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Cadmium exposure and incidence of heart failure and atrial fibrillation
- Results from the Malmö Diet and Cancer Study

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Short running title: Cadmium exposure and heart failure and atrial fibrillation

ABSTRACT

Objectives

Cadmium is a non-essential toxic metal with multiple adverse health effects. Cadmium has shown to be associated with cardiovascular diseases, but few studies have investigated heart failure (HF) and none of them atrial fibrillation (AF). We examined whether cadmium exposure is associated with incidence of HF or AF.

Design

A prospective, observational cohort study with a 17-year follow-up.

Setting

The city of Malmö, Sweden.

Participants

Blood cadmium levels were measured in 4378 subjects without a history of HF or AF (aged 46 to 67 years, 60 % women), who participated in the Malmö Diet and Cancer (MDC) study during 1992-1994.

Primary and secondary outcome measures

Incidence of HF and AF were identified from the Swedish hospital discharge register.

Results

143 subjects (53% men) were diagnosed with new-onset HF and 385 individuals (52% men) were diagnosed with new-onset AF during follow-up for 17 years. Blood cadmium in the sex-specific 4th quartile of the distribution was significantly associated with incidence of HF. Hazards ratio, (HR, 4th vs 1st quartile) was 2.64 (95% CI: 1.60-4.36), adjusted for age; and 1.95 (1.02-3.71) after adjustment also for conventional risk factors and biomarkers. Blood cadmium level was not significantly associated with risk of incident AF.

Conclusion Blood cadmium levels in the 4th quartile were associated with increased incidence of HF in this cohort with comparatively low exposure to cadmium. Incidence of AF was not associated with cadmium.

Keywords: blood cadmium levels, heart failure, atrial fibrillation, cohort study, epidemiology

Article summary

Article focus

- Cadmium has shown to be associated with cardiovascular diseases, but few studies have investigated heart failure (HF) and none of them atrial fibrillation (AF).
- The research question was whether cadmium exposure is associated with incidence of HF or AF.

Key messages

- Blood cadmium levels in the 4th quartile were associated with increased incidence of HF in this cohort with comparatively low exposure to cadmium.
- Incidence of AF was not associated with cadmium.

Strengths and limitations of this study

- A large number of subjects and events during a long follow-up period. The cardiovascular endpoints were retrieved from national hospital registers in Sweden with high validity for HF and AF.
- We were unable to detect cases of HF that were diagnosed in the out-patient setting or undiagnosed. Therefore, it is unclear whether the present results could be generalized to less severe forms of HF.

INTRODUCTION

Cadmium is a non-essential toxic metal, which can be absorbed through smoking, diet and occupational exposure in certain industries. The elimination of cadmium from the human body is a slow process - the biological half-life has been estimated to 10-40 years [1, 2]. Multiple adverse health effects of cadmium exposure have been reported, such as increased risk of renal dysfunction [1, 3, 4, 5], osteoporosis [5, 6, 7] and cancer [8, 9, 10]. Recent studies also found that cadmium was associated with the prevalence and future growth of atherosclerotic plaques [11] and with cardiovascular disease (CVD) [12, 13]. However, to our knowledge, only two studies, both in US, investigated the association between cadmium and heart failure (HF)[12, 14] Peters et al conducted a cross-sectional study in a sample from the US general population, and found both blood and urinary cadmium to be associated with self-reported prevalence of HF, and the association was similar among men and women [14]. Tellez-Plaza et al reported an association between urinary cadmium and HF in a prospective cohort study of 3348 American Indian adults aged 45-74 years [12]. There is experimental support for a direct toxic effect of cadmium on the heart muscle cells and possibly also the cardiac conduction system [15, 16, 17, 18, 19, 20] . In humans, autopsy studies have shown that the myocardial content of cadmium increases with age, up to late middle age and mirrors the exposure to cadmium [21].

To our best knowledge, there is no previous study exploring the relationship between cadmium and incidence of atrial fibrillation (AF). AF is a comorbid condition among HF patients and they share risk factors such as hypertension, diabetes, ischemic heart disease and valvular disease [22, 23, 24].

Thus, the purpose of this population-based study was to explore whether elevated blood cadmium levels are associated with incidence of HF or AF in middle-aged subjects.

METHODS

Study population

The Malmö Diet and Cancer (MDC) is a prospective cohort study from the city of Malmö, Sweden with baseline examination between March 1991 and September 1996 [25, 26]. Participants filled out a self-administered questionnaire, underwent physical examination and sampling of peripheral venous blood. The participant rate was 41%. A random sample of the MDC cohort, the MDC cardiovascular cohort (MDC-CC) (N=6,103), was invited to take part in a study of the epidemiology of carotid artery disease between 1991 to 1994 [27]. Blood samples were donated by 5,543 of them after overnight fast, and cadmium could be measured in 4,952 participants (aged 46-68 years, 60% women). Individuals with history of HF (n=8) or AF (n=42) at baseline were excluded from the analysis of the respective outcomes. The final study population included 4 378 subjects after exclusion of subjects with missing values on conventional cardiovascular risk factors and biomarkers.

The study was approved by the regional Ethics Committee (LU51/90) and all participants provided written informed consent. The study complies with the Declaration of Helsinki.

Measurements and definitions

The self-administered questionnaire provided information on current use of antihypertensive, lipid- lowering, and anti-diabetic medication, smoking habits, marital status and educational status [25]. Waist circumference (in cm) was measured midway between the lowest rib margin and iliac crest. Blood pressure was measured once using a mercury-column sphygmomanometer after 10 minutes of rest in the supine position. Diabetes mellitus was defined as self-reported physician's diagnosis of diabetes, use of anti-diabetic medications or fasting whole blood glucose level greater than 109 mg/dL (e.g. ≥6.1 mmol/L). Subjects were

categorized into current smokers (those who smoked regularly or occasionally), former smokers or never-smokers. High alcohol consumption was defined as >40 g alcohol per day for men and >30 g per day for women. Marital status was categorized as being married or not. Educational level was divided into less than 9 years, 9 to 11 years, and 12 years or more of education.

Laboratory measurements

The blood cadmium concentrations were calculated using erythrocyte concentrations of cadmium adjusted for hematocrit. Cadmium was analyzed using inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS, Agilent Technologies). All samples were analyzed in three different rounds with external quality control samples included. Two QC samples were used (Seronorm™ Trace Elements Whole Blood L-1, Lot No. 1103128 and Seronorm™ Trace Elements Whole Blood L-2, Lot No.1103129, Sero AS, Billingstad Norway). The results from all rounds versus recommended limits were 0.34 ± 0.02 µg /L (N=70) versus 0.32-0.40 µg/L and 5.7 ±0.18 µg/L (N=70) versus 5.4-6.2 µg/L. The results were similar for the three different rounds. Furthermore 20 erythrocyte samples (range 0.2-0.96 µg/L) were used to compare with another laboratory (Occupational and Environmental Medicine, Lund, Sweden). The results showed good agreement with a Pearson's correlation coefficient of 0.99 and a slope of 1.04 (standard error 0.04).

Measurements of high- density lipoprotein (HDL) were performed on fresh blood samples according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. Low- density lipoprotein cholesterol (LDL) concentration was calculated according to Friedewald's formula. Plasma biomarkers were measured from fasting plasma samples that had been frozen at −80°C immediately after collection. High-sensitivity C-

reactive protein (hsCRP) was analyzed using the Tina-quant® CRP latex assay (Roche Diagnostics, Basel, Switzerland). Plasma creatinine (µmol/L) was analyzed with the Jaffé method, standardized according to the International Standardization with isotope dilution mass spectometry (IDMS).

Follow-up and ascertainment of cardiovascular events

All cases were retrieved from the Swedish hospital discharge register. This register has been operating in south of Sweden since 1970 and became nationwide in 1987. HF was defined according to International Classification of Diseases (ICD) code 427.00, 427.10 and 428.99 (ICD-8); 428 (ICD-9); and I50, I11 (ICD-10) as the primary diagnosis [28]. AF was defined using diagnosis codes 427.92 (ICD-8), 427D (ICD-9) and I48 (ICD-10) [29]. Validation studies have shown that the Swedish hospital discharge register has a case validity of 95% for a primary diagnosis of HF [28] and a case validity of 98% for AF[29].

Statistical analysis

Individuals with history of HF or AF at baseline were excluded from the respective analyses. All subjects were followed from the baseline examination until emigration death or December 31, 2010, whichever came first. In addition, follow-up time was stopped at a first event of HF for the study of incidence of HF, and at a first event of AF for the study of incidence of AF. hsCRP showed a right-skewed distribution and was log-transformed. Cross-sectional relations of sex-specific cadmium quartiles (i.e., with the same proportions of men and women in each quartile) to cardiovascular risk factors were assessed using one-way ANOVA for continuous variables and logistic regression for dichotomous variables. Cox proportional hazards regression was used to examine the association between cadmium (in sex-specific quartiles) and incidence of HF or AF. Hazard ratios (HR), with 95% confidence intervals (CI) were

calculated. The basic model was adjusted for age. In model 2, we also adjusted for other established cardiovascular risk factors systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking habits, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status. The fit of the proportional hazards model was checked visually by plotting the incidence rates over time and by entering time-dependent variables into the model. Possible interactions between cadmium and cardiovascular risk factors on incident HF or AF were explored by introducing interaction terms in the multivariate model. The Kaplan-Meier curve was used to illustrate incidence of HF or AF in relation to cadmium. The association between incident HF and cadmium level was also assessed using restricted cubic spline with 5 knots of blood cadmium, adjusted for sex and age. $P<0.05$ was considered statistically significant. All analyses were performed using IBM SPSS version 20 (IBM Corp.) or Stata software version 12.0 (StataCorp).

RESULTS

Baseline characteristics

Cardiovascular risk factors at the baseline examination in relation to the sex-specific quartiles of cadmium are presented in Table 1. Age, hsCRP, HDL, plasma creatinine, systolic blood pressure, history of diabetes, history of a coronary event, current smoking, high alcohol intake, being unmarried and low educational level were significantly associated with the cadmium level, Table 1.

Incidence of first HF or AF in relation to cadmium

During a mean follow-up of 16.8 years, 143 subjects (53% men) were diagnosed with new-onset HF. A total of 385 individuals (52% men) were diagnosed with new-onset AF during the follow-up.

Subjects in the 4th compared to the 1st quartile of cadmium had a significantly higher risk for incident HF (HR: 2.64, 95% CI: 1.60-4.36) after adjustment for age, Table 2 and Figure 1. This association remained significant after further adjustment for possible confounders, HR 1.95 (1.02-3.72). However, the relationship across quartiles of cadmium was non-linear (*p* for trend 0.21). The restricted cubic spline model of age and sex adjusted relative risk of incident HF across cadmium level, is shown in Figure 2. The risk for HF was not linear across the cadmium level, and 4th quartile of blood cadmium (> 0.49 µg/L) marked for increased risk for HF.

Elevated blood cadmium level was not significantly associated with increased incidence of AF, when adjusted for age (HR: 1.25, 0.93-1.68), Table 2. The associations were further attenuated when adjusting for other risk factors.

In the multivariate adjusted model for incident HF, age, use of blood pressure-lowering medication, diabetes, waist circumference, hsCRP and history of a coronary event were independently associated with an increased risk for HF. Age, waist circumference, use of lipid-lowering medication, antihypertensive medication and low LDL were significantly associated with AF in the multivariate analysis. No significant interaction was observed between cadmium level and other cardiovascular risk factors on incident HF or AF, respectively.

Sensitivity analysis was performed separately for men and women to examine the effects of cadmium on incidence of HF. The significant association between cadmium and incident HF

was only found among men, Table 3. Additional adjustment for menopausal status in women did not change the association, data not shown.

Since cadmium exposure is higher in smokers, we also performed a separate analysis of incidence of HF in never smoking subjects. The point-estimate was similar to that in all subjects, although not statistically significant (Table 4).

DISCUSSION

This large, prospective Swedish population-based study showed a significantly higher incidence of HF in subjects in the 4th quartile of blood cadmium compared to the 1st quartile. However, the relationship was non-linear and no increased risk was found for subjects in the 3rd quartile of cadmium. Our results extend data from two previous studies from the US which were based on self-reported HF in a cross-sectional study [14] and the incidence of HF in an ethnic minority with much higher levels of exposure to cadmium than in Sweden [12]. The results support the hypothesis of a relationship between increased cadmium levels and risk of HF. However, we found no significant association between blood cadmium and incidence of AF in the present study.

The mechanisms underlying the association between cadmium and HF are unclear. Cadmium has been associated with many cardiovascular risk factors, such as hypertension [30, 31], smoking and kidney disease [3, 4], which partly might explain the association between cadmium and HF. Cadmium was significantly associated with baseline blood pressure, current smoking, history of a coronary event and plasma creatinine in our study. However, cadmium was associated with increased risk of HF after adjustment for the baseline levels of these risk factors. It has been reported that cadmium could have cardio-toxic effects, which might

explain the association between cadmium and HF. Chronic exposure to cadmium causes degenerative changes to myocardial cells in rats, and in mice cardiac depressant effects following low dose cadmium exposure have been reported[15, 16]. Cadmium may affect tissue structure and integrity of the heart muscle by oxidative stress and increased reactive oxygen species (ROS) production or by DNA methylation [17, 18]. Cadmium also affects cardiac conduction system by interfering with calcium-mediated physiologic and biochemical processes [19]. Blockade of L-type calcium channels and altered outward potassium current in ventricular myocytes were also observed in vivo [20, 32].

HF is a heterogeneous syndrome characterized by impairment of the heart to fill and/or eject blood commensurate with the metabolic needs of the body, resulting in pulmonary or venous congestion [33]. Traditionally HF has been associated with reduced left ventricular ejection fraction (HF with reduced ejection fraction – HFREF). During the last decades it has been widely recognized that HF also occurs with preserved ejection fraction (HFPEF)[34], characterized by normal left ventricular systolic function and abnormal diastolic function in combination with clinical signs or symptoms of HF [35]. The prevalence of HF increases strongly after middle age and is about 10% among those above the age of 75 years [34]. Recent studies have shown that half of the cases with HF have HFPEF [34]. In comparison with HFREF, patients with HFPEF are older, more often women, more often hypertensive and have higher prevalence of AF but a lower prevalence of coronary artery disease [34]. Non-cardiovascular co-morbidities seem to be more prevalent and include renal impairment, chronic lung diseases, anaemia and other diseases [34]. A meta-analysis has shown that HFPEF is associated with a 50% lower hazard ratio for mortality compared with HFREF and a higher likelihood of non-cardiovascular death [36]. A limitation of the present study is that we were unable to distinguish between different types of HF. Since the mean age of the cohort

was rather young (57 years) and the relationship between cadmium and HF was only found in men it could be speculated that the cadmium level mainly is associated with HFREF.

Smoking is a major source of cadmium exposure and an established cardiovascular risk factor. In our study the association between cadmium and incident HF remained after adjustment for smoking status. We did not have information on serum cotinine and we did not adjust for pack-years due to incomplete data. Hence, it is still possible that residual confounding with respect to smoking increased the point estimates. However, in a previous study, adjustments for pack-years in addition to smoking status did not change the association between cadmium and cardiovascular outcomes [12]. Furthermore, the relationship between cadmium and incidence of HF was similar in a separate analysis of never smokers. The major source of cadmium in non-smokers is high dietary intake of contaminated healthy food items, such as whole grains and vegetables [1, 37]. High intake of whole grains and vegetables could have protective effects and reduce the risk of cardiovascular disease [38, 39]. Hence, a more healthy diet could counterbalance the adverse effects of cadmium in non-smokers, and this could hypothetically explain the non-linear association between cadmium and HF.

The strength of the present study included a large number of subjects and events during a long follow-up period. The cardiovascular endpoints were retrieved from national hospital registers in Sweden with high validity for HF [28] and AF[29]. Since all recorded events of HF had HF as the primary diagnosis and were hospitalized, we can assume that the diagnosis was valid and that HF was quite severe in most cases. A limitation is that we were unable to detect cases of HF that were diagnosed in the out-patient setting or undiagnosed. Therefore, it is unclear whether the present results could be generalized to less severe forms of HF.

In conclusion, elevated level of cadmium in blood was associated with increased incidence of HF. Incidence of AF was not associated with cadmium. The results from this population-based study give support to the hypothesis of a relationship between cadmium and increased risk of HF.

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Conflict of interest: none declared.

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Figure legends

Figure 1: Incidence of heart failure in relation to sex-specific quartiles (Q1-Q4) of cadmium.

Figure 2: Cubic splines model with the age and sex adjusted hazard ratios for incident heart failure as a function of cadmium levels in blood.

Table 1. Baseline Characteristics of the MDC study cohort in relation to sex-specific quartiles (Q1- Q4) of blood cadmium level.

	QUARTILES OF BLOOD CADMIIUM				
MDC (N=4378)	**Q1** (n=1094)	**Q2** (n=1095)	**Q3** (n=1095)	**Q4** (n=1094)	*P* value
Blood cadmium range, men (µg/L) Median (range)	0.12 (0.02-0.15)	0.19 (0.15-0.24)	0.31 (0.24-0.49)	0.98 (0.49-5.07)	
Blood cadmium range, women (µg/L) Median (range)	0.14 (0.03-0.18)	0.22 (0.18-0.27)	0.34 (0.27-0.49)	0.97 (0.49-4.83)	
Age (years)	56.9±5.9	57.9±6.0	58.3±5.8	56.8±5.9	<0.001
Male sex (%)	40.1	40.2	40.2	40.1	1.00
Waist circumference (cm)	83.2±12.5	83.6±13.2	83.8±12.6	82.7±12.9	0.16
SBP (mmHg)	140±18	141±19	142±19	140±19	0.03
LDL	4.20±1.0	4.18±1.0	4.13±1.0	4.23 ±1.0	0.13
HDL	1.39 ±0.37	1.42 ±0.38	1.41 ±0.37	1.36 ±0.37	0.001
hsCRP	2.28±4.3	2.18±3.8	2.41±3.8	3.24±5.3	<0.001
Plasma creatinine (µmol/L)	85.5±13	85.5±17	86.2±17	82.5±16	<0.001
Blood pressure-lowering medication (%)	13.5	17.3	18.1	14.1	0.62
Lipid-lowering medication (%)	2.0	1.7	3.1	2.3	0.28
Diabetes (%)	5.4	3.0	3.0	3.1	0.01
History of a coronary event (%)	0.4	1.3	2.1	1.8	0.001
Smoking status					<0.001
Current smoker (%)	3.7	4.8	15.1	80.7	
Former smoker (%)	31.9	41.9	46.8	13.3	
Never smoker (%)	64.4	53.3	38.1	6.0	
High alcohol intake (%)	3.0	2.9	2.9	5.2	0.008
Married (%)	71.5	74.7	68.4	59.0	<0.001
Low education (%)	39.7	43.8	46.6	52.4	<0.001

All values are mean±standard deviation, unless otherwise stated. LDL: low-density lipoprotein , HDL: high-density lipoprotein, hsCRP: high-sensitivity C-reactive protein.

Table 2. Incidence of heart failure (HF) or atrial fibrillation (AF) in relation to sex-specific quartiles of cadmium.

	Sex-specific Quartiles of cadmium				P,trend
	Q1	Q2	Q3	Q4	
HF, n, per 1000 p-y	22 (1.2)	38 (2.0)	33 (1.8)	50 (2.8)	
HR (95%CI), model 1	1.00	1.56 (0.92-2.63)	1.28 (0.75-2.20)	2.64 (1.60-4.36)	<0.001
HR (95%CI), model 2	1.00	1.59 (0.92-2.72)	1.04 (0.59-1.84)	1.95 (1.02-3.72)	0.21
AF, n, per 1000 p-y	83 (4.5)	90 (4.9)	122 (6.7)	90 (5.2)	
HR (95%CI), model 1	1.00	0.99 (0.74-1.34)	1.32 (1.00-1.75)	1.25 (0.93-1.68)	0.08
HR (95%CI), model 2	1.00	0.98 (0.73-1.33)	1.19 (0.89-1.59)	1.02 (0.69-1.51)	0.45

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking habits, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.

p-y, person-year. Cl, confidence interval.

Table 3. Incidence of heart failure (HF) in relation to sex-specific quartiles of cadmium among men and women.

	Quartiles of cadmium				P,trend
	Q1	Q2	Q3	Q4	
Men					
HF, n, per 1000 p-y	6(0.8)	23(3.2)	18(2.5)	29(4.2)	
HR (95%CI), model 1	1.00	3.61 (1.47-8.86)	2.58(1.02-6.50)	5.39 (2.24-13.00)	<0.001
HR (95%CI), model 2	1.00	3.37 (1.29-8.78)	1.76(0.66-4.73)	3.91 (1.32-11.54)	0.18
Women					
HF, n, per 1000 p-y	16(1.4)	15(1.3)	15(1.3)	21(1.9)	
HR (95%CI), model 1	1.00	0.82 (0.40-1.65)	0.80 (0.39-1.61)	1.58 (0.82-3.02)	0.20
HR (95%CI), model 2	1.00	0.90 (0.44-1.83)	0.74 (0.35-1.54)	1.18 (0.49-2.82)	0.99

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking habits, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.

p-y, person-year. Cl,confidence interval.

Table 4 . Additional analysis on incidence of heart failure (HF) in relation to sex-specific quartiles of cadmium among never smokers.

	Quartiles of cadmium				P,trend
	Q1	Q2	Q3	Q4	
HF, n, per 1000 p-y	8(1.7)	23(2.7)	9(3.4)	2(6.0)	
HR (95%CI), model 1	1.00	3.05 (1.36-6.83)	1.48(0.57-3.82)	2.23 (0.47-10.50)	0.37
HR (95%CI), model 2	1.00	3.72 (1.63-8.51)	1.51(0.56-4.03)	2.87 (0.60-13.85)	0.11

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.* P value >0.05, p-y, person-year. Cl, confidence interval.

Figure 1: Incidence of heart failure in relation to sex-specific quartiles (Q1-Q4) of cadmium.

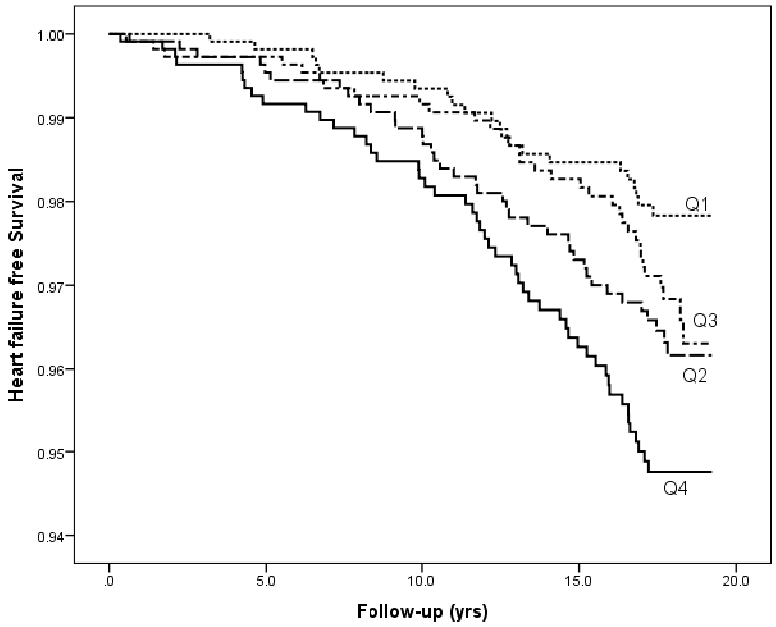
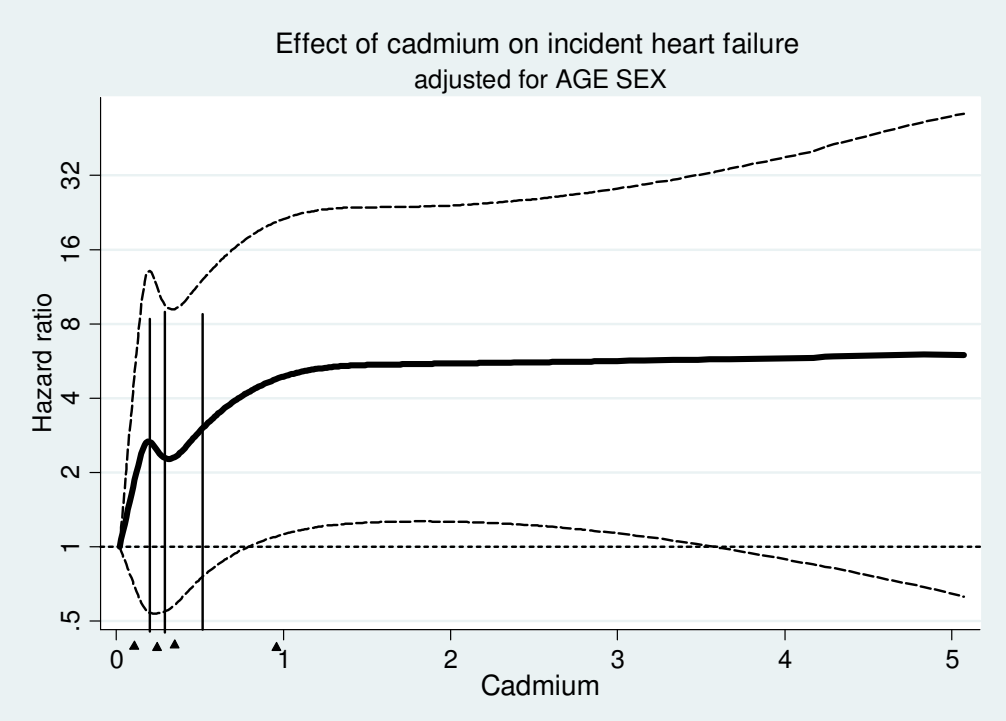


Figure 2: Cubic splines model with the age and sex adjusted hazard ratios for incident heart failure as a function of cadmium levels in blood. Triangels indicate median cadmium concentrations for Q1-Q4.



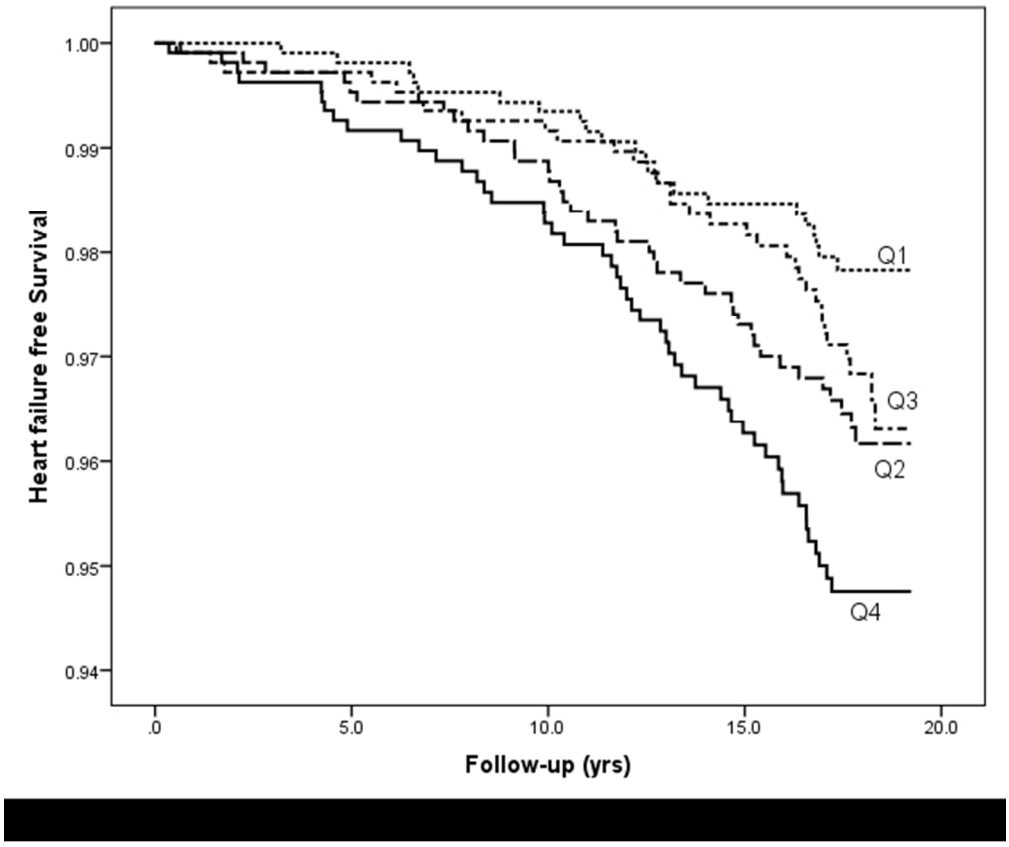


Figure 1: Incidence of heart failure in relation to sex-specific quartiles (Q1-Q4) of cadmium.
165x139mm (96 x 96 DPI)

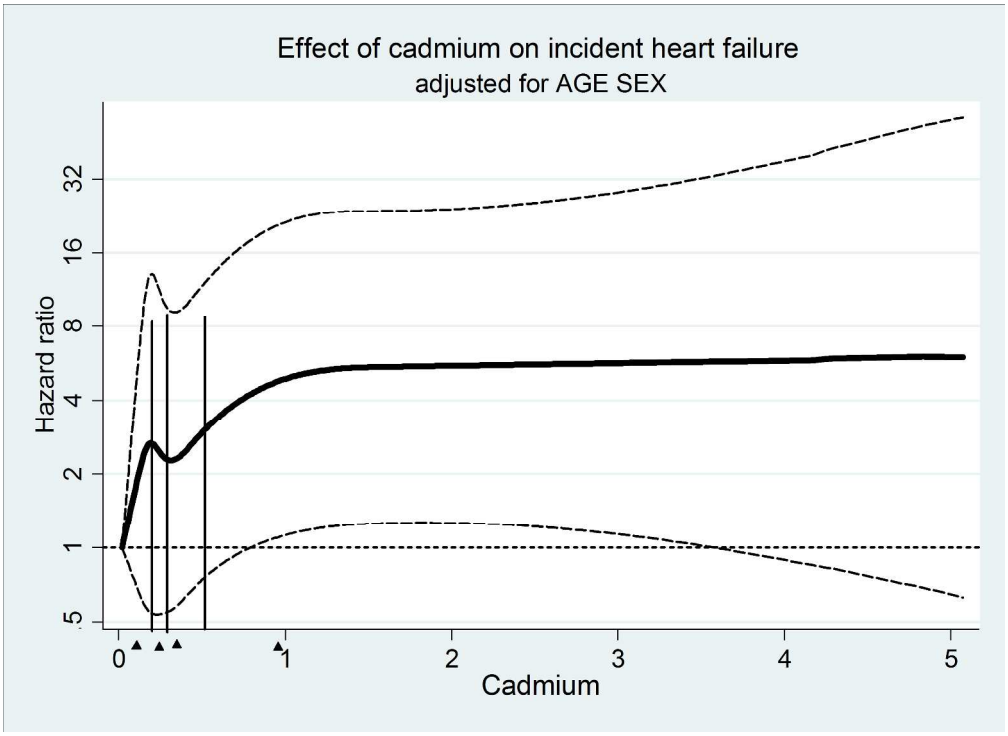


Figure 2: Cubic splines model with the age and sex adjusted hazard ratios for incident heart failure as a function of cadmium levels in blood. Triangels indicate median cadmium concentrations for Q1-Q4.
875x635mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2,3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1,2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6,7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6,7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6,7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6,7
Bias	9	Describe any efforts to address potential sources of bias	5,6,7,8
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8,9,10
		(b) Indicate number of participants with missing data for each variable of interest	5
		(c) Summarise follow-up time (eg, average and total amount)	9,10
Outcome data	15*	Report numbers of outcome events or summary measures over time	8,9,10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8,9,10
		(*b*) Report category boundaries when continuous variables were categorized	8,9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8,9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12,13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Cadmium exposure and incidence of heart failure and atrial fibrillation - a population-based prospective cohort study

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Cadmium exposure and incidence of heart failure and atrial fibrillation - a population-based prospective cohort study

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Short running title: Cadmium exposure and heart failure and atrial fibrillation

ABSTRACT

Objectives

Cadmium is a non-essential toxic metal with multiple adverse health effects. Cadmium has shown to be associated with cardiovascular diseases, but few studies have investigated heart failure (HF) and none of them atrial fibrillation (AF). We examined whether cadmium exposure is associated with incidence of HF or AF.

Design

A prospective, observational cohort study with a 17-year follow-up.

Setting

The city of Malmö, Sweden.

Participants

Blood cadmium levels were measured in 4378 subjects without a history of HF or AF (aged 46 to 67 years, 60 % women), who participated in the Malmö Diet and Cancer (MDC) study during 1992-1994.

Primary and secondary outcome measures

Incidence of HF and AF were identified from the Swedish hospital discharge register.

Results

143 subjects (53% men) were diagnosed with new-onset HF and 385 individuals (52% men) were diagnosed with new-onset AF during follow-up for 17 years. Blood cadmium in the sex-specific 4th quartile of the distribution was significantly associated with incidence of HF. Hazards ratio (HR, 4th vs 1st quartile) was 2.64 (95% CI: 1.60-4.36), adjusted for age; and 1.95 (1.02-3.71) after adjustment also for conventional risk factors and biomarkers. Blood cadmium level was not significantly associated with risk of incident AF.

Conclusion

Blood cadmium levels in the 4th quartile were associated with increased incidence of HF in this cohort with comparatively low exposure to cadmium. Incidence of AF was not associated with cadmium.

Keywords: blood cadmium levels, heart failure, atrial fibrillation, cohort study, epidemiology

Article summary

Article focus

- Cadmium has shown to be associated with cardiovascular diseases, but few studies have investigated heart failure (HF) and none of them atrial fibrillation (AF).
- The research question was whether cadmium exposure is associated with incidence of HF or AF.

Key messages

- Blood cadmium levels in the 4th quartile were associated with increased incidence of HF in this cohort with comparatively low exposure to cadmium.
- Incidence of AF was not associated with cadmium.

Strengths and limitations of this study

- A large number of subjects and events during a long follow-up period. The cardiovascular endpoints were retrieved from national hospital registers in Sweden with high validity for HF and AF.
- We were unable to detect cases of HF that were diagnosed in the out-patient setting or undiagnosed. Therefore, it is unclear whether the present results could be generalized to less severe forms of HF.

INTRODUCTION

Cadmium is a non-essential toxic metal, which can be absorbed through smoking, diet and occupational exposure in certain industries. The elimination of cadmium from the human body is a slow process - the biological half-life has been estimated to 10-40 years [1, 2]. Previous studies have shown that exposure to cadmium is low in the Swedish population, as compared to e.g., studies from the US [3, 4] and the Far East [5]. Smoking and diets including vegetables, whole grain and potatoes are important sources of cadmium in Sweden [6]. Occupational exposures have historically caused very high exposures, however, this is now an unusual reason for high cadmium in Sweden [7].

Multiple adverse health effects of cadmium exposure have been reported, such as increased risk of renal dysfunction [1, 6, 8, 9], osteoporosis [9, 10, 11] and cancer [12, 13, 14]. Recent studies also found that cadmium was associated with the prevalence and future growth of atherosclerotic plaques [15] and with cardiovascular disease (CVD) [16, 17]. However, to our knowledge, only two studies, both in US, investigated the association between cadmium and heart failure (HF) [3, 16] Peters et al conducted a cross-sectional study in a sample from the US general population, and found both blood and urinary cadmium to be associated with self-reported prevalence of HF, and the association was similar among men and women [3]. Tellez-Plaza et al reported an association between urinary cadmium and HF in a prospective cohort study of 3348 American Indian adults aged 45-74 years [16]. There is experimental support for a direct toxic effect of cadmium on the heart muscle cells and possibly also the cardiac conduction system [18, 19, 20, 21, 22, 23] . In humans, autopsy studies have shown that the myocardial content of cadmium increases with age, up to late middle age and mirrors the exposure to cadmium [24].

To our best knowledge, there is no previous study exploring the relationship between cadmium and incidence of atrial fibrillation (AF). AF is a comorbid condition among HF patients and they share risk factors such as hypertension, diabetes, ischemic heart disease and valvular disease [25, 26, 27].

Thus, the purpose of this population-based study was to explore whether elevated blood cadmium levels are associated with incidence of HF or AF in middle-aged subjects.

METHODS

Study population

The Malmö Diet and Cancer (MDC) is a prospective cohort study from the city of Malmö, Sweden with baseline examination between March 1991 and September 1996 [28, 29]. Participants filled out a self-administered questionnaire, underwent physical examination and sampling of peripheral venous blood. The participant rate was 41%. A random sample of the MDC cohort, the MDC cardiovascular cohort (MDC-CC) (N=6,103), was invited to take part in a study of the epidemiology of carotid artery disease between 1991 to 1994 [30]. Blood samples were donated by 5,540 of them after overnight fast, and cadmium could be measured in 4,952 participants (aged 46-68 years, 60% women). Individuals with history of HF (n=8) or AF (n=42) at baseline were excluded from the analysis of the respective outcomes. The final study population included 4 378 subjects after exclusion of subjects with missing values on conventional cardiovascular risk factors and biomarkers.

The study was approved by the regional Ethics Committee (LU51/90) and all participants provided written informed consent. The study complies with the Declaration of Helsinki.

Measurements and definitions

The self-administered questionnaire provided information on current use of antihypertensive, lipid- lowering, and anti-diabetic medication, smoking habits, marital status and educational status [28]. Waist circumference (in cm) was measured midway between the lowest rib margin and iliac crest. Blood pressure was measured once using a mercury-column sphygmomanometer after 10 minutes of rest in the supine position. Diabetes mellitus was defined as self-reported physician's diagnosis of diabetes, use of anti-diabetic medications or fasting whole blood glucose level greater than 109 mg/dL (e.g. ≥6.1 mmol/L) [31]. History of a coronary event at the baseline examination was retrieved from the Swedish Hospital Discharge Register. Subjects were categorized into current smokers (those who smoked regularly or occasionally), former smokers or never-smokers. Pack-years of smoking were calculated the years of smoking X the number of daily cigarettes, divided by 20. As never smokers were marked as zero, pack-years were available in 3597 subjects and 781 subjects missing information on pack-years. High alcohol consumption was defined as >40 g alcohol per day for men and >30 g per day for women. Marital status was categorized as being married or not. Educational level was divided into less than 9 years, 9 to 11 years, and 12 years or more of education.

Laboratory measurements

The blood cadmium concentrations were calculated using erythrocyte concentrations of cadmium adjusted for hematocrit. Cadmium was analyzed using inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS, Agilent Technologies). All samples were analyzed in three different rounds with external quality control samples included. Two QC samples were used (Seronorm™ Trace Elements Whole Blood L-1, Lot No. 1103128 and Seronorm™ Trace Elements Whole Blood L-2, Lot No.1103129, Sero AS, Billingstad Norway). The results from all rounds versus recommended limits were 0.34 ± 0.02 µg /L

(N=70) versus 0.32-0.40 µg/L and 5.7 ±0.18 µg/L (N=70) versus 5.4-6.2 µg/L. The results were similar for the three different rounds. Furthermore 20 erythrocyte samples (range 0.2-0.96 µg/L) were used to compare with another laboratory (Occupational and Environmental Medicine, Lund, Sweden). The results showed good agreement with a Pearson's correlation coefficient of 0.99 and a slope of 1.04 (standard error 0.04).

Measurements of high- density lipoprotein (HDL) were performed on fresh blood samples according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. Low- density lipoprotein cholesterol (LDL) concentration was calculated according to Friedewald's formula. Plasma biomarkers were measured from fasting plasma samples that had been frozen at −80°C immediately after collection. High-sensitivity C-reactive protein (hsCRP) was analyzed using the Tina-quant® CRP latex assay (Roche Diagnostics, Basel, Switzerland). Plasma creatinine (µmol/L) was analyzed with the Jaffé method, standardized according to the International Standardization with isotope dilution mass spectometry (IDMS).

Follow-up and ascertainment of cardiovascular events

All cases were retrieved from the Swedish hospital discharge register. This register has been operating in south of Sweden since 1970 and became nationwide in 1987. HF was defined according to International Classification of Diseases (ICD) code 427.00, 427.10 and 428.99 (ICD-8); 428 (ICD-9); and I50, I11 (ICD-10) as the primary diagnosis [32]. AF was defined using diagnosis codes 427.92 (ICD-8), 427D (ICD-9) and I48 (ICD-10) [33]. Validation studies have shown that the Swedish hospital discharge register has a case validity of 95% for a primary diagnosis of HF [32] and a case validity of 98% for AF [33].

Statistical analysis

Individuals with history of HF or AF at baseline were excluded from the respective analyses. All subjects were followed from the baseline examination until death, emigration from Sweden or December 31, 2010, whichever came first. In addition, follow-up time was stopped at a first event of HF for the study of incidence of HF, and at a first event of AF for the study of incidence of AF. A total of 30 (0.7%) participants emigrated from Sweden during the follow-up.

hsCRP showed a right-skewed distribution and was log-transformed. Cross-sectional relations of sex-specific cadmium quartiles (i.e., with the same proportions of men and women in each quartile) to cardiovascular risk factors were assessed using one-way ANOVA for continuous variables and logistic regression for dichotomous variables. Cox proportional hazards regression was used to examine the association between cadmium (in sex-specific quartiles) and incidence of HF or AF. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated. The basic model was adjusted for age. In model 2, we also adjusted for other established cardiovascular risk factors, i.e., systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking status, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital- and educational status. The fit of the proportional hazards model was checked visually by plotting the incidence rates over time and by entering time-dependent variables into the model. Possible interactions between cadmium and cardiovascular risk factors on incident HF or AF were explored by introducing interaction terms in the multivariate model. The Kaplan-Meier curve was used to illustrate incidence of HF in relation to cadmium. The association between incident HF and cadmium level was also assessed using restricted cubic spline with 5 knots of blood cadmium, models adjusted for age, sex and

smoking status. P<0.05 was considered statistically significant. All analyses were performed using IBM SPSS version 20 (IBM Corp.) or Stata software version 12.0 (StataCorp).

RESULTS

Baseline characteristics

Cardiovascular risk factors at the baseline examination in relation to the sex-specific quartiles of cadmium are presented in Table 1. Age, hsCRP, HDL, plasma creatinine, systolic blood pressure, history of diabetes, history of a coronary event, current smoking, high alcohol intake, being unmarried and low educational level were significantly associated with the cadmium level, Table 1.

Incidence of HF and AF in relation to cadmium

During a mean follow-up of 16.8 years, 143 subjects (53% men) had a first hospital diagnosis of HF. A total of 385 individuals (52% men) were diagnosed with AF during the follow-up.

Subjects in the 4th compared to the 1st quartile of cadmium had a significantly higher risk for incident HF (HR: 2.64, 95% CI: 1.60-4.36) after adjustment for age, Table 2 and Figure 1. This association remained significant after further adjustment for possible confounders, HR 1.95 (1.02-3.72). However, the relationship across quartiles of cadmium was non-linear (*p* for trend 0.21). The restricted cubic spline model of incident HF across cadmium levels, adjusted for age, sex and smoking status, is shown in Figure 2. The risk for HF was not linear across the cadmium level, and the 4th quartile of blood cadmium (> 0.49 µg/L) indicated an increased risk for HF.

A sensitivity analysis with additional adjustment for pack-years was performed. The result was similar (HR for 4th vs 1st quartile: 1.98: 1.04-3.78, p=0.038). We also performed a

sensitivity analysis using erythrocyte cadmium instead of blood cadmium, and the results were essentially the same, supplement table 1. In addition, we analysed the association between blood cadmium and HF using quartile limits based on the entire sample instead of sex specific cutoffs. The HR (Q4 vs Q1) was 1.83 (1.00-3.36, p=0.052) for the adjusted model when sex was added to the covariates. The relationship between cadmium and HF was only observed in subjects above 57 years of age. HR for Q4 vs Q1 for sex-specific quartiles cadmium was 1.11 (0.26-4.73, p=0.888) among 46-57 years old and 2.20 (1.07-4.50, p=0.031) among above 57 to 67 years old. However, only 24 HF cases occurred in subjects 46-57 years. Sensitivity analysis was performed separately for men and women to examine the effects of cadmium on incidence of HF. The significant association between cadmium and incident HF was only found among men, Table 3. Additional adjustment for menopausal status in women did not change the association, data not shown. Since cadmium exposure is higher in smokers, we also performed a separate analysis of incidence of HF in never smoking subjects. The point-estimate was similar to that in all subjects, although not statistically significant (Table 4).

Elevated blood cadmium level was not significantly associated with increased incidence of AF, when adjusted for age (HR: 1.25, 0.93-1.68), Table 2. The associations were further attenuated when adjusting for other risk factors.

In the multivariate adjusted model for incident HF, age, use of blood pressure-lowering medication, diabetes, waist circumference, hsCRP and history of a coronary event were independently associated with an increased risk for HF. Age, waist circumference, use of lipid-lowering medication, antihypertensive medication and low LDL were significantly associated with AF in the multivariate analysis. No significant interaction was observed

between cadmium level and other cardiovascular risk factors on incident HF or AF, respectively.

DISCUSSION

This large, prospective Swedish population-based study showed a significantly higher incidence of HF in subjects in the 4th quartile of blood cadmium compared to the 1st quartile. However, the relationship was non-linear and no increased risk was found for subjects in the 3rd quartile of cadmium. Our results extend data from two previous studies from the US which were based on self-reported HF in a cross-sectional study [3] and the incidence of HF in an ethnic minority with much higher levels of exposure to cadmium than the median levels in this study (0.24 µg/L in men and 0.27 µg/L in women) [16]. The results support the hypothesis of a relationship between increased cadmium levels and risk of HF. However, we found no significant association between blood cadmium and incidence of AF in the present study.

The mechanisms underlying the association between cadmium and HF are unclear. Cadmium has been associated with many cardiovascular risk factors, such as hypertension [5, 34], smoking and kidney disease [6, 8], which partly might explain the association between cadmium and HF. Cadmium was significantly associated with baseline blood pressure, current smoking, history of a coronary event and plasma creatinine in our study. However, cadmium was associated with increased risk of HF after adjustment for the baseline levels of these risk factors.

Cadmium could potentially replace and interact with the homeostasis of several essential metals, such as zinc, iron and calcium [35]. It has been reported that cadmium could have

cardio-toxic effects, which might explain the association between cadmium and HF. Chronic exposure to cadmium causes degenerative changes to myocardial cells in rats, and in mice cardiac depressant effects following low dose cadmium exposure have been reported [18, 19]. Cadmium may affect tissue structure and integrity of the heart muscle by oxidative stress and increased reactive oxygen species (ROS) production or by DNA methylation [20, 21]. Cadmium also affects cardiac conduction system by interfering with calcium-mediated physiologic and biochemical processes [22]. Blockade of L-type calcium channels and altered outward potassium current in ventricular myocytes were also observed in vivo [23, 36].

HF is a heterogeneous syndrome characterized by impairment of the heart to fill and/or eject blood commensurate with the metabolic needs of the body, resulting in pulmonary or venous congestion [37]. Traditionally HF has been associated with reduced left ventricular ejection fraction (HF with reduced ejection fraction – HFREF). During the last decades it has been widely recognized that HF also occurs with preserved ejection fraction (HFPEF) [38], characterized by normal left ventricular systolic function and abnormal diastolic function in combination with clinical signs or symptoms of HF [39]. The prevalence of HF increases strongly after middle age and is about 10% among those above the age of 75 years [38]. Recent studies have shown that half of the cases with HF have HFPEF [38]. In comparison with HFREF, patients with HFPEF are older, more often women, more often hypertensive and have higher prevalence of AF but a lower prevalence of coronary artery disease [38]. Non-cardiovascular co-morbidities seem to be more prevalent and include renal impairment, chronic lung diseases, anaemia and other diseases [38]. A meta-analysis has shown that HFPEF is associated with a 50% lower hazard ratio for mortality compared with HFREF and a higher likelihood of non-cardiovascular death [40]. A limitation of the present study is that we were unable to distinguish between different types of HF. Since the mean age of the cohort

was rather young (57 years) and the relationship between cadmium and HF was only found in men it could be speculated that the cadmium level mainly is associated with HFREF.

Smoking is a major source of cadmium exposure and an established cardiovascular risk factor. In our study the association between cadmium and incident HF remained after adjustment for smoking status. We did not have information on serum cotinine but adjustment for pack-years which were available in a major part of the cohort did not change the results. In addition, the point-estimate of the association between cadmium and incidence of HF was similar in a separate analysis of never smokers, although not attaining statistical significance. The major source of cadmium in non-smokers is high dietary intake of contaminated healthy food items, such as whole grains and vegetables [1, 41]. High intake of whole grains and vegetables could have protective effects and reduce the risk of cardiovascular disease [42, 43]. Hence, a more healthy diet could counterbalance the adverse effects of cadmium in non-smokers, and this could hypothetically explain the non-linear association between cadmium and HF.

The strength of the present study included a large number of subjects and events during a long follow-up period. The cardiovascular endpoints were retrieved from national hospital registers in Sweden with high validity for HF [32] and AF [33]. Since all recorded events of HF had HF as the primary diagnosis and were hospitalized, we can assume that the diagnosis was valid and that HF was quite severe in most cases. A limitation is that we were unable to detect cases of HF that were diagnosed in the out-patient setting or undiagnosed. Therefore, it is unclear whether the present results could be generalized to less severe forms of HF.

In conclusion, elevated level of cadmium in blood was associated with increased incidence of HF. Incidence of AF was not associated with cadmium. The results from this population-

based study give support to the hypothesis of a relationship between cadmium and increased risk of HF.

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Conflict of interest: none declared.

Contributorship

YB was involved in the design, data collection, analysis and write-up of the research. LB was involved in the design, data collection, analysis and write-up of the research. MP was involved in the design, data collection, analysis and write-up of the research. BH was involved in the design, data collection, analysis and write-up of the research. BF was involved in the design, data collection, analysis and write-up of the research. GE was involved in the design, data collection, analysis and write-up of the research. All authors read and approved the final manuscript.

Data sharing

No additional data are available.

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Figure legends

Figure 1: Incidence of heart failure in relation to sex-specific quartiles (Q1-Q4) of blood cadmium levels.

Figure 2: Cubic splines model for incident heart failure as a function of cadmium levels in blood. Hazards ratio is adjusted for age and sex and smoking status. Triangles indicate median cadmium concentrations for Q1-Q4.

Table 1. Baseline Characteristics of the MDC study cohort in relation to sex-specific quartiles (Q1- Q4) of blood cadmium level.

	OVERALL	QUARTILES OF BLOOD CADMIIUM				
MDC (N=4378)		**Q1** (n=1094)	**Q2** (n=1095)	**Q3** (n=1095)	**Q4** (n=1094)	*P* value
Blood cadmium range, men (µg/L), Median (range)	0.24 (0.02-5.07)	0.12 (0.02-0.15)	0.19 (0.15-0.24)	0.31 (0.24-0.49)	0.98 (0.49-5.07)	
Blood cadmium range, women (µg/L), Median (range)	0.27 (0.03-4.83)	0.14 (0.03-0.18)	0.22 (0.18-0.27)	0.34 (0.27-0.49)	0.97 (0.49-4.83)	
Age (years)	57.4±5.9	56.9±5.9	57.9±6.0	58.3±5.8	56.8±5.9	<0.001
Male sex (%)	40.2	40.1	40.2	40.2	40.1	1.00
Waist circumference (cm)	83.2±12.8	83.2±12.5	83.6±13.2	83.8±12.6	82.7±12.9	0.16
SBP (mmHg)	141±19	140±18	141±19	142±19	140±19	0.03
LDL	4.18±0.99	4.20±1.0	4.18±1.0	4.13±1.0	4.23 ±1.0	0.13
HDL	1.39 ±0.37	1.39 ±0.37	1.42 ±0.38	1.41 ±0.37	1.36 ±0.37	0.001
hsCRP	2.53±4.3	2.28±4.3	2.18±3.8	2.41±3.8	3.24±5.3	<0.001
Plasma creatinine (µmol/L)	84.7±16	85.5±13	85.5±17	86.2±17	82.5±16	<0.001
Blood pressure-lowering medication (%)	15.7	13.5	17.3	18.1	14.1	0.62
Lipid-lowering medication (%)	2.3	2.0	1.7	3.1	2.3	0.28
Diabetes (%)	3.6	5.4	3.0	3.0	3.1	0.01
History of a coronary event (%)	1.4	0.4	1.3	2.1	1.8	0.001
Smoking status						<0.001
Current smoker (%)	26.1	3.7	4.8	15.1	80.7	
Former smoker (%)	33.5	31.9	41.9	46.8	13.3	
Never smoker (%)	40.5	64.4	53.3	38.1	6.0	
pack-years	10.7±18.6	2.4±10.2	4.7±12.6	8.6±18.6	25.0±20.7	
High alcohol intake (%)	3.5	3.0	2.9	2.9	5.2	0.008
Married (%)	68.4	71.5	74.7	68.4	59.0	<0.001
Low education (%)	45.6	39.7	43.8	46.6	52.4	<0.001

All values are mean±standard deviation, unless otherwise stated. LDL: low-density lipoprotein , HDL: high-density lipoprotein, hsCRP: high-sensitivity C-reactive protein.

Table 2. Incidence of heart failure (HF) or atrial fibrillation (AF) in relation to sex-specific quartiles of blood cadmium.

	Sex-specific Quartiles of blood cadmium				P,trend
	Q1	Q2	Q3	Q4	
HF, n, per 1000 p-y	22 (1.2)	38 (2.0)	33 (1.8)	50 (2.8)	
HR (95%CI), model 1	1.00	1.56 (0.92-2.63)	1.28 (0.75-2.20)	2.64 (1.60-4.36)	<0.001
HR (95%CI), model 2	1.00	1.59 (0.92-2.72)	1.04 (0.59-1.84)	1.95 (1.02-3.72)	0.21
AF, n, per 1000 p-y	83 (4.5)	90 (4.9)	122 (6.7)	90 (5.2)	
HR (95%CI), model 1	1.00	0.99 (0.74-1.34)	1.32 (1.00-1.75)	1.25 (0.93-1.68)	0.08
HR (95%CI), model 2	1.00	0.98 (0.73-1.33)	1.19 (0.89-1.59)	1.02 (0.69-1.51)	0.45

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking status, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.

p-y, person-year. Cl, confidence interval.

Table 3. Incidence of heart failure (HF) in relation to sex-specific quartiles of blood cadmium among men and women.

	Quartiles of blood cadmium				P,trend
	Q1	Q2	Q3	Q4	
Men					
HF, n, per 1000 p-y	6(0.8)	23(3.2)	18(2.5)	29(4.2)	
HR (95%CI), model 1	1.00	3.61 (1.47-8.86)	2.58(1.02-6.50)	5.39 (2.24-13.00)	<0.001
HR (95%CI), model 2	1.00	3.37 (1.29-8.78)	1.76(0.66-4.73)	3.91 (1.32-11.54)	0.18
Women					
HF, n, per 1000 p-y	16(1.4)	15(1.3)	15(1.3)	21(1.9)	
HR (95%CI), model 1	1.00	0.82 (0.40-1.65)	0.80 (0.39-1.61)	1.58 (0.82-3.02)	0.20
HR (95%CI), model 2	1.00	0.90 (0.44-1.83)	0.74 (0.35-1.54)	1.18 (0.49-2.82)	0.99

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking status, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.

p-y, person-year. Cl,confidence interval.

Table 4 . Additional analysis on incidence of heart failure (HF) in relation to sex-specific quartiles of blood cadmium among never smokers.

	Quartiles of blood cadmium				P,trend
	Q1	Q2	Q3	Q4	
HF, n, per 1000 p-y	8(1.7)	23(2.7)	9(3.4)	2(6.0)	
HR (95%CI), model 1	1.00	3.05 (1.36-6.83)	1.48(0.57-3.82)	2.23 (0.47-10.50)	0.37
HR (95%CI), model 2	1.00	3.72 (1.63-8.51)	1.51(0.56-4.03)	2.87 (0.60-13.85)	0.11

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status. p-y, person-year. Cl, confidence interval.

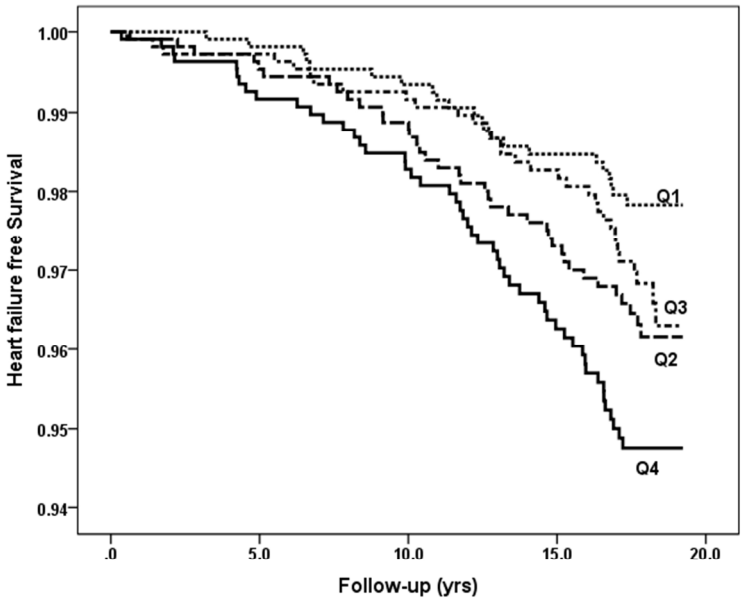

Figure 1: Incidence of heart failure in relation to sex-specific quartiles (Q1-Q4) of blood cadmium levels.
254x190mm (300 x 300 DPI)

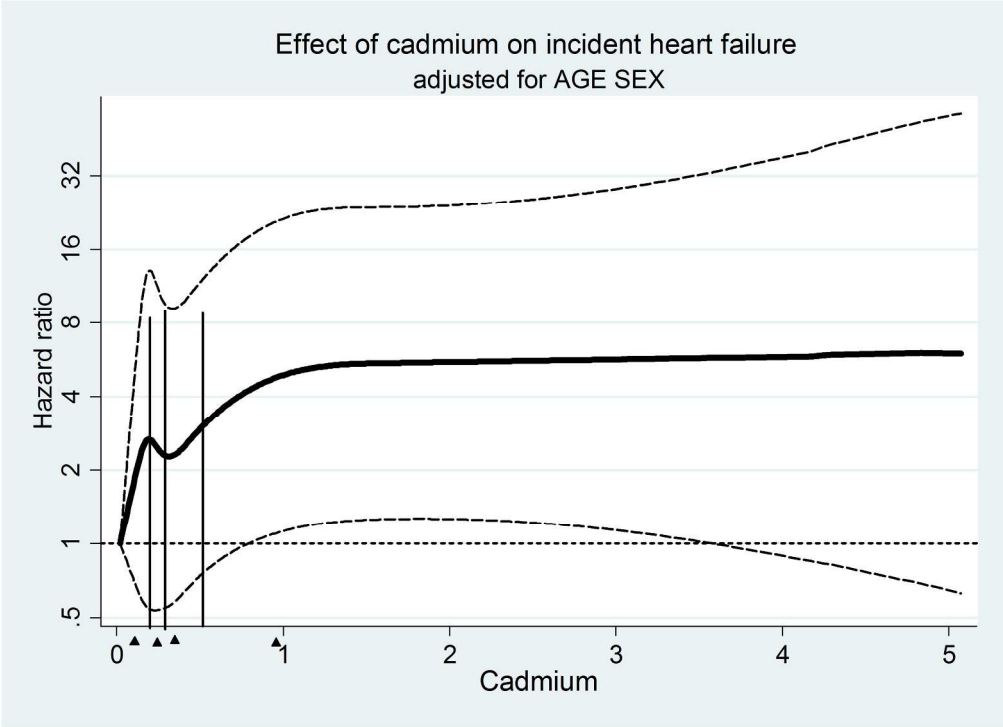

Figure 2: Cubic splines model for incident heart failure as a function of cadmium levels in blood. Hazards ratio is adjusted for age and sex and smoking status. Triangles indicate median cadmium concentrations for Q1-Q4.
254x190mm (300 x 300 DPI)

Supplement

Table 1. Incidence of heart failure (HF) or atrial fibrillation (AF) in relation to sex-specific quartiles of erythrocyte cadmium.

	Sex-specific Quartiles of erythrocyte cadmium				P,trend
HF	Q1	Q2	Q3	Q4	
HR (95%CI), model 1	1.00	1.32 (0.79-2.23)	1.28 (0.78-2.15)	2.37 (1.45-3.86)	0.001
HR (95%CI), model 2	1.00	1.55 (0.91-2.64)	1.19 (0.70-2.03)	1.92 (1.03-3.59)	0.131
AF					
HR (95%CI), model 1	1.00	0.88 (0.65-1.18)	1.19 (0.90-1.56)	1.17 (0.88-1.57)	0.086
HR (95%CI), model 2	1.00	0.91 (0.67-1.22)	1.10 (0.87-1.46)	0.99 (0.68-1.46)	0.629

Hazard ratio model 1 adjusted for age.

HR model 2 adjusted for age, systolic blood pressure, use of blood pressure-lowering or lipids-lowering medications, presence of diabetes mellitus, history of a coronary event, waist circumference, smoking status, alcohol intake, LDL, HDL, hsCRP, plasma creatinine, marital and educational status.

p-y, person-year. Cl, confidence interval.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2,3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1,2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6,7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6,7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6,7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6,7
Bias	9	Describe any efforts to address potential sources of bias	5,6,7,8
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8,9,10
		(b) Indicate number of participants with missing data for each variable of interest	5
		(c) Summarise follow-up time (eg, average and total amount)	9,10
Outcome data	15*	Report numbers of outcome events or summary measures over time	8,9,10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8,9,10
		(*b*) Report category boundaries when continuous variables were categorized	8,9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8,9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12,13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.