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Cadmium exposure, intercellular adhesion molecule-1 and peripheral artery disease: a cohort and an experimental study

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ABSTRACT

Objectives: Cadmium exposure has been found to be associated with atherosclerotic plaques in the carotid arteries and with circulating levels of the proatherogenic intercellular adhesion molecule-1 (ICAM-1). The research questions were (1) if blood and urinary cadmium levels are associated with low ankle-brachial index (ABI) as a measure of peripheral artery disease in a longitudinal study and (2) if ICAM-1 mediates proatherogenic effects of cadmium exposure.

Design: A prospective, observational cohort study with a 5-year follow-up and an experimental study of cultured human aortic endothelial cells exposed to cadmium.

Setting: Research unit at a university hospital.

Participants: A cohort of 64-year-old women (n=489) recruited by stratified sampling of similarly sized groups with normal, impaired and diabetic glucose tolerance as assessed in a population-based screening examination.

Primary and secondary outcome measures: ABI (ratio of the systolic blood pressures in the tibial and brachial arteries ≤0.9 in any artery) in relation to cadmium exposure; ICAM-1 concentrations in the cell culture medium after cadmium incubation.

Results: High (tertile 3 vs 1) concentrations of blood (B-Cd) or creatine-adjusted urinary cadmium (U-Cd) at baseline were found to predict low ABI after adjustment for smoking and other cardiovascular risk factors at baseline. For U-Cd the OR was 2.5 (95% CI 1.1 to 5.8). After exclusion of participants with ultrasound-assessed femoral atherosclerosis at baseline the OR for U-Cd was unchanged, and for B-Cd it was 3.7 (95% CI 1.05 to 13.3). Inclusion of serum ICAM-1 levels did not affect the cadmium-related ORs in multivariate analyses. The experimental study did not show any cadmium-induced increase of the production of ICAM-1 from human endothelial cells.

Conclusions: Cadmium exposure was associated with future peripheral artery disease, supporting the concept that cadmium exposure in the population has proatherogenic effects, although ICAM-1 mediated effects do not seem to be involved.

ARTICLE SUMMARY

Article focus
- Cadmium exposure is associated with atherosclerotic plaques in the carotid arteries and with circulating levels of the proatherogenic intercellular adhesion molecule-1 (ICAM-1).
- The research questions were (1) if low ankle-brachial index as a measure of peripheral artery disease in a longitudinal study is associated with cadmium exposure and (2) if ICAM-1 mediates proatherogenic effects of cadmium exposure.

Key messages
- Cadmium exposure was associated with peripheral artery disease at the 5-year follow-up.
- Multivariate statistical analyses did not indicate that ICAM-1 mediates a proatherogenic effect of cadmium.
- In cultured human aortic endothelial cells, cadmium exposure did not induce the expression of ICAM-1.

Strengths and limitations of this study
- Further evidence of the proatherogenic effect of cadmium exposure was obtained and the hypothesis of ICAM-1 as a potential mediating molecule was not confirmed.
- As only women and individuals at similar age were studied, further studies of larger cohorts with both sexes are warranted.

INTRODUCTION

Cadmium is a non-essential metal widely distributed in the environment. Environmental exposure to cadmium occurs primarily through consumption of food and smoking.[1] [2] Women have higher cadmium uptake than men, mainly explained by increased intestinal absorption of dietary cadmium at low iron stores secondary to menstruation and pregnancy.[3] [4] Cadmium concentrations in urine and whole blood concentration have long biological half-lives and are valid biomarkers of exposure, irrespective of source.[1]

Results from cross-sectional and prospective studies have indicated an association between cadmium exposure and cardiovascular disease,[5–9] although these data are not consistent and results differ between countries and between men and women.[5–10] Experimental studies have shown that cadmium causes endothelium dysfunction in vitro and promotes the formation of atherosclerotic plaques in vivo.[5, 11–14] We have recently shown that in a cohort of 64-year-old women cadmium concentrations in whole blood and urine were associated with the prevalence and size of atherosclerotic plaques in the carotid arteries, independently of smoking and other cardiovascular risk factors.[15] In addition, we observed that the change in plaque area during more than 5-year follow-up was associated with cadmium exposure at baseline. A further finding was that circulating intercellular adhesion molecule-1 (ICAM-1) levels were associated with both degree of cadmium exposure and plaque occurrence and area.[15] ICAM-1 is expressed by endothelium and other cell types and involves both monocyte and lymphocyte adhesion to the endothelium which is believed to be an important component in the atherosclerotic process.[16] In endothelial cells from mouse brain microvessels cadmium has been reported to stimulate the expression of ICAM-1 via NF-κB activation.[17]

Against this background we hypothesised that cadmium-induced expression of ICAM-1 and proinflammatory cytokines in human endothelium might be a mechanistic link between cadmium-exposure and atherosclerosis. If that would be the case, circulating ICAM-1 levels might also be associated with the occurrence of peripheral artery disease. Such disease can be assessed as a low ankle-brachial index, which is both a valid measure of atherosclerotic disease in the arteries of the legs and a powerful indicator of future cardiovascular disease.[18] Hence, the aims of the present study were (1) to examine if cadmium exposure was associated with future peripheral artery disease measured as ankle-brachial index and (2) if ICAM-1 mediates proatherogenic effects of cadmium exposure.

METHODS
Subjects
As previously described in detail, all 64-year-old women in Gothenburg were identified in the County Register and were invited during 2001–2003 to Sahlgrenska University Hospital for a screening examination with oral glucose tolerance tests.[19] Exclusion criteria were cancer, chronic inflammatory disease and severe mental disorder. A stratified sampling procedure was used to recruit similar-sized groups of women with normal, impaired and diabetic glucose tolerance.[15 19] After a baseline examination that included 599 women with measurements of cadmium exposure, a follow-up examination was performed in 489 participants after a median time of 5.4 years (minimum 5.1, maximum 6.7 years). Reasons for non-participation have been

described elsewhere.[15] As also previously described, women lost to follow-up did not differ from the participants in the re-examination in terms of cadmium exposure, but had a more adverse cardiovascular risk factor profile including elevated serum ICAM-1 levels.[15] However, the prevalence of femoral artery plaques at baseline did not differ between participants (29.9%) and non-participants (30.6%). The study was approved by the regional ethics committee and all participating individuals gave informed consent.

Information was lacking on pack-years of smoking (n=21), statin treatment (n=7), HbA1c (n=3), ICAM-1 (n=1) and apoliprotein B/A-I (n=3). Data on both blood cadmium and creatine-adjusted cadmium concentrations were available in 422 women while one of these measures of cadmium exposure was available in 36 women.

Examinations
At baseline, questionnaires were used to obtain information on previous and present diseases, current medication and smoking history.[19] Pack-years were calculated as the number of cigarette packs smoked daily multiplied with the number of years of smoking. Body weight, height and blood pressure were measured, and blood samples were drawn after an overnight fast with preparation for storing in freezer at –80°C within 4 h. The participants collected urine for 12 h over night. The urine volume was measured and 20 ml was stored in freezer at –80°C.

As previously described in greater detail, the right femoral arteries were examined with ultrasound.[20] The examination was performed with an ultrasound scanner (Acuson Sequoia, Siemens, Mountain View, California, USA). The femoral artery was scanned distally to the inguinal ligament at the site where the artery divides into the superficial femoral artery and the profound femoral artery. The femoral artery was scanned ≈40 mm proximal to the bifurcation and further in the superficial and profound femoral arteries about 10 mm distal to the bifurcation, with the aim to identify and record the occurrence of atherosclerotic plaques. A plaque was defined as a distinct area with an intima-media thickness more than 50% thicker as compared with neighbouring sites (visually judged). The occurrence of femoral plaques is associated with the ankle-brachial index in both the left and right legs.[21]

At follow-up blood pressure in the posterior and anterior tibial arteries were measured twice bilaterally using a handheld Doppler (Nicolet, Vascular, Elite model no.100, Vialysis Helthcare, Madison, Wisconsin) and the mean values were calculated, respectively.[22] Mean systolic blood pressure was calculated from duplicate measurements in the right brachial artery using a cuff that was appropriate to the size of the arm. The ankle-brachial index was calculated by dividing the blood pressure for each ankle measurement with systolic blood pressure of the right arm. Presence of peripheral artery disease was

defined as an ankle-brachial index ≤0.9 in any of the four lower limb arteries.[18 20 21–23]

Biochemistry

As previously described in detail, cadmium concentrations were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK) at the department of Occupational and Environmental Medicine, Lund University, Sweden.[15] The detection limits were 0.05 µg/l for blood cadmium and 0.03 µg/l for urine cadmium, respectively. Imprecision as calculated from duplicate analyses was 4% (coefficient of variation; CV) for both blood and urine cadmium. High-sensitive ELISA kits were used to measure ICAM-1 (R&D System Europe Ltd, Abingdon, UK). Intra-assay and interassay CV for ICAM-1 were 3.3% and 6%. All other analyses were performed with well-documented routine methods as previously described.[19] WHO criteria were used for the definitions of diabetes mellitus.[24]

Cell culture and cadmium incubation

Fresh human aortic endothelial cells (Lonza, Basel, Switzerland) were cultured in EGM2 medium supplemented with 3% fetal bovine serum (Lonza, Basel, Switzerland), and seeded onto six-well tissue culture plates after two passages. The culture medium did not contain any phosphate-buffered saline. Cells were incubated for 48 h with 100 or 500 µg/l cadmium ($CdCl_2$). Cytokine levels in the cell culture medium were analysed with a SECTOR Imager 2400 reader (MesoScale Discovery, Gaithersburg, Maryland, USA). Total RNA was extracted from the cells using RNeasy Kit (QIAGEN, Hilden, Germany) and cDNA was synthesised using the high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA). mRNA expression of ICAM was analysed with TaqMan real-time PCR in an ABI Prism 7900 HT Detection System (Applied Biosystems).

Prior to this experiment we explored the effects of lower concentrations of cadmium. The human aortic endothelial cells were incubated for 72 h with and without $CdCl_2$ at three concentrations: 1, 10 and 100 µg/l. Aortic cellular expression of ICAM-1 was examined with fluorescence-activated cell sorting (FACS) (BD Accuri C6 Flow Cytometer, San Jose, California, USA) and ICAM-1 concentration was measured in the culture medium. We further tested with lower and higher concentrations of $CdCl_2$ (0.1 and 500 µg/l, respectively) and incubated the cells with and without the cadmium carrier metallothionein (25 ng/ml).

Statistics

Statistical analyses were performed with SPSS V.18.0 (SPSS Inc, Chicago, Illinois, USA). Results are presented as number (%), means (SD) or median (5–95 percentile) for skewed data. χ^2, analysis of variance and trend-tests were used to compare characteristics of participants by cadmium tertiles. Student t test was used for comparison of continuous variables. Pearson's correlation coefficients were calculated and linear multiple regression with log-transformation of skewed variables was used. Logistic regression analyses were performed to calculate the OR for occurrence of low ankle-brachial index. The 95% CI were calculated. The variables introduced in the statistical model were risk factors for cardiovascular disease at baseline (pack-years of smoking, current smoking, systolic blood pressure, HbA1c and apolipoprotein B/A-I as measures of dyslipidaemia) as well as glucose tolerance status at baseline (normal or impaired or diabetic glucose tolerance) and statin treatment. Serum ICAM-I was introduced in the final model. Femoral plaque occurrence at baseline was used as a proxy for prevalent stenotic artery disease in the legs. In a sensitivity analysis the calculation was repeated after exclusion of women with femoral plaques at baseline. Two-tailed p<0.05 was considered statistically significant. For analyses of non-linear association between cadmium levels and occurrence of low ankle-brachial index (see online supplementary material).

RESULTS

Cadmium exposure and occurrence of low ankle-brachial index

In this cohort the geometric means of blood cadmium and creatine-adjusted urinary cadmium were 0.37 µg/l and 0.36 µg/g creatine, respectively. As demonstrated in figure 1 increased levels of blood and urinary cadmium at baseline were associated with more cases with low ankle-brachial index in one or both legs at follow-up.

The baseline characteristics of the participants are described in table 1. Fifty-five women (12%) were found to have low ankle-brachial index at the follow-up examination. These women differed from the women with normal ankle-brachial index in characteristics at baseline. They were more often smokers, had more pack-years; the mean systolic blood pressure and the proportion of women on treatment with antihypertensive drugs were higher. HbA1c tended also to be higher and blood cadmium and urinary cadmium levels as well as serum ICAM-1 concentrations were elevated (table 1). This group had more often atherosclerotic plaques in the right femoral artery (table 1).

Logistic regression analysis with occurrence of low ankle-brachial index at follow-up as a dependent variable and baseline characteristics as independent variables was performed. In comparison with tertile 1 of blood cadmium levels, tertile 3 was associated with an unadjusted OR of 2.8 (95% CI 1.4 to 5.7) for occurrence of low ankle-brachial index, and 2.4 (0.9 to 6.3) after adjustment. This OR did not change after further adjustment for ICAM-1 (table 2). In the sensitivity analysis that only included women who were plaque free at baseline, the OR for low ankle-brachial index was 3.8 (1.1 to 13.3) after full adjustment (table 2). Corresponding analyses
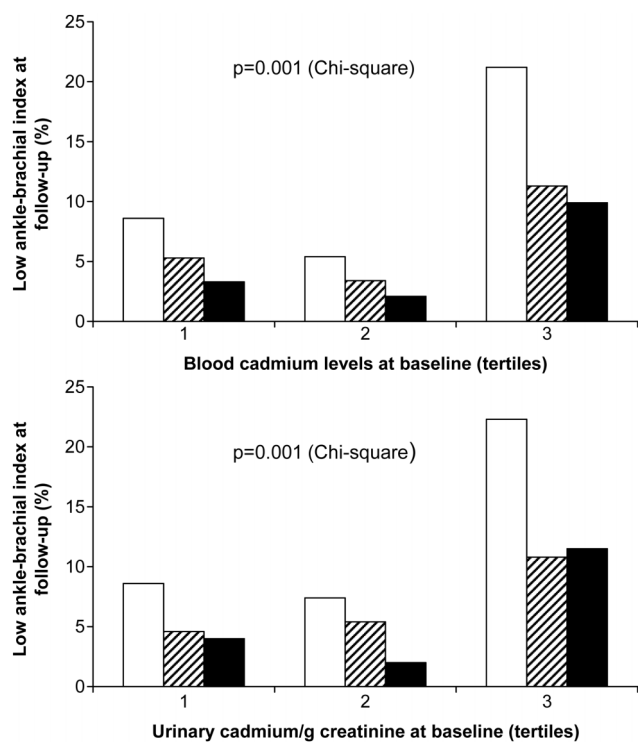
Figure 1 Baseline data on how tertiles of blood cadmium levels (upper panel) and creatine-adjusted urinary cadmium (lowel panel) were related to the occurrence of low ankle-brachial index (≤0.9) at the follow-up examination after 5.4 years. Unfilled bars represent any case with low ankle-brachial index, striped bars represent cases with low ankle-brachial index in one leg and black bars those with low ankle-brachial index in both legs.

for tertiles of creatine-adjusted urinary cadmium levels showed an unadjusted OR of 3.1 (1.5 to 6.1), and 2.5 (1.1 to 5.8) after adjustment with no major difference after further adjustment for ICAM-1 (table 3). In the sensitivity analysis the OR was 2.5 (0.8 to 7.4).

Logistic regression analyses were also performed with log2 transformed blood cadmium and urinary cadmium levels with adjustment for pack-years, and current smoking as independent variables. The corresponding ORs were 1.3 (0.9 to 2.1) and 1.0 (0.98 to 1.03), respectively. Taken together, these analyses indicate a non-linear relationship between cadmium exposure and occurrence of low ankle-brachial index. This observation is further supported by an analysis based on B-Cd and U-Cd treated as continuous variables using splines (see online supplementary material).

Serum ICAM-1 in relation to low ankle--brachial index, cadmium levels and smoking

At baseline ICAM-1 levels were gradually increased from women who had normal ankle-brachial index to those with low index at follow-up in one leg, and both legs, respectively (246 (percentiles 5–95: 178–381), 282 (133–563), and 294 (195–593) ng/ml, respectively, p=0.001 for trend). Log ICAM-1 correlated to log blood cadmium (r=0.24, p<0.001), log creatine-adjusted urinary cadmium (r=0.22, p<0.001), pack-years (r=0.21, p<0.001) and current smoking (r=0.26, p<0.001). In a multiple regression analysis the associations between log ICAM-1 and log creatine-corrected urinary cadmium levels remained after adjustment for current smoking and pack-years, whereas this was not the case for log blood cadmium (data not shown). The corresponding geometric mean ratios (95% CI) of ICAM-1 per unit increase in log urine or log blood cadmium levels were 1.13 (1.03 to 1.26) and 1.09 (0.96 to 1.24), respectively. Log ICAM-1 levels were mainly associated with current smoking in multivariate analysis (p<0.008).

Cell culture and cadmium incubation

As we had found that serum ICAM levels were associated with both circulating cadmium concentrations and occurrence of low ankle-brachial index, we tested the hypothesis that cadmium has proinflammatory effects on endothelium. Fresh human aortic endothelial cells were incubated for 48 h in a medium supplemented with 100 or 500 μg/l cadmium (table 4). The mRNA expression of ICAM and cytokine levels in the culture medium were then analysed. The results showed that cadmium did neither induce increased expression of ICAM or increased cytokine levels in the culture medium (table 2).

In the initial exploring experiment with lower concentrations of cadmium in the culture medium the results did not indicate any effects of CdCl2. The FACS results showed that the aortic endothelial cell expression of ICAM-1 (median) were 50 658, 52 099, 50 134 and 48 554 fluoroscence units in untreated cells and in those incubated with 1, 10 and 100 μg/l. Neither did the levels of ICAM-1 in the culture medium show any clear differences between the different cadmium concentrations (data not shown). In the additional tests with lower and higher concentrations of CdCl2 (0.1 and 500 μg/l, respectively) and incubation with and without the cadmium carrier metallothionein (25 ng/ml) no increased expression of ICAM-1 was observed (data not shown).

DISCUSSION

We found that blood cadmium concentrations above 0.44 μg/l and urinary cadmium levels above 0.46 μg/g creatine were associated with OR>2 for peripheral artery disease in comparison with participants in the lowest tertiles of cadmium exposure after 5.4 years of follow-up. This increase in risk remained for blood cadmium levels after exclusion of participants with plaques in the right femoral artery and adjustment for pack-years, current smoking and other cardiovascular risk factors at baseline. Previously published studies from the National Health and Nutrition Examination Survey have shown

Table 1 Characteristics at baseline of the cohort of 64-year-old women by occurrence of low ankle-brachial index (ABI) at follow-up after 5.4 years (n=458)

Baseline characteristics	At the follow-up examination			p Value (ABI groups)
	ABI ≤0.9 (n=55)	ABI >0.9 (n=403)	Total (n=458)	
Body mass index (kg/m^2) (mean (SD))*	27.7 (4.1)	27.6 (4.5)	27.6 (4.4)	0.87
Smoking				
Never, n (%)	19 (35)	197 (49)	216 (47)	0.001
Previous, n (%)	16 (29)	148 (37)	164 (36)	
Current, n (%)	20 (36)	58 (14)	78 (17)	
Pack-years, n (median (5–95 percentiles))	14 (0–47)	1 (0–35)	1.4 (0–40)	0.001
Known hypertension, n (%)	20 (36)	104 (26)	124 (27)	0.10
Diabetes mellitus, n (%)	23 (42)	118 (29)	141 (31)	0.06
Hypertension treatment, n (%)	25 (46)	119 (30)	144 (31)	0.017
Statin treatment, n (%)	11 (20)	47 (12)	58 (13)	0.081
Systolic blood pressure (mm Hg)	151 (23)	142 (18)	144 (19)	0.008
Serum apolipoproteins B/A-I (median (5–95 percentiles))	0.75 (0.44–1.10)	0.71 (0.46–1.15)	0.72 (0.48–1.14)	0.74
HbA1c (%) (median (5–95 percentiles))	4.8 (4.2–9.1)	4.7 (4.1–6.3)	4.7 (4.1–6.6)	0.051
Serum ICAM-1 (ng/ml) (median (5–95 percentiles))	292 (163–541)	246 (178–381)	249 (178–390)	0.001
Blood cadmium (μg/l) (median (5–95 percentiles)) (51/389) †	0.53 (0.14–2.19)	0.31 (0.14–1.15)	0.33 (0.14–1.65)	0.001
Urinary cadmium (μg/g creatine) (median (5–95 percentiles)) (55/385)†	0.57 (0.14–1.47)	0.34 (0.14–0.95)	0.35 (0.14–0.96)	0.001
Plaque in the right femoral artery, n (%)	23 (42)	112 (28)	135 (30)	0.034

*SD-standard deviation.
†Number of cases with available data in the ABI ≤0.9/>0.9 groups.
HbA1c, glycated haemoglobin; ICAM-1, proatherogenic intercellular adhesion molecule-1.

Table 2 OR (95% CI) for low ankle-brachial index (ABI) after 5 years of follow-up in women who were 64-years old at baseline and in relation to tertiles of blood cadmium levels, serum ICAM-1 and prevalence of atherosclerotic plaques in the femoral artery at baseline

All (n=440)					No femoral plaque at baseline (n=312)			
Tertiles (B-Cd, μg/l)*	1 (0.08 to 0.25)	2 (0.25 to 0.44)	3 (0.44 to 4.07)	p Value (tertile 3/1)	1 (0.08 to 0.25)	2 (0.25 to 0.44)	3 (0.44 to 4.07)	p Value (tertile 3/1)
ABI ≤ 0.9, n (%)†	13 (8.6)	8 (5.4)	30 (21.1)		7 (6.1)	6 (5.5)	17 (19.1)	
Unadjusted	1	0.6 (0.3 to 1.5)	2.8 (1.4 to 5.7)	0.003	1	0.9 (0.3 to 2.7)	3.6 (1.4 to 9.1)	0.007
Adjustment‡	1	0.6 (0.2 to 1.6)	2.4 (0.9 to 6.3)	0.068	1	0.9 (0.3 to 3.0)	3.7 (1.05 to 13.3)	0.042
Adjustment‡ + ICAM-1	1	0.6 (0.2 to 1.6)	2.4 (0.9 to 6.3)	0.068	1	0.9 (1.3 to 3.1)	3.8 (1.1 to 13.3)	0.041

*Minimum–maximum.
†Number (%) of low ankle-brachial index in each tertile.
‡Adjustment for pack-years of smoking, current smoking, systolic blood pressure, HbA1c, apolipoprotein B/A-I, statin treatment, stratification group at baseline (normal glucose tolerance, impaired glucose tolerance, diabetes).
HbA1c, glycated haemoglobin; ICAM-1, proatherogenic intercellular adhesion molecule-1.

Table 3 OR (95% CI) for low ankle-brachial index (ABI) after 5 years of follow-up in women who were 64-year old at baseline and in relation to tertiles of creatine-adjusted urinary cadmium levels, serum ICAM-1 and prevalence of atherosclerotic plaques in femoral artery at baseline

All (n=440)					No femoral plaque at baseline (n=309)			
Tertiles (U-Cd, μg/g creatine)*	1 (0.06 to 0.28)	2 (0.29 to 0.46)	3 (0.46 to 2.06)	p Value (tertile 3/1)	1 (0.06 to 0.28)	2 (0.29 to 0.46)	3 (0.47 to 1.89)	p Value (tertile 3/1)
ABI ≤0.9 (n)†	13 (8.6)	11 (7.4)	31 (22.3)		8 (7.1)	8 (7.6)	16 (17.6)	
Unadjusted	1	0.9 (0.4 to 2.0)	3.1 (1.5 to 6.1)	0.002	1	1.1 (0.4 to 3.0)	2.8 (1.1 to 6.9)	0.025
Adjustment‡	1	0.8 (0.3 to 1.8)	2.5 (1.1 to 5.8)	0.037	1	1.0 (0.3 to 2.8)	2.5 (0.8 to 7.4)	0.11
Adjustment‡ + ICAM-1	1	0.9 (0.6 to 1.5)	2.4 (1.0 to 5.5)	0.050	1	0.9 (0.3 to 2.8)	2.4 (0.8 to 7.2)	0.12

*Minimum–maximum.
†Number (%) of low ankle-brachial index in each tertile.
‡Adjustment for pack-years of smoking, current smoking, systolic blood pressure, HbA1c, apolipoprotein B/A-I, statin treatment, stratification group at baseline (normal glucose tolerance, impaired glucose tolerance, diabetes).
HbA1c, glycated haemoglobin; ICAM-1, proatherogenic intercellular adhesion molecule-1.

Table 4 Expression of ICAM-1 (normalised ICAM/18s) and proinflammatory and anti-inflammatory cytokines by fresh human aortic endothelial cells after incubation for 48 h in medium supplemented with cadmium at two concentrations in comparison with controls (mean±SEM)

	Controls n=12	Cadmium (100 µg/l) n=10	Cadmium (500 µg/l) n=10
ICAM-1	0.91±0.37	0.63±0.19	0.83±0.19
Granulocyte-macrophage colony-stimulating factor	19.9±8.7	15.7±6.1	12.8±3.0
Interferon-γ	34.1±9.6	30.0±6.6	29.1±5.8
Interleukin-10	5.1±0.78	5.0±1.1	5.5±2.1
Interleukin 12 p70	8.2±1.8	7.0±0.87	7.6±1.48
Interleukin-1β	4.1±0.94	4.8±1.6	4.2±1.0
Interleukin-2	22.8±12.0	23.0±18.4	15.4±5.6
Interleukin-6	419±35	422±43	385±54
Interleukin-8	3204±269	3181±480	2862±354
Tumor necrosis factor-α	11.1±2.1	10.1±1.3	9.3±0.87

ICAM-1, proatherogenic intercellular adhesion molecule-1.

associations between cadmium exposure and low ankle-brachial index in cross-sectional studies.[7 25 26]

We also observed that circulating ICAM-1 concentrations were associated with peripheral artery disease in the legs, and that ICAM-1 correlated with urinary cadmium independently of smoking. We have recently reported that ICAM-1 was associated with the occurrence and area of atherosclerotic plaques in the carotid arteries in the same cohort as in the present study.[15] However, the results from our experimental study failed to show that cadmium exposure induces expression of ICAM-1 from human aortic endothelial cells. In addition, the OR for low ankle-brachial index did not change when ICAM-1 was included in the statistical models in a final step in the present study. Hence, we find no support for the hypothesis that cadmium-induced expression of ICAM-1 in human endothelium might be a mechanistic link between cadmium-exposure and atherosclerosis. Neither did we find any indication that cadmium increased the production of proinflammatory cytokines from aortic endothelial cells. The concentrations of cadmium used (100—500 µg/l) are expected to increase intracellular cadmium concentrations in endothelial cells.[27 28] Tentatively, smoking may have confounded the results as tobacco smoke is both proatherogenic and known to induce ICAM-1 expression in endothelial cells.[29] The subgroup of never-smokers was small and their cadmium levels were very low. Therefore we cannot exclude that effects shown after adjustment for smoking may in fact be caused by a combination of cadmium and some other component in cigarette smoke. Finally, it must also be kept in mind that endothelial cells differ, depending on type of vessel, vessel diameter, flow conditions and surrounding tissue.[30] Hence, the endothelial cells we used may not have been representative of in vivo mechanisms. Bernhard et al[31] have reported that cadmium downregulates a number of proinflammatory genes in arterial endothelial cells. Other mechanisms may also be involved. Cadmium is known to affect physiological signal transduction and to disrupt endothelial integrity. One

mechanism is that of the disruption of endothelial cadherin–cadherin bonds leading to increased permeability.[12] Messner et al[13] have previously shown that cadmium also increases vascular endothelial permeability by inhibiting endothelial cell proliferation and inducing cell-death. Precise information on uptake of cadmium in vascular cells and effects on the atherosclerotic process are still lacking.

Limitations of the present cohort study are that only women and individuals of similar age were studied. However, this is also advantageous as the effects of sex and age are kept constant. The study findings have to be confirmed in prospective studies in population samples including both men and women and with a broader age range. The lack of data from individuals lost to follow-up may have introduced selection bias, even if they did not differ in occurrence of femoral artery plaques, cadmium levels in blood and urine at baseline. The size of the study limits the precision of analyses in subgroups, but on the other hand our precision to diagnose peripheral artery disease was high as we measured ankle-brachial index in both anterior and posterior tibial arteries in both legs. As previously shown, measurements restricted to only the posterior tibial arteries results in a failure to identify 30–40% of those with peripheral artery disease.[22] Previous studies have not measured ankle-brachial index in both anterior and posterior tibial arteries.[7 25–26] We did not measure ankle-brachial index at baseline but used the occurrence of atherosclerotic plaques in the right femoral artery as a proxy for prevalence of lower limb atherosclerosis. As previously reported, plaque occurrence in the right common femoral artery is significantly associated with ankle-brachial index both in the right and left legs.[21]

In conclusion, cadmium exposure at levels often found in the population was associated with peripheral artery disease, assessed as low ankle-brachial index at follow-up after 5.4 years. ICAM-1 mediated effects do not seem to be involved. Taken together the study gives further support to the concept that cadmium exposure

of the population may be an important environmental factor with proatherogenic effects. Future studies are needed to confirm the findings and to clarify the underlying proatherogenic mechanisms.

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REFERENCES

1. Nordberg GF, Nogawa K, Nordberg M, *et al*. Cadmium. In: Nordberg GF, Fowler GF, Nordberg M, Friberg L. *Handbook on the toxicology of metals*. Amsterdam: Elsevier, 2007:445–86.
2. EFSA, European Food Safety Authority. Scientific opinion of the panel on contaminants in the food chain. *EFSA J* 2009;980:1–139. http://www.efsa.europa.eu/en/efsajournal/doc/980.pdf (accessed 30 Jan 2009).
3. Berglund M, Akesson A, Nermell B, *et al*. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ Health Perspect* 1994;102:1058–66.
4. Akesson A, Berglund M, Schütz A, *et al*. Cadmium exposure in pregnancy and lactation in relation to iron status. *Am J Public Health* 2002;92:284–7.
5. Järup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* 2009;238:201–8.
6. Menke A, Muntner P, Silbergeld EK, *et al*. Cadmium levels in urine and mortality among U.S. adults. *Environ Health Perspect* 2009;117:190–6.
7. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, *et al*. Cadmium and peripheral arterial disease: gender differences in the 1999–2004 US National Health and Nutrition Examination Survey. *Am J Epidemiol* 2010;172:671–81.
8. Peters JL, Perlstein TS, Perry MJ, *et al*. Cadmium exposure in association with history of stroke and heart failure. *Environ Res* 2010;110:199–206.
9. Tellez-Plaza M, Navas-Acien A, Menke A, *et al*. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. *Environ Health Perspect* 2012;120:1017–22.
10. Gallagher CM, Kovach JS, Meliker JR. Urinary cadmium and osteoporosis in U.S. women ≥50 years of age: NHANES 1988–1994 and 1999–2004. *Environ Health Perspect* 2008;116:1338–43.
11. Prozialeck WC, Edwards JR, Woods JM. The vascular endothelium as a target of cadmium toxicity. *Minirev Life Sci* 1998;79:1493–506.
12. Messner B, Bernhard D. Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance. *Biometals* 2010;23:811–22.
13. Messner B, Knoflach M, Seubert A, *et al*. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. *Arterioscler Thromb Vasc Biol* 2009;29:1392–8.
14. Knoflach M, Messner B, Shen YH, *et al*. Non-toxic cadmium concentrations induce vascular inflammation and promote atherosclerosis. *Circ J* 2011;75:2491–5.
15. Fagerberg B, Bergström G, Borén J, *et al*. Cadmium exposure is accompanied by increased prevalence and future growth of atherosclerotic plaques in 64-year-old women. *J Intern Med* 2012;272:601–10.
16. Schmidt C, Hulthe J, Fagerberg B. Baseline ICAM-1 and VCAM-1 are increased in initially healthy middle-aged men who develop cardiovascular disease during 6.6 years of follow-up. *Angiology* 2009;60:108–14.
17. Jeong EM, Moon CH, Kim CS, *et al*. Cadmium stimulates the expression of ICAM-1 via NF-kappaB activation in cerebrovascular endothelial cells. *Biochem Biophys Res Commun* 2004;320:887–92.
18. Resnick HE, Lindsay RS, McDermott MM, *et al*. Relationship of high and low ankle brachial index to all-cause and cardiovascular disease mortality: the Strong Heart Study. *Circulation* 2004;109:733–9.
19. Brohall G, Behre CJ, Hulthe J, *et al*. Prevalence of diabetes and impaired glucose tolerance in 64-year-old Swedish women: experiences of using repeated oral glucose tolerance tests. *Diabetes Care* 2006;29:363–7.
20. Schmidt C, Fagerberg B. ApoB/apoA-I ratio is related to femoral artery plaques in 64-year-old women also in cases with low LDL cholesterol. *Atherosclerosis* 2008;196:817–22.
21. Suurküla M, Fagerberg B, Wendelhag I, *et al*. Atherosclerotic disease in the femoral artery in hypertensive patients at high cardiovascular risk. The value of ultrasonographic assessment of intima-media thickness and plaque occurrence. Risk Intervention Study (RIS) Group. *Arterioscler Thromb Vasc Biol* 1996;16:971–7.
22. Johansson K, Behre CJ, Bergström G, *et al*. Ankle-brachial index should be measured in both the posterior and the anterior tibial arteries in studies of peripheral arterial disease. *Angiology* 2010;61:780–3.
23. Schroder F, Diehm N, Kareem S, *et al*. A modified calculation of ankle-brachial pressure index is far more sensitive in the detection of peripheral arterial disease. *J Vasc Surg* 2006;44:531–6.
24. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539–53.
25. Navas-Acien A, Selvin E, Sharrett AR, *et al*. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation* 2004;109:3196–201.
26. Navas-Acien A, Silbergeld EK, Sharrett R, *et al*. Metals in urine and peripheral arterial disease. *Environ Health Perspect* 2005;113:164–9.
27. Von Zglinicki T, Edwall C, Ostlund E, *et al*. Very low cadmium concentrations stimulate DNA synthesis and cell growth. *J Cell Sci* 1992;103:1073–81.
28. Kaji T, Mishima A, Koyanagi E, *et al*. Possible mechanism for zinc protection against cadmium cytotoxicity in cultured vascular endothelial cells. *Toxicology* 1992;76:257–70.
29. Kalra VK, Ying Y, Deemer K, *et al*. Mechanism of cigarette smoke condensate induced adhesion of human monocytes to cultured endothelial cells. *J Cell Physiol* 1994;160:154–62.
30. Garlanda C, Dejana E. Heterogeneity of endothelial cells. Specific markers. *Arterioscler Thromb Vasc Biol* 1997;17:1193–202.
31. Bernhard D, Rossmann A, Henderson B, *et al*. Increased serum cadmium and strontium levels in young smokers: effects on arterial endothelial cell gene transcription. *Arterioscler Thromb Vasc Biol* 2006;26:833–8.