Cadmium exposure and atherosclerotic carotid plaques – Results from the Malmö diet and Cancer study

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**Abstract**

**Background:** Epidemiological studies indicate that cadmium exposure through diet and smoking is associated with increased risk of cardiovascular disease. There are few data on the relationship between cadmium and plaques, the hallmark of underlying atherosclerotic disease.

**Objectives:** To examine the association between exposure to cadmium and the prevalence and size of atherosclerotic plaques in the carotid artery.

**Methods:** A population sample of 4639 Swedish middle-aged women and men was examined in 1991–1994. Carotid plaque was determined by B-mode ultrasound. Cadmium in blood was analyzed by inductively coupled plasma mass spectrometry.

**Results:** Comparing quartile 4 with quartile 1 of blood cadmium, the odds ratio (OR) for prevalence of any plaque was 1.9 (95% confidence interval 1.6–2.2) after adjustment for sex and age; 1.4 (1.1–1.8) after additional adjustment for smoking status; 1.4 (1.1–1.7) after the addition of education level and life style factors; 1.3 (1.03–1.8) after additional adjustment for risk factors and predictors of cardiovascular disease. No effect modification by sex was found in the cadmium-related prevalence of plaques. Similarly, ORs for the prevalence of small and large plaques were after full adjustment 1.4 (1.0–2.1) and 1.4 (0.9–2.0), respectively. The subgroup of never smokers showed no association between cadmium and atherosclerotic plaques.

**Conclusions:** These results extend previous studies on cadmium exposure and clinical cardiovascular events by adding data on the association between cadmium and underlying atherosclerosis in humans. The role of smoking remains unclear. It may both cause residual confounding and be a source of proatherogenic cadmium exposure.

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1. Introduction

Cadmium is a non-essential, toxic metal that is widely distributed in the environment. It has been used in batteries, paints, and plastic stabilizers, and is a common contaminant in fertilizers. The impact of environmental contamination of cadmium is a major concern (Nordberg et al., 2007; EFSA. European Food Safety Authority, 2009; Agency for Toxic Substances and Disease Registry (ATSDR), 2012). Grains, and leafy and root vegetables as well as tobacco plants bio-concentrate cadmium, leading to exposure through diet and smoking. Cadmium in tobacco smoke is effectively absorbed in the lungs. Cadmium levels are higher in women than men, mainly because of increased intestinal absorption of dietary cadmium at low iron stores secondary to menstruation and pregnancy. Whole blood and urine concentrations of cadmium have long biological half-lives and are valid biomarkers of cumulative exposure in the general population (Nordberg et al., 2007; EFSA. European Food Safety Authority, 2009).

Cadmium is a very cytotoxic metal and results from experimental studies indicate that cadmium exposure may cause...
atherosclerosis (Messner et al., 2009; Knoflach et al., 2011; Subramanyam et al., 1992; Almenar et al., 2013; Prozialek et al., 2008). Tentative causal mechanisms are disruption of endothelium and increase in reactive oxygen species formation. In humans, cadmium is enriched in arterial vessel walls (Abu-Hayyeh et al., 2001). As summarized in a recent review, several epidemiological studies, mostly from the US, have examined the associations between low to moderate exposure to cadmium and cardiovascular disease (Tellez-Plaza et al., 2013). Exposure was measured as cadmium concentrations in blood or urine. In the mentioned review, a meta-analysis of twelve studies of good quality, cadmium exposure was reported to increase the risk of cardiovascular disease by 36%. The pooled relative risks for cardiovascular disease in men, women and never smokers were 1.29 (95% confidence interval 1.12–1.48), 1.20 (0.92–1.56) and 1.27 (0.97–1.67), respectively.

Atherosclerotic plaque is the culprit lesion of atherosclerotic disease. High resolution ultrasound technique makes it possible to study the occurrence and size of such plaques in the carotid arteries. Data are still lacking on the relationship between cadmium exposure and development of atherosclerosis in humans. To our knowledge, there is only one published study that has examined associations between cadmium exposure and plaque status. In that study, of a cohort of 64-year-old Swedish women, blood and urinary cadmium levels were associated with carotid plaques in terms of occurrence, size, and growth, after 6 years of follow-up (Fagerberg et al., 2012). The results were not conclusive in the subgroup of never smokers and there are no data available for men. Accordingly, the present study was undertaken with the aim to examine if cadmium exposure is associated with the prevalence and size of atherosclerotic plaques, not only in women but also in men, and if this association can be found in never smoking subjects.

2. Material and methods

2.1. Study population

As previously described in detail, all men and women living in the city of Malmö in Sweden, born between 1923 and 1945, were invited between 1991 and 1996 to participate in the Malmö Diet and Cancer Study (MDCS) (Berglund et al., 1993). The aim was to investigate the relation between dietary factors and cancer. Between 1991 and 1994 a random sample of the MDC cohort, the MDC cardiovascular cohort, was selected for a substudy with the aim of studying the epidemiology of carotid artery disease (Rosvall et al., 2000). A total of 6103 subjects underwent an ultrasound examination at the first visit in the study and where rescheduled for fasting blood sampling (with a median timelag of 7 months) under standardized conditions (Rosvall et al., 2005).

Inclusion criteria in the present study were available data on ultrasound-assessed plaque status in the carotid artery, blood cadmium concentration, and smoking status. Data were missing in 254, 1155, and 315 subjects, respectively, and 4639 subjects were included in the present study. The mean age of subjects in the total subcohort and subjects participating in the present study was almost identical (57.5 and 57.4 years, respectively). Women constituted 57.9% and 59.6%, respectively, of the study population. Carotid plaques were found in 34.6% of those in the total subcohort and in 35.4% of the subjects in the present study.

2.2. Cardiovascular risk factors

The subjects completed established questionnaires concerning life style, socioeconomic status, including occupation, health, and medication (Rosvall et al., 2000). The subjects were categorized into never, ex-, and current smokers. The last category also included those who smoked occasionally. Pack-years of smoking were calculated as the product of the years of smoking and the number of daily smoked cigarettes, divided by 20. Data on pack-years were available for 1160 current smokers, 789 ex-smokers, and all non-smokers (n=1,851), who were given the value of zero (in total n=3,800). The daily alcohol intake was calculated. Leisure time physical activity was a composite measure of 18 different leisure time activities during the preceding year. A summary score of all physical activities was obtained by using intensity factors for each activity, combined with information on time spent on the activity (Li et al., 2009). Low physical activity was defined as the lowest quartile of the summary score. Educational level was classified into three categories: “Primary education” – < 9 years of education; “some secondary education” – 9 and up to 11 years of education; and “completed secondary education” – 12 years or more of education. Anti-hypertensive and lipid-lowering treatment, postmenopausal status, and hormone replacement therapy were assessed by questionnaire. Height, waist circumference, and body mass were recorded and body mass index (BMI) was calculated. Systolic and diastolic blood pressure was measured after supine rest for 10 min.

Overnight fasting blood samples were drawn for determination of high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, hemoglobin A1c (HbA1c), and whole blood glucose according to standard procedures at the Department of Clinical Chemistry, Malmö University Hospital. High sensitivity C-reactive protein (hsCRP) (mg/L) was measured from frozen samples (–80 °C) and analyzed using the Tina-quant CPR latex high sensitivity assay (Roche Diagnostics, Basel, Switzerland) on an ADVIA 1650 Chemistry System (Bayer Healthcare, Tarrytown, NY, US). Subjects were classified as having diabetes mellitus if they reported the diagnosis in the questionnaire, used anti-diabetic medication, or had a fasting venous whole blood glucose ≥ 6.1 mmol/L.

2.3. Ultrasound examination

As previously reported, subjects underwent B-mode ultrasoundography (Acuson128 CT system, Siemens, Mountain View, Ca, USA) of the right carotid artery, according to a standardized protocol, performed by trained, certified sonographers (Rosvall et al., 2000). In short, the bifurcation area of the right common carotid artery was scanned within a pre-defined “window” comprising 3 cm of the distal common carotid artery, the bifurcation, and 1 cm of the internal and external carotid artery, respectively, for the occurrence of plaques (defined as a focal thickening of the intima-media complex > 1.2 mm and with an area ≥ 10 mm²). The degree of stenosis by visually judging to what extent the plaque protruded into the lumen was possible to assess in 1152 subjects. Methods of quality control were used, as described previously (Rosvall et al., 2000).

2.4. Blood cadmium concentrations

In whole blood, cadmium is located in red blood cells with only marginal levels in plasma (Nordberg et al., 2007). Cadmium concentrations were analyzed in erythrocytes which had been kept frozen in −80 °C since the baseline examination. These concentrations were adjusted for hematocrit to obtain the calculated cadmium concentrations in blood.

Cadmium in erythrocytes was analyzed by inductively coupled plasma mass spectrometry with an octopole reaction system (Agilent 7700x ICP-MS; Agilent Technologies, Santa Clara, Ca, USA). The reaction system was operated in the helium collision cell.
mode to eliminate interference from isobaric polyatomic species via kinetic energy discrimination. Samples were introduced using a peristaltic pump and nebulization was carried out with a Mira-Mist nebulizer (Agilent Technologies, Santa Clara, CA, USA). The spray chamber used was a Scott-type double pass spray chamber operated at 2 °C. A standard quartz torch with 2.5 mm internal diameter (ID) injector was used. The instrument was equipped with an Agilent I-AS integrated autosampler (Agilent Technologies, Santa Clara, CA, USA).

All samples were diluted 20 times with a basic diluent containing 1-butanol (2% w/v), ethylenediamine tetraacetic acid (EDTA) (0.05% w/v), Triton X-100 (0.05% w/v), and ammonium hydroxide (1% w/v). Indium was used as internal standard and was added from an internal standard mix (Agilent Technologies) containing 10 mg/L, to give a final concentration of 25 μg/L.

The imprecision was 9.6%, calculated as the coefficient of variation for 50 duplicate samples (mean 0.43 μg/L). The limit of detection (LOD), calculated as three times the standard deviation of the blank, was 0.02 μg/L. None of the samples were below the LOD.

All samples were analyzed in three different rounds with external quality control (QC) samples included. Two QC samples were used (Seronorm™ Trace Elements Whole Blood I-1, Lot no. 1103128, and Seronorm™ Trace Elements Whole Blood I-2, Lot no. N1103129; Sero AS, Billingsstad, Norway). The results from all rounds vs. recommended limits were 0.34 ± 0.02 μg/L (N = 70) vs. 0.32–0.40 μg/L, and 5.7 ± 0.18 μg/L (N = 70) vs. 5.4–6.2 μg/L. The results for the three rounds were similar. Furthermore, a comparison including 20 erythrocyte samples (range 0.2–0.96 μg/L) was made 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 (SE) 0.04).

2.5. Statistical analyses

PASSW 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The prevalence of any plaque was the primary response variable followed by the prevalence of small and large plaques, respectively. Results are presented as means (standard deviation (SD) for continuous variables, and as percentage for categorical variables. Blood cadmium concentrations and other skewed variables are presented as geometric mean (5th–95th percentiles). Differences between groups were analyzed with a Student’s t-test, and trend test after log transformation of skewed variables. Differences in proportions were analyzed with chi-square test. Logistic regression was used to examine the odds ratios (ORs) for plaque occurrence by quartiles of blood cadmium levels. Since we found no effect modification by sex in the cadmium-related prevalence of plaques, the primary analysis was performed in the total sample, including both sexes. To adjust for the influence of the potential confounders in the analyses, we first adjusted for age and sex and then used three models. Model 1 included also smoking status. Model 2 additionally adjusted for <9 years of schooling, lowest quartile of physical activity score, and alcohol intake. Model 3 additionally adjusted for waist circumference, systolic blood pressure, LDL and HDL cholesterol, triglycerides, HbA1c, hsCRP, antihypertensive treatment, lipido-lowering treatment, diabetes, postmenopausal status, hormone replacement therapy.

In separate analyses, pack-years were substituted for smoking status. Subgroup analyses were performed by evaluating differential associations by sex and smoking subgroups. The odds ratio for plaque occurrence was also estimated as a smooth function of blood cadmium (adjusted for sex, smoking and age), using the procedure gam in the ‘mgcv’ package in R version 3.0.2, where the smooth term was represented using a penalized regression spline (with 4.262 edf). Two-sided p < 0.05 was considered statistically significant.

3. Results

The geometric mean blood cadmium concentration was 0.31 μg/L (0.32 μg/L (5th–95th percentiles 0.11–161)) for women and 0.29 μg/L (5th–95th percentiles 0.09–160) for men. Two subjects had occupations with potential exposure for cadmium, but blood cadmium levels were not high (data not shown). As shown in Table 1, blood cadmium levels were positively associated with female sex, age, serum triglycerides, HbA1c, hsCRP, and smoking burden in terms of smoking status and pack-years. There were negative associations with BMI, waist circumference, educational level, and physical activity.

3.1. Prevalence of plaques

The prevalence of atherosclerotic plaques in each of the 1st–4th cadmium quartiles was 31.5% (n = 365), 31.3% (n = 363), 34.3% (n = 398), and 44.3% (n = 514), respectively (p < 0.0001 for both chi-square test and trend analysis). Fig. 1 demonstrates the estimated risk of plaque as a smooth function of blood cadmium after adjustment for sex, age and smoking status and supports the use of quartiles of cadmium in the further analyses. As shown in Table 2, comparing quartile 4 with quartile 1 of blood cadmium levels, the OR for plaque prevalence was 1.9 (95% CI 1.6–2.2) after adjustment for sex and age, 1.4 (1.1–1.8) after additional adjustment for smoking status, 1.4 (1.1–1.7) after further adjustment for educational level, life style factors and finally 1.3 (1.03–1.8) after additional adjustment for all potential clinical confounders. The results remained principally unchanged in Model 1 and 2 when the analyses were performed in the subjects with complete data in Model 3 (data not shown). In additional analyses, pack-years were substituted for smoking status, showing similar results (ORs for plaque prevalence after adjustment for age and sex, Model 1, 2 and 3, were 1.9 (1.6–2.2), 1.6 (1.3–1.9), 1.5 (1.2–1.9), and 1.6 (1.3–2.1), respectively).

3.2. Plaque size

The degree of luminal stenosis was possible to determine in 551 women and 601 men with plaques. Small plaques were defined as a degree of stenosis below the median (<20% stenosis) and large plaques as above the median (stenosis 20–80%). As shown in Fig. 2, the prevalences of these small and large plaques increased by blood cadmium quartiles in both sexes. Logistic regression analyses showed that when cadmium quartiles 4 and 1 were compared, the OR for occurrence of small plaques was 2.0 (1.5–2.6) after adjustment for age and sex, and 1.5 (1.04–2.1) after adjustment for smoking, educational level and life style factors and 1.4 (1.0–2.1) after full adjustment (Table 3). For large plaques, the corresponding ORs were 2.4 (1.9–3.2), 1.5 (1.1–2.1), and 1.4 (0.9–2.0) (Table 3). In additional analyses, when pack-years were substituted for smoking status, the OR was 1.8 (1.3–2.5) for small plaques and 1.7 (1.2–2.3) for large plaques, after full adjustment according to Model 3.

3.3. Subgroup analyses

In the subgroup analyses, the interaction term for sex and cadmium concentrations was not statistically significant with regard to plaque prevalence in the logistic regressions (p = 0.15 in Model 3). The distributions of small and large plaques by cadmium quartiles for each sex are shown in Fig. 2. Fig. 3 presents the
Table 1
Associations between factors related to cardiovascular disease and blood cadmium concentrations in middle aged women \( (n=2,764) \) and men \( (n=1,875) \).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quartiles of blood cadmium</th>
<th>P for trend</th>
<th>P for quartile 4 vs. quartile 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  ( (n=1,159) )</td>
<td>2  ( (n=1,360) )</td>
<td>3  ( (n=1,160) )</td>
</tr>
<tr>
<td>Blood cadmium, ( \mu g/L ) ( GM^a ) (minimum–maximum)</td>
<td>0.12 (0.03–0.17)</td>
<td>0.21 (0.17–0.26)</td>
<td>0.34 (0.26–0.50)</td>
</tr>
<tr>
<td>Women, ( n ) (%)</td>
<td>577 (49.8)</td>
<td>728 (62.8)</td>
<td>768 (66.2)</td>
</tr>
<tr>
<td>Age, y (mean ± SD)</td>
<td>56.9 ± 5.9</td>
<td>57.8 ± 6.0</td>
<td>58.2 ± 5.8</td>
</tr>
<tr>
<td>BMI, ( \text{mean} ± \text{SD}, \text{kg/m}^2 )</td>
<td>25.9 ± 3.9</td>
<td>25.7 ± 3.7</td>
<td>25.7 ± 3.8</td>
</tr>
<tr>
<td>Waist, ( \text{cm} ) ( GM^a ) (5th–95th percentiles)</td>
<td>84.1 [66–106]</td>
<td>81.9 [65–104]</td>
<td>81.5 [65–104]</td>
</tr>
<tr>
<td>Low educational level (&lt; 9 years of schooling), ( n ) (%)</td>
<td>477 (41.2)</td>
<td>502 (43.4)</td>
<td>535 (48.3)</td>
</tr>
<tr>
<td>Low physical activity, ( n ) (%)</td>
<td>255 (22.2)</td>
<td>275 (23.9)</td>
<td>285 (24.8)</td>
</tr>
<tr>
<td>Postmenopausal status, ( n ) (%) ( (576/726/765/689) )</td>
<td>429 (74.5)</td>
<td>536 (73.8)</td>
<td>569 (74.4)</td>
</tr>
<tr>
<td>Hormonal replacement therapy, ( n ) (%)</td>
<td>107 (21.8)</td>
<td>125 (19.5)</td>
<td>136 (19.7)</td>
</tr>
<tr>
<td>Smoking status, ( n ) (%)</td>
<td>Never smoker 726 (62.6)</td>
<td>596 (51.4)</td>
<td>459 (39.6)</td>
</tr>
<tr>
<td></td>
<td>Ex-smoker 390 (33.6)</td>
<td>499 (43.0)</td>
<td>526 (45.3)</td>
</tr>
<tr>
<td></td>
<td>Current smoker 43 (3.7)</td>
<td>65 (5.6)</td>
<td>175 (15.1)</td>
</tr>
<tr>
<td></td>
<td>Pack-years of smoking ( \text{mean} ± \text{SD} ) ( (n=961/903/873/1,063) )</td>
<td>2.8 (10.2)</td>
<td>4.9 (12.6)</td>
</tr>
<tr>
<td></td>
<td>Lipid lowering treatment, ( n ) (%)</td>
<td>26 (2.2)</td>
<td>22 (1.9)</td>
</tr>
<tr>
<td></td>
<td>Treatment for hypertension, ( n ) (%)</td>
<td>265 (14.2)</td>
<td>200 (17.2)</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus, ( n ) (%)</td>
<td>112 (9.7)</td>
<td>82 (7.1)</td>
</tr>
<tr>
<td></td>
<td>Alcohol intake, ( \text{mean} ± \text{SD}, \text{g/day} )</td>
<td>10 ± 11</td>
<td>10 ± 12</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure, ( \text{mean} ± \text{SD}, \text{mmHg} )</td>
<td>141 ± 18</td>
<td>141 ± 19</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure, ( \text{mean} ± \text{SD}, \text{mmHg} )</td>
<td>87 ± 9</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>LDL cholesterol, ( \text{mean} ± \text{SD}, \text{mmol/L} ) ( (n=1,122/1,132/1,129/1,130) )</td>
<td>4.15 ± 1.00</td>
<td>4.19 ± 0.96</td>
<td>4.12 ± 0.96</td>
</tr>
<tr>
<td>HDL cholesterol, ( \text{mean} ± \text{SD}, \text{mmol/L} )</td>
<td>1.35 ± 0.36</td>
<td>1.43 ± 0.38</td>
<td>1.43 ± 0.38</td>
</tr>
<tr>
<td>Serum triglycerides, ( \text{mmol/L} )</td>
<td>1.22 (0.60–2.98)</td>
<td>1.17 (0.98–2.59)</td>
<td>1.15 (0.58–2.55)</td>
</tr>
<tr>
<td>( \text{GM}^{a} ) [5th–95th percentiles]</td>
<td>40 [32–51]</td>
<td>40 [32–48]</td>
<td>40 [32–48]</td>
</tr>
<tr>
<td>HbA1c(^c), mmol/mol ( \text{GM [5th–95th percentiles]} )</td>
<td>5.8 [5.7–5.9]</td>
<td>5.8 [5.7–5.9]</td>
<td>5.8 [5.7–5.9]</td>
</tr>
<tr>
<td>hsCRP(^d), mg/L ( \text{GM [5th–95th percentiles]} )</td>
<td>1.22 [0.2–6.94]</td>
<td>1.23 [0.2–7.1]</td>
<td>1.37 [0.3–7.8]</td>
</tr>
</tbody>
</table>

\( ^a \text{GM} \) = geometric mean.  
\( ^b n \) in each quartile.  
\( ^c \text{LDL} \) = low density lipoprotein.  
\( ^d \text{HDL} \) = high density lipoprotein.  
\( ^e \text{HbA1c} \) = hemoglobin A1c.  
\( ^f \text{hsCRP} \) = high sensitivity C-reactive protein.

**Fig. 1.** Estimated blood cadmium concentration-response curve and 95% confidence interval for plaque occurrence (the model is adjusted for sex, smoking and age). Individual values of blood cadmium are indicated on the X-axis, and the arrows show the geometric means of blood cadmium in quartile 1–4.
In the present cross-sectional study of a large population-based sample, we observed that blood cadmium concentrations were associated with atherosclerotic plaques in the carotid artery, in terms of both prevalence and size. The concentration of cadmium in blood was used as an established measure of cadmium exposure and the observed level corresponded well to that in a previously published study from Sweden (Fagerberg et al., 2012). The plaque status was assessed with high resolution ultrasound technique (Rosvall et al., 2000). In the present study, statistical adjustment was made for a wide array of risk factors for cardiovascular disease, ranging from socioeconomic and life style factors to traditional and novel cardiovascular risk factors.

Our results are in line with findings in a previous, smaller study of 64-year-old Swedish women, in whom blood as well as urinary cadmium concentrations were associated with carotid plaques in terms of prevalence and size (Fagerberg et al., 2012). In that study, cadmium exposure was related to growth of plaques during follow-up. Hence, the result from the present study supports the hypothesis that cadmium exposure is associated with the occurrence and size of carotid plaques. Not only the prevalence of plaques, but also the size of carotid plaques has been shown to improve prediction of future cardiovascular disease in addition to cardiovascular risk factors (Rosvall et al., 2005; Wyman et al., 2006; Spence et al., 2002). In a clinical context this means that the cadmium exposure corresponding to the highest 25% in the present population sample is associated with an odds ratio of 1.3 for carotid plaque, and that those with plaques have an 80% increase in relative risk for future coronary events, on top of the risk associated with usual cardiovascular risk factors (Rosvall et al., 2005). In addition, comparing large carotid plaques with no, or very small plaques is associated with a three-fold increase in the risk of future cardiovascular disease after adjustment for traditional cardiovascular risk factors (Spence et al., 2002).

We also estimated the risk of plaque as a smooth function of blood cadmium, adjusted for sex, smoking and age. The result showed that in the range that encompassed the geometric means of the quartiles of blood cadmium (0.12–1.04 μg/L) there was a fairly linear association with plaque prevalence. Hence, we find it justifiable to use blood cadmium levels divided into quartiles in the statistical analyses.

In the present study, an interaction analysis did not reveal any effect modification by sex in the cadmium-related prevalence of plaques. Still, in the subgroup analysis of the association between blood cadmium levels and plaque prevalence, statistical significance was not attained for men in the fully adjusted model, except when smoking status was substituted by pack-years.

Previous epidemiological studies more often report an association between cadmium exposure and cardiovascular disease in men than in women (Tellez-Plaza et al., 2013). It is important to keep in mind that the occurrence of clinical atherosclerotic disease, such as symptomatic carotid stenosis, is dependent on the process of a stable plaque transforming into an unstable plaque.
on life-long smoking exposure are based on self-reported information, whereas measurement of cotinine only informs on recent smoking. In three of the studies in the meta-analysis mentioned above, adjustment was made concomitantly for smoking status, pack-years of smoking, and cotinine, and the association between cadmium exposure and cardiovascular disease remained (Tellez-Plaza et al., 2013). Never smokers are of particular interest and in the meta-analysis, the association between cadmium exposure and cardiovascular disease did not reach statistical significance in never smoking groups, although the point estimate (OR 1.27) suggested an effect. In the present study, we adjusted for smoking status in terms of never, ex-, and current smoking or for pack-years in a subgroup that did not include all subjects. In never smoking subjects, there was no indication that increasing cadmium levels were associated with increased risk of atherosclerotic plaques.

The effect of dietary exposure to cadmium has been examined in two Swedish population-based studies of 33,000 women and 37,000 men (Julin et al., 2013a; Julin et al., 2013b). The validity of this method of calculating cadmium intake from a food frequency questionnaire has been investigated and is further supported by the observations of significant positive associations between this measure of exposure from food and risk of fractures (Engström et al., 2012; Thomas et al., 2011) and prostate (Julin et al., 2012a), endometrial (Åkesson et al., 2008), and breast cancer (Julin et al., 2012b) in these cohorts. However, in recent reports on the same cohorts, no association was found between dietary cadmium intake and cardiovascular disease, although there were more than 8000 clinical events during 12 years of follow-up (Julin et al., 2013a; Julin et al., 2013b). We suggest that this inconsistency can be explained by two factors. Firstly, in the Swedish population, the exposure to cadmium through diet is not high enough to have discernable pro-atherogenic effects. Secondly, in never smokers, dietary intake of cadmium, as well as biochemical markers such as blood cadmium, are also measures of anti-atherogenic components in the diet. Regarding the first issue, it should be noted that the cadmium concentrations in blood or urine indicate that exposure is lower in Sweden (Fagerberg et al., 2012) than in many of

(Blanken et al., 2006). It is still unclear if, and how, cadmium contributes to this process.

The key issue is smoking, since it is both a strong cardiovascular risk factor and a very important source of cadmium exposure. Data

Table 3
Odds ratios for prevalences of smalla (<20% luminal stenosis) and largeb (>20–80% luminal stenosis) atherosclerotic plaques in the right carotid artery by quartiles of blood cadmium concentrations.

<table>
<thead>
<tr>
<th>Model (n in each quartile)</th>
<th>Odds ratios (95% confidence intervals) by quartiles of blood cadmium concentrations</th>
<th>P for trend</th>
<th>P for quartile 4 vs. quartile 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 [905] 1.0 (0.7–1.3) [913] 1.2 (0.9–1.6) [893] 2.0 (1.5–2.6) [812]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2 [908] 1.0 (0.7–1.3) [915] 1.2 (0.9–1.5) [893] 2.4 (1.9–3.2) [857]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3 [906] 1.0 (0.7–1.3) [916] 1.2 (0.9–1.5) [894] 2.4 (1.9–3.2) [856]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4 [907] 1.0 (0.7–1.3) [917] 1.2 (0.9–1.5) [895] 2.4 (1.9–3.2) [857]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* There were 551 subjects with small plaques, 601 subjects with large plaques and 2972 subjects with no plaques or stenoses.

b Model 1: Sex, age, smoking status (never smoker, ex-smoker, current smoker).

b Model 2: Additionally adjusted for <9 years of schooling, lowest quartile of physical activity score, and alcohol intake.

b Model 3: Additionally adjusted for waist circumference, systolic blood pressure, low density lipoprotein and high density lipoprotein cholesterol, triglycerides, HbA1c, C-reactive protein, antihypertensive treatment, lipid-lowering treatment, diabetes, postmenopausal status, hormone replacement therapy.

Fig. 3. Percentage of subjects according to smoking status in each quartile of blood cadmium in women (upper panel) and men (lower panel).
the studies from the US (Peters et al., 2010; Agarwal et al., 2011; Tellez-Plaza et al., 2010; Tellez-Plaza et al., 2012), Belgium (Naurot et al., 2008), and Korea (Lee et al., 2011). In the present study, we found that only 3.8% of the never smokers had a blood cadmium level corresponding to quartile 4, and the 95th percentile of blood cadmium in never smokers was < 0.5 μg/L. Our suggestion is that cadmium exposure from diet below this level is not associated with increased risk of carotid artery atherosclerosis. Further, it is well known that high dietary intake of cadmium is largely based on high intake of otherwise healthy food items, such as whole grains and vegetables, and that non-smokers consume more of these food items than ever-smokers (Nordberg et al., 2007; Julin et al., 2013a; Julin et al., 2013b). Finally, it is well documented in many studies that high intake of whole grains reduces the risk of cardiovascular disease (Jacobs and Gallaher, 2004). As a supporting observation of this paradoxical health promoting effect of cadmium containing food, we found that among never smokers in the present study, blood cadmium concentrations showed beneficial correlations with blood glucose \((r = -0.12, p < 0.0005)\), serum triglycerides \((r = -0.07, p < 0.0005)\), and HDL cholesterol \((r = 0.14, p < 0.0005)\). However, in the ex-smoking-smokers, we found no correlations to blood glucose or HDL cholesterol \((r = 0.00) for both) and a positive correlation to triglycerides \((r = 0.07, p < 0.0005)\).

A limitation of the present study is that the information on pack-years of smoking was incomplete, although it was available for 3800 subjects. We did not have access to urinary cadmium concentrations which are regarded as a better measure of long-term exposure in ex-smokers. However, recent studies have raised concerns about the usefulness of urine cadmium as a biomarker of long-term cadmium exposure in populations exposed to low to moderate cadmium levels (Akerstrom et al., 2013; Chaumont et al., 2013).

5. Conclusions

The conclusion is that our results extend previous studies of cadmium exposure and cardiovascular events by adding data on an association between cadmium and underlying atherosclerosis in humans. In the subgroups-analyses the association between dietary cadmium exposure and cardiovascular events by adding data on long-term exposure in ex-smokers. However, recent studies have raised concerns about the usefulness of urine cadmium as a biomarker of long-term exposure in populations exposed to low to moderate cadmium levels (Akerstrom et al., 2013; Chaumont et al., 2013).

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.11.004.

**References**


EFSA. European Food Safety Authority. 2009. Scientific opinion of the panel on contaminants in the food chain. EFSA J. 980, 1–139 (Available at), (http://www.


