.

Riederer, A.M., Belova, A., George, B.J., Anastas, P.T., 2013. Urinary cadmium in the 1999–2008 U.S. National Health and Nutrition Examination Survey (NHANES). Environ Sci Technol 47, 1137–1147. https://doi.org/10.1021/es303556n.

Rothman, K.J., Greenland, S., Lash, T.L., 2008. Modern Epidemiology, third ed. Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia, PA.

Sahmoun, A.E., Case, L.D., Jackson, S.A., Schwartz, G.G., 2005. Cadmium and prostate cancer: a critical epidemiologic analysis. Cancer Invest 23 (3), 256–263. https://doi.org/10.1081/cnv-200055968.

Schmidt, M., Pedersen, L., Sørensen, H.T., 2014. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 29 (8), 541–549. https://doi.org/10.1007/s10654-014-9930-3.

Schmidt, M., Schmidt, S.A.J., Sandegaard, J.L., Ehrenstein, V., Pedersen, L., Sørensen, H. T., 2015. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 7, 449–490. https://doi.org/10.2147/CLEP.S91125.

Singh, B.R., 1994. Trace element availability to plants in agricultural soils, with special emphasis on fertilizer inputs. Environ Rev 2 (2), 133–146. https://doi.org/10.1139/a94-009.

Smolders, R., Koch, H.M., Moos, R.K., Cocker, J., Jones, K., Warren, N., et al., 2014. Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 1: metals. Toxicol Lett 231 (2), 249–260. https://doi.org/10.1016/j.toxlet.2014.08.014.

Suwazono, Y., Åkesson, A., Alfvén, T., Järup, L., Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10 (2–3), 117–126. https://doi.org/10.1080/13547500500159001.

Tellez-Plaza M, Guallar E, Howard BV, Umans JG, Francesconi KA, Goessler W, et al. 2013a. Cadmium exposure and incident cardiovascular disease: Epidemiology 24: 421–429; doi:10.1097/EDE.0b013e31828b0631.

Tellez-Plaza, M., Jones, M.R., Dominguez-Lucas, A., Guallar, E., Navas-Acien, A., 2013b. Cadmium exposure and clinical cardiovascular disease: A systematic review. Curr Atheroscler Rep 15 (356). https://doi.org/10.1007/s11883-013-0356-2.

Tellez-Plaza, M., Navas-Acien, A., Menke, A., Crainiceanu, C.M., Pastor-Barriuso, R., Guallar, E., 2012. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. Environ Health Perspect 120 (7), 1017–1022. https://doi.org/10.1289/ehp.1104352.

Thiébaut, A.C.M., Bénichou, J., 2004. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. Statist Med 23, 3803–3820. https://doi.org/10.1002/sim.2098.

Tinkov, A.A., Filippini, T., Ajsuvakova, O.P., Skalnaya, M.G., Aaseth, J., Bjørklund, G., et al., 2018. Cadmium and atherosclerosis: A review of toxicological mechanisms and a meta-analysis of epidemiologic studies. Environ Res 162, 240–260. https://doi.org/10.1016/j.envres.2018.01.008.

Tjønneland, A., Olsen, A., Boll, K., Stripp, C., Christensen, J., Engholm, G., et al., 2007. Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: A population-based prospective cohort study of 57,053 men and women in Denmark. Scand J Public Health 35 (4), 432–441. https://doi.org/10.1080/14034940601047986.

Vacchi-Suzzi, C., Eriksen, K.T., Levine, K., McElroy, J., Tjønneland, A., Raaschou-Nielsen, O., et al., 2015. Dietary intake estimates and urinary cadmium levels in Danish postmenopausal women. PloS One 10 (9), e0138784. https://doi.org/10.1371/journal.pone.0138784.

Vacchi-Suzzi, C., Kruse, D., Harrington, J., Levine, K., Meliker, J.R., 2016. Is urinary cadmium a biomarker of long-term exposure in humans? A review. Curr Environ Health Rep 3 (4), 450–458. https://doi.org/10.1007/s40572-016-0107-y.

Vacchi-Suzzi, C., Porucznik, C.A., Cox, K.J., Zhao, Y., Ahn, H., Harrington, J.M., et al., 2017. Temporal variability of urinary cadmium in spot urine samples and first morning voids. J Expo Sci Environ Epidemiol 27 (3), 306–312. https://doi.org/10.1038/jes.2016.28.

Vahter, M., Åkesson, A., Lidén, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ Res 104 (1), 85–95. https://doi.org/10.1016/j.envres.2006.08.003.

VanderWeele, T.J., 2019. Principles of confounder selection. Eur J Epidemiol 34 (3), 211–219. https://doi.org/10.1007/s10654-019-00494-6.

Voinescu, G.C., Shoemaker, M., Moore, H., Khanna, R., Nolph, K.D., 2002. The relationship between urine osmolality and specific gravity. Am J Med Sci 323 (1), 39–42. https://doi.org/10.1097/00000441-200201000-00007.

Wang, X., Dong, F., Wang, F., Yan, S., Chen, X., Tozawa, H., et al., 2018. Low dose cadmium upregulates the expression of von Willebrand factor in endothelial cells. Toxicol Lett 290, 46–54. https://doi.org/10.1016/j.toxlet.2018.03.020.

Woods, J.M., Leone, M., Klosowska, K., Lamar, P.C., Shaknovsky, T.J., Prozialeck, W.C., 2008. Direct antiangiogenic actions of cadmium on human vascular endothelial cells. Toxicol in Vitro 22 (3), 643–651. https://doi.org/10.1016/j.tiv.2007.12.009.

Yamamoto, C., Kaji, T., Sakamoto, M., Kozuka, H., 1993. Cadmium stimulation of plasminogen activator inhibitor-1 release from human vascular endothelial cells in culture. Toxicology 83 (1–3), 215–223. https://doi.org/10.1016/0300-483X(93)90103-Y.

Yuan, Y., Xiao, Y., Feng, W., Liu, Y., Yu, Y., Zhou, L., et al., 2017. Plasma Metal Concentrations and Incident Coronary Heart Disease in Chinese Adults: The Dongfeng-Tongji Cohort. Environ Health Perspect 125 (10), 107007. https://doi.org/10.1289/EHP1521.

Zavon, M.R., Meadows, C.D., 1970. Vascular sequelae to cadmium fume exposure. Amer Ind Hyg Assoc 31, 180–182.