Cadmium Exposure and Incident Peripheral Arterial Disease

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Background—Cadmium has been associated with peripheral arterial disease (PAD) in cross-sectional studies, but prospective evidence is lacking. Our goal was to evaluate the association of urine cadmium concentrations with incident PAD in a large population-based cohort.

Methods and Results—A prospective cohort study was performed with 2864 adult American Indians 45 to 74 years of age from Arizona, Oklahoma, and North and South Dakota who participated in the Strong Heart Study from 1989 to 1991 and were followed through 2 follow-up examination visits in 1993 to 1995 and 1997 to 1999. Participants were free of PAD, defined as an ankle brachial index <0.9 or >1.4 at baseline, and had complete baseline information on urine cadmium, potential confounders, and ankle brachial index determinations in the follow-up examinations. Urine cadmium was measured using inductively coupled plasma mass spectrometry and corrected for urinary dilution by normalization to urine creatinine. Multivariable-adjusted hazard ratios were computed using Cox-proportional hazards models for interval-censored data. A total of 470 cases of incident PAD, defined as an ankle brachial index <0.9 or >1.4, were identified. After adjustment for cardiovascular disease risk factors including smoking status and pack-years, the hazard ratio comparing the 80th to the 20th percentile of urine cadmium concentrations was 1.41 (1.05–1.81). The hazard ratio comparing the highest to the lowest tertile was 1.96 (1.32–2.81). The association persisted after excluding participants with ankle brachial index >1.4 only as well as in subgroups defined by sex and smoking status.

Conclusions—Urine cadmium, a biomarker of long-term cadmium exposure, was independently associated with incident PAD, providing further support for cadmium as a cardiovascular disease risk factor. (Circ Cardiovasc Qual Outcomes. 2013;6:626-633.)

Key Words: cadmium ■ peripheral arterial disease
WHAT IS KNOWN

• Humans are exposed to cadmium through smoking, diet, and ambient air.
• Cadmium induces atherosclerosis and vascular dysfunction in experimental settings.
• Cadmium is associated with prevalent atherosclerotic disease in studies from the United States and other countries.
• Cadmium has been prospectively associated with peripheral arterial disease (PAD) in 64-year-old women from Sweden.

WHAT THE STUDY ADDS

• The article provides prospective evidence supporting cadmium exposure as a risk factor for the development of PAD in men and women independently of smoking status.

as the SHS. Nonetheless, individuals with either ABI <0.90 or >1.40 can be considered at increased risk of incident cardiovascular events and mortality independently of the presence of symptoms of PAD and other cardiovascular risk factors.24 As a consequence, we also studied ABI <0.9 in at least 1 leg only or >1.4 in at least 1 leg only as secondary outcomes. Cumulative cadmium exposure was evaluated by measuring cadmium in urine, a biomarker of long-term exposure with a half-life of decades.2 Because a cross-sectional study from a US population exposed to low levels of cadmium suggested interactions by sex and smoking,25 we also evaluated the associations in subgroups defined by sex and smoking status.

Methods

Study Population

SHS is a large population-based prospective cohort study of cardiovascular disease in American Indian communities in Arizona, Oklahoma, and the Dakotas.26-27 Conducted in a population with high rates of cardiovascular disease, SHS is one of the major cardiovascular cohorts funded by the National Heart, Lung, and Blood Institute in the United States and has served as a model for the evaluation of physiological and environmental risk factors in populations with a high burden of cardiovascular disease. From 1989 to 1991, men and women 45 to 75 years of age from 13 American Indian communities in Arizona, Oklahoma, and North and South Dakota were invited to participate in the SHS. In Arizona and Oklahoma, every eligible person was invited; in North and South Dakota, a cluster sampling technique was used.26,28 The overall participation rate was 62%.26 From the original cohort, 3974 individuals had frozen urine specimens available for metal determinations. At baseline, the number of individuals with prevalent primary end point (ABI <0.9 or >1.40 in at least 1 leg) and secondary endpoints (ABI <0.9 in at least 1 leg only and >1.40 in at least 1 leg only) was 415, 182, and 234, respectively. One individual at baseline showed 1 leg with ABI <0.9 and the other leg with ABI >1.4.

For this study, we used data from 3559 SHS participants with urine samples available for metal determinations who were free of PAD at baseline (defined as ABI <0.9 or >1.40). We further excluded 396 participants missing follow-up ABI determinations, 2 participants missing urine cadmium determinations because of insufficient sample, 129 participants missing smoking information, and 168 participants missing other variables of interest, leaving 2864 participants for the primary analysis. Included participants were similar to excluded participants in age and body mass index but were predominantly women and had self-reported diabetes mellitus and hypertension (data not shown). In secondary analyses, alternative PAD definitions included ABI <0.9 only and >1.40 only (N=2950 and 2937 participants, respectively, after excluding prevalent PAD cases and participants with missing corresponding follow-up variables and baseline data). The study protocol was approved by the Institutional and Indian Health Service Review Boards and the participating American Indian communities. All the participants provided oral and written informed consent.

Baseline Data Collection

Sociodemographic data (age, sex, geographical location, education), postmenopausal status, smoking history (smoking status and pack-years), and history of diabetes mellitus and medication use were obtained through baseline SHS questionnaire administered by trained and certified staff.28 Body mass index was calculated as measured weight in kilograms divided by measured height in meters-squared. Three consecutive blood pressure determinations were taken from the right arm in the sitting position after 5 minutes of rest using a Baum mercury sphygmomanometer,26 and the last 2 determinations were averaged. Hypertension was defined as a mean systolic blood pressure ≥140 mm Hg, a mean diastolic blood pressure ≥90 mm Hg, or antihypertensive medication use.

Total cholesterol, plasma glucose, and plasma creatinine were measured in fasting serum samples using enzymatic methods (reagent kits from Boehringer Mannheim Diagnostics, Indianapolis, IN). Low-density lipoprotein cholesterol was estimated by the Friedewald equation.29 High cholesterol was defined as estimated low-density lipoprotein cholesterol ≥130 mg/dL. HbA1c was measured by high-performance liquid chromatography.30 Diabetes mellitus was defined as a fasting glucose ≥126 mg/dL, a nonfasting glucose ≥200 mg/dL, an HbA1c ≥6.5%, or the use of insulin or oral hypoglycemic agents. Estimated glomerular filtration rate was calculated from calibrated creatinine, age, and sex using the Modification of Diet in Renal Disease (MDRD) study formula.31 The MDRD equation factor for ethnicity was dropped for all participants.

Urine Cadmium Determinations

Spot urine samples were collected in polypropylene tubes, frozen within 1 to 2 hours of collection, shipped on dry ice, and stored at −70°C in the Penn Medical Laboratory, MedStar Research Institute, Washington, DC. In 2009, up to 1.0 mL of urine from each participant was transported on dry ice to the Trace Element Laboratory of the Institute of Chemistry—Analytical Chemistry, Graz University, Graz, Austria. We measured cadmium using inductively coupled plasma mass spectrometry (Agilent 7700x ICPMS; Agilent Technologies, Waldbronn, Germany), a multielement technique that also provided information on other metals such as As, Mo, Pb, Sb, Se, W, and Zn. In this article, we focused on cadmium because we have strong experimental and epidemiological evidence supporting the study hypothesis. For other metals, except As, limited evidence is available supporting a possible role in cardiovascular disease development. The analytical methods used and the associated quality control criteria have been previously described.32 The limit of detection for urine cadmium was 0.015 µg/L. Only 1 participant had urine cadmium concentration below the limit of detection. Cadmium concentration in this participant was imputed as the limit of detection divided by the square root of 2. The Seronorm Trace Elements Urine blank (Sero AS, Billingstad, Norway) with a recommended cadmium concentration of 0.35 µg/L was used for quality control. We found a mean of 0.38 (SD 0.07) µg/L (n=66). Additionally, cadmium concentration in the certified reference material NIST 1643e trace elements in water (NIST, Gaithersburg, MD) was determined in each run. We obtained a mean of 6.42 (SD 0.38) µg/L (n=79), whereas the certified concentration mean was 6.57 (SD 0.07) µg/L. Following the methods by Jarrett et al.,13 urine cadmium concentrations were corrected for molybdenum oxide interference using the formula [Cd]corr=[Cd]−0.0016×[Mo].
To account for urine dilution, urine cadmium concentrations were expressed in micrograms per gram of urine creatinine. Urine creatinine was measured at the Laboratory of the National Institute of Diabetes and Digestive and Kidney Disease, Epidemiology and Clinical Research Branch, Phoenix, AZ, by an alkaline picrate methodology conducted in a rapid flow analyzer.26

**Incident PAD Follow-Up**

Right arm blood pressure and bilateral ankle blood pressure for ABI determinations were measured twice, with the subject in supine position, using a hand-held Doppler device (Imex Medical Systems, Golden, CO). ABI for each leg was computed as the mean systolic blood pressure in the ankle (posterior tibial artery) divided by the mean systolic blood pressure in the right arm (brachial artery). In the main analysis, incident cases of PAD were defined as a newly diagnosed ABI <0.90 or >1.40 in at least 1 leg in at least 1 of the 2 follow-up clinic visits conducted in 1993 to 1995 and 1997 to 1999. Stenotic PAD and vascular calcification are generally long-term progressive processes.24,34 Consequently, participants missing ABI determinations in the 1993 to 1995 visit, but free of PAD in 1997 to 1999 (N=161), were imputed as not having PAD at the 1993 to 1995 visit. Participants missing ABI determinations in the baseline visit but free of PAD in the 1993 to 1995 visit (N=30) were imputed as not having PAD in the baseline visit.

Time to event was calculated from the date of baseline examination in 1989 to 1991 to the date of follow-up examination with newly diagnosed PAD for participants with incident PAD and to the date of the last examination available or the date of death, whichever occurred first, for participants without incident PAD. The mean follow-up time among participants who did and did not develop PAD (primary outcome) was 4.3 and 6.9 years, respectively.

**Statistical Methods**

Urine cadmium levels were markedly right-skewed and log-transformed for statistical analyses. The prospective association of urine cadmium concentrations with cardiovascular disease incidence was evaluated using Cox-proportional hazard models for interval-censored data using the R-package intcox35 using years since baseline enrollment as the time scale. The assumption of hazards proportionality was visually assessed based on the smoothed association between time and log(−log[S(t)]), with S(t) being the survival at time t, by urine cadmium categories, with no major departures from proportionality.

We used 3 statistical models with progressive adjustment. In model 1, we adjusted for sex, age at baseline (continuous as restricted cubic splines with knots at 50 and 60 years), education (≥12 years education completed, <12 years education completed), and geographical location (Arizona, Dakotas, Oklahoma). In model 2, we additionally adjusted for postmenopausal status for women (no, yes), body mass index (continuous), hypertension (no, yes), diabetes mellitus (no, yes), low-density lipoprotein cholesterol (continuous), and estimated glomerular filtration rate (continuous). In model 3, we additionally adjusted for smoking status (never, former, current) and cumulative smoking dose (pack-years modeled as restricted cubic splines with knots at 10, 20, and 30 pack-years). Urine cadmium was introduced in the models as cadmium tertiles (categorical) or log-transformed cadmium concentrations (continuous). We also modeled nonlinear relationships between cadmium levels and incidence of cardiovascular endpoints by using restricted quadratic splines with knots at the 5th, 50th, and 95th percentiles of the creatinine-corrected urine cadmium (which corresponded to 0.33, 0.94, and 2.65 μg/g, respectively).

Based on previous studies,25 we conducted subgroup analyses, including interaction terms for log-transformed urine cadmium concentrations with the corresponding indicator variables for subgroups defined by sex (men, women) and smoking status (never, former, current) in separate models. Bootstrap confidence intervals for the hazard ratios were estimated as bias-corrected and accelerated percentile intervals. Probability values for linear and nonlinear trend and probability values for interaction were estimated using Wald tests based on the bootstrap standard errors. We conducted sensitivity analysis by modeling urine cadmium concentrations in micrograms per liter and adjusting for urine creatinine concentrations instead of dividing urine cadmium by urine creatinine concentrations, with consistent findings. An additional sensitivity analysis was conducted using Weibull regression models for interval-censored data with proportional hazards parameterization, with similar results (data not shown).

**Results**

The overall geometric mean of urine cadmium concentrations at baseline was 0.94 μg/g creatinine (0.83, 0.86, and 1.16 μg/g for Arizona, Oklahoma, and North and South Dakota, respectively). A total of 470 incident PAD (primary outcome) cases were identified through 1999 (248 in visit 2 and 222 in visit 3). Incident PAD status was associated with lower levels of education; higher age, body mass index, and cigarette pack-years; and increasing prevalence of ever smoking status, diabetes mellitus, and hypertension (Table 1).

The adjusted hazard ratio of PAD comparing the highest to the lowest tertile of urine cadmium concentrations was 1.96 (1.32, 2.81; Table 2, model 3). In detailed dose–response analyses using restricted quadratic splines, the association between cadmium concentrations and incident PAD did not show, overall, significant departures from linearity (P for non-linear components, 0.31). Among smokers, the association was clearly linear throughout the whole range of cadmium levels (Figure). In never-smokers, a linear association was also observed in the range of cadmium concentrations between 0.4 and 1.7 μg/g (Figure). Assuming a linear relationship, the fully adjusted hazard ratios (95% confidence intervals) for incident PAD comparing the 80th to the 20th percentile of urine cadmium distribution in the overall sample, in ever-smokers, and in never-smokers were 1.41 (1.05–2.05), 150 (1.05–2.05), and 1.25 (0.83–1.80), respectively (Table 3). The probability value for the interaction of cadmium by smoking status was 0.45. The associations were consistent for incident PAD defined as either ABI <0.9 only or >1.4 only (Table 2; Figures I and II in the online-only Data Supplement). The adjusted hazard ratios of PAD comparing the highest to the lowest tertile of urine cadmium concentrations were 2.10 (1.16–3.53) for ABI <0.9 in at least 1 leg only and 2.32 (0.91–4.59) for ABI >1.4 in at least 1 leg only (Table 2).

**Discussion**

Cadmium exposure, measured by urine cadmium concentrations, was prospectively associated with increased risk of new-onset PAD in adult men and women followed for up to 10 years. Mechanistic evidence has linked cadmium to atherosclerosis development,4,6,36 and cross-sectional studies have also evidenced an increased prevalence of atherosclerotic vascular disease with increased cadmium exposure in the US general population31,32 and in populations from other countries.8,12,23 Our results add prospective evidence of the importance of cadmium exposure as a risk factor for the development of PAD. The results were similar for PAD defined as ABI <0.90 or >1.40 combined and for PAD defined as ABI <0.90 or >1.40 separately, consistent with the fact that chances of coexistent PAD in patients with ABI >1.4 could range from 60% to 80%.24 Importantly, cadmium was associated with...
incident PAD in smoking-adjusted models and separately in smokers and never-smokers, suggesting that cadmium is an important cardiovascular disease risk factor independent of the source of exposure.

Cadmium is a byproduct from mining, smelting, and refining zinc, lead, and copper ores. Over the last decades, its use and production in consumer products, particularly in nickel–cadmium batteries, has increased substantially. Cadmium has also become an important soil contaminant from industrial releases, fuel combustion, and cadmium-containing fertilizers. Exposure to cadmium in the population occurs through tobacco smoke, food and ambient air, particularly near hazardous waste sites, and industrial and occupational settings. We do not have information on specific sources of cadmium exposure in our study population. Nonetheless, urine cadmium concentrations in the SHS participants were higher than in the general US population. Although the prevalence of smoking is higher in American Indians than in other US ethnic groups, their intensity of smoking is markedly lower, and sources of exposure other than smoking, such as occupational and environmental exposures, may be involved in the increased urine cadmium concentrations in our study population.

Our prospective analysis evaluated the association between cadmium and incident PAD. The findings were consistent with the limited number of cross-sectional studies evaluating the association between cadmium exposure and prevalent PAD. In the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES), the odds ratio for PAD comparing the 75th to the 25th percentile of urine cadmium distribution was 3.05 (0.97–9.58). In the same study population, the odds ratio for PAD comparing the highest to the lowest quartiles of blood cadmium was 2.42 (1.13–5.15). In a subsample of 428 participants from the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), blood cadmium was also associated with PAD, and the point estimates were similar to the ones reported in NHANES 1999 to 2000. However, the association between PAD and 24-hour urine cadmium concentrations was not statistically significant, but the point estimates were not reported, and thus, we could not evaluate the power of the study to detect an association. Studies with other endpoints, including increased carotid atherosclerosis, prevalent cardiovascular disease, incident cardiovascular morbidity, and mortality, all support a role for cadmium exposure in atherosclerosis development in humans.

Table 1. Baseline Characteristics of Study Participants by Incident Peripheral Arterial Disease Status*†

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PAD‡ (N=470)</th>
<th>No PAD (N=2394)</th>
<th>Overall (N=2864)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.4 (0.4)</td>
<td>55.6 (0.2)</td>
<td>55.9 (0.1)</td>
</tr>
<tr>
<td>Women, %</td>
<td>61.5 (2.2)</td>
<td>61.0 (1.0)</td>
<td>61.1 (0.9)</td>
</tr>
<tr>
<td>Postmenopausal women§, %</td>
<td>81.3 (2.3)</td>
<td>74.1 (1.1)</td>
<td>75.3 (1.0)</td>
</tr>
<tr>
<td>Location, %</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Arizona</td>
<td>38.5 (2.2)</td>
<td>30.6 (0.9)</td>
<td>31.9 (0.9)</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>30.2 (2.1)</td>
<td>35.4 (1.0)</td>
<td>34.6 (0.9)</td>
</tr>
<tr>
<td>Dakotas</td>
<td>31.3 (2.1)</td>
<td>34.0 (1.0)</td>
<td>33.5 (0.9)</td>
</tr>
<tr>
<td>Education below high school, %</td>
<td>52.1 (2.3)</td>
<td>44.4 (1.0)</td>
<td>45.7 (0.9)</td>
</tr>
<tr>
<td>Smoking, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>31.7 (2.1)</td>
<td>30.8 (0.9)</td>
<td>30.9 (0.9)</td>
</tr>
<tr>
<td>Current</td>
<td>36.4 (2.2)</td>
<td>34.9 (1.0)</td>
<td>35.1 (0.9)</td>
</tr>
<tr>
<td>Cumulative smoking, pack-years</td>
<td>11.8 (0.8)</td>
<td>10.5 (0.4)</td>
<td>10.7 (0.3)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.2 (0.3)</td>
<td>30.8 (0.1)</td>
<td>30.9 (0.1)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>192.9 (1.7)</td>
<td>189.4 (0.8)</td>
<td>190.0 (0.7)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>118.8 (1.5)</td>
<td>116.6 (0.7)</td>
<td>116.9 (0.6)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>45.1 (2.3)</td>
<td>43.1 (1.0)</td>
<td>45.8 (0.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>59.8 (2.3)</td>
<td>43.1 (1.0)</td>
<td>45.8 (0.9)</td>
</tr>
<tr>
<td>GFR &lt;60 mL/min per 1.73 m², %</td>
<td>11.9 (1.5)</td>
<td>7.9 (0.5)</td>
<td>8.5 (0.5)</td>
</tr>
<tr>
<td>Urine cadmium, μg/L</td>
<td>1.03 (0.96–1.10)</td>
<td>1.03 (1.00–1.07)</td>
<td>1.03 (1.00–1.06)</td>
</tr>
<tr>
<td>Urine cadmium, μg/g creatinine</td>
<td>0.96 (0.90–1.02)</td>
<td>0.94 (0.91–0.96)</td>
<td>0.94 (0.92–0.96)</td>
</tr>
</tbody>
</table>

GFR indicates glomerular filtration rate; LDL, low-density lipoprotein; and PAD, peripheral arterial disease.

*Percentages (standard errors) are given for categorical variables or arithmetic means (standard errors) for continuous variables, except for urine cadmium and creatinine-adjusted urine cadmium, for which geometric means (95% confidence intervals) are reported.

†To convert urine cadmium to nanomoles per liter, multiply by 8.896; to convert creatinine-corrected urine cadmium to nanomoles/millimoles creatinine, multiply by 1.006.

‡PAD defined as an ankle brachial index <0.9 or >1.4.
§Subsample of women (N overall, 1749; N PAD, 289; N no PAD, 1460).
women mostly reflected the association in never-smokers.\textsuperscript{25} In the present study, the associations were similar for men and women, and the interaction by sex was not statistically significant. It is possible that the sex differences respond to random sampling variability, but it will be necessary to reproduce the findings in other prospective studies in general population samples exposed to relatively low levels of exposure.

Residual confounding by smoking is a concern in epidemiological studies of cadmium because smoking is a well-known and strong risk factor for PAD\textsuperscript{51} and a major determinant of cadmium exposure.\textsuperscript{3} To address confounding by smoking, we performed separate analyses by smoking status. In our study, the association for never-smokers was weaker than for smokers and nonstatistically significant. Although the probability value for the interaction of smoking status and cadmium was not statistically significant, the power of this test may be limited. Positive residual confounding by intensity or duration of smoking is possible for both never- and ever-smokers because smoking status and pack-years were defined by self-report. Among never-smokers, the association of cadmium with PAD is also subject to positive confounding because of potential exposure to secondhand smoke. In NHANES analyses, however, adjustment for serum cotinine, an objective biomarker of tobacco internal dose, did not change the association between cadmium and PAD,\textsuperscript{21,25} supporting that cadmium is a risk factor for PAD independent of tobacco smoke.

Other reasons could also explain differential associations by smoking status. For instance, potential effect modification of cadmium with other atherogenic components of tobacco smoke could explain stronger associations between cadmium and PAD among smokers compared with nonsmokers. Among never-smokers, negative confounding by vegetable intake, a major source of cadmium exposure, is possible. In a recent systematic review of epidemiological studies,\textsuperscript{52} 8 studies evaluated associations between cadmium exposure and clinical cardiovascular disease endpoints among never-smokers. The clinical endpoints were heterogeneous and could not be pooled, but 7 of 8 studies showed positive associations between cadmium and clinical cardiovascular disease, although only 2 of them were statistically significant. Altogether, cadmium is clearly associated with cardiovascular disease among smokers. Among nonsmokers, there is supportive evidence that cadmium is a cardiovascular risk factor, but this evidence is not conclusive.

The associations between cadmium and PAD observed in SHS are consistent with experimental evidence supporting a variety of potential mechanisms for cadmium as an atherogenic factor, including promoting the formation of reactive oxygen species,\textsuperscript{6} interfering with antioxidative stress responses,\textsuperscript{5,53} promoting hypertension\textsuperscript{54,55} and kidney disease,\textsuperscript{66} and causing endothelial dysfunction and damage.\textsuperscript{5,6,57} Cadmium could also act through epigenetic\textsuperscript{58} and endocrine disruption\textsuperscript{59–61} pathways. The mechanistic studies add biological plausibility to the evidence from studies in human populations, suggesting a dose–response relationship in the association between cadmium exposure and PAD.

<table>
<thead>
<tr>
<th>Table 2. Hazard Ratio (95% CI) for Incident Peripheral Arterial Disease by Urine Cadmium Tertiles</th>
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</thead>
<tbody>
<tr>
<td>Urine Cadmium, μg/g</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Ankle brachial index &lt;0.9 or &gt;1.4 in at least 1 leg</td>
</tr>
<tr>
<td>Tertiles</td>
</tr>
<tr>
<td>≤0.71</td>
</tr>
<tr>
<td>0.71–1.23</td>
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<tr>
<td>&gt;1.23</td>
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<tr>
<td>Linear P-trend</td>
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<tr>
<td>Ankle brachial index &lt;0.9 in at least 1 leg</td>
</tr>
<tr>
<td>Tertiles</td>
</tr>
<tr>
<td>≤0.71</td>
</tr>
<tr>
<td>0.71–1.23</td>
</tr>
<tr>
<td>&gt;1.23</td>
</tr>
<tr>
<td>Linear P-trend</td>
</tr>
<tr>
<td>Ankle brachial index &gt;1.4 in at least 1 leg</td>
</tr>
<tr>
<td>Tertiles</td>
</tr>
<tr>
<td>≤0.71</td>
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<tr>
<td>0.71–1.23</td>
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<tr>
<td>&gt;1.23</td>
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<tr>
<td>Linear P-trend</td>
</tr>
</tbody>
</table>

Model 1: Sex, age at baseline (restricted cubic splines with knots at 50 and 65 y), education, and location. Model 2: Model 1 further adjusted for body mass index, postmenopausal status, total cholesterol, estimated LDL cholesterol, hypertension, diabetes mellitus, and glomerular filtration rate. Model 3: Model 2 further adjusted for smoking status and cumulative smoking dose (restricted cubic splines with knots at 10, 20, and 30 pack-years). Sample sizes were N=2864 for an ankle brachial index <0.9 or >1.4; N=2950 for an ankle brachial index <0.9; and N=2937 an ankle brachial index >1.4. CI indicates confidence interval; HR, hazards ratio; and LDL, low-density lipoprotein.
Our study has some limitations. First, we used a single baseline urine cadmium determination as a biomarker of exposure. Nondifferential measurement error may, thus, have resulted in an underestimation of the associations. Second, we imputed 191 individuals who were known to be PAD-free at the end of the follow-up but did not have ABI measurements during the follow-up as being PAD-free also at intermediate follow-up visits. ABI measurements, however, can undergo daily fluctuations. In our study population, only 74 and 59 individuals with measured ABIs between 0.9 and 1.4 at the first and third examination visits, respectively, had values above or below that range at the second examination. Accordingly, depending on the PAD definition, we would expect between 2.5% and 5% of the imputed observations to be misclassified (a total of 5–10 individuals). Therefore, the expected number of misclassified participants with imputed data is small and unlikely to impact the findings. Third, 10% of participants were lost to follow-up. However, the distribution of cadmium concentrations and demographic variables at baseline did not differ comparing those who missed and not PAD determinations, making selection bias less likely. Finally, American Indian communities, compared with other populations, have increased burdens of diabetes mellitus and obesity, as well as mortality, because of noncardiovascular causes. Generalizability to populations with a different cardiovascular risk factor profile is unknown, although cadmium toxicity pathways are likely to be common for human populations.

Important strengths of this study include the prospective design and long follow-up, the high number of events, the availability of detailed information on relevant confounders, and the careful standardization and quality control of data collection, laboratory analyses, and identification of incident cardiovascular events. In addition, our study contributed to the characterization of a potentially important environmental cardiovascular risk factor in American Indian populations, an understudied ethnic group.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Cases/Noncases</th>
<th>HR (95% CI)</th>
<th>P-int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>181/934</td>
<td>1.32 (0.84–1.90)</td>
<td>0.73</td>
</tr>
<tr>
<td>Women</td>
<td>289/1460</td>
<td>1.44 (1.01–1.99)</td>
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<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smokers</td>
<td>150/822</td>
<td>1.25 (0.83–1.80)</td>
<td>0.45</td>
</tr>
<tr>
<td>Ever-smokers</td>
<td>320/1572</td>
<td>1.50 (1.05–2.05)</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>470/2394</td>
<td>1.41 (1.05–1.81)</td>
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</tbody>
</table>

The 80th and 20th percentiles of urine cadmium distribution were 1.62 and 0.55 μg/g, respectively. Models were adjusted for sex, age at baseline (restricted cubic splines with knots at 50 and 65 y), education, location, body mass index, postmenopausal status, total cholesterol, estimated LDL cholesterol, hypertension, diabetes mellitus, glomerular filtration rate, smoking status, and cumulative smoking dose (restricted cubic splines with knots at 10, 20, and 30 pack-years). Hazard ratios and associated P for interaction were obtained from Cox-proportional hazard models with log-transformed cadmium as a continuous variable. CI indicates confidence interval; and HR, hazard ratio.

Our study has some limitations. First, we used a single baseline urine cadmium determination as a biomarker of exposure. Nondifferential measurement error may have, thus, resulted in an underestimation of the associations. Second, we imputed 191 individuals who were known to be PAD-free at the end of the follow-up but did not have ABI measurements during the follow-up as being PAD-free also at intermediate follow-up visits. ABI measurements, however, can undergo daily fluctuations. In our study population, only 74 and 59 individuals with measured ABIs between 0.9 and 1.4 at the first and third examination visits, respectively, had values above or below that range at the second examination. Accordingly, depending on the PAD definition, we would expect between 2.5% and 5% of the imputed observations to be misclassified (a total of 5–10 individuals). Therefore, the expected number of misclassified participants with imputed data is small and unlikely to impact the findings. Third, 10% of participants were lost to follow-up. However, the distribution of cadmium concentrations and demographic variables at baseline did not differ comparing those who missed and not PAD determinations, making selection bias less likely. Finally, American Indian communities, compared with other populations, have increased burdens of diabetes mellitus and obesity, as well as mortality, because of noncardiovascular causes. Generalizability to populations with a different cardiovascular risk factor profile is unknown, although cadmium toxicity pathways are likely to be common for human populations.

Important strengths of this study include the prospective design and long follow-up, the high number of events, the availability of detailed information on relevant confounders, and the careful standardization and quality control of data collection, laboratory analyses, and identification of incident cardiovascular events. In addition, our study contributed to the characterization of a potentially important environmental cardiovascular risk factor in American Indian populations, an understudied ethnic group.

Table 3. Hazard Ratio (95% CI) for Incident Peripheral Arterial Disease (Ankle Brachial Index <0.9 or >1.4) Comparing the 80th to the 20th Percentile of Urine Cadmium Distribution, by Sex and Smoking

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Cases/Noncases</th>
<th>HR (95% CI)</th>
<th>P-int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>181/934</td>
<td>1.32 (0.84–1.90)</td>
<td>0.73</td>
</tr>
<tr>
<td>Women</td>
<td>289/1460</td>
<td>1.44 (1.01–1.99)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smokers</td>
<td>150/822</td>
<td>1.25 (0.83–1.80)</td>
<td>0.45</td>
</tr>
<tr>
<td>Ever-smokers</td>
<td>320/1572</td>
<td>1.50 (1.05–2.05)</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>470/2394</td>
<td>1.41 (1.05–1.81)</td>
<td></td>
</tr>
</tbody>
</table>

The 80th and 20th percentiles of urine cadmium distribution were 1.62 and 0.55 μg/g, respectively. Models were adjusted for sex, age at baseline (restricted cubic splines with knots at 50 and 65 y), education, location, body mass index, postmenopausal status, total cholesterol, estimated LDL cholesterol, hypertension, diabetes mellitus, glomerular filtration rate, smoking status, and cumulative smoking dose (restricted cubic splines with knots at 10, 20, and 30 pack-years). Hazard ratios and associated P for interaction were obtained from Cox-proportional hazard models with log-transformed cadmium as a continuous variable. CI indicates confidence interval; and HR, hazard ratio.

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In conclusion, cadmium exposure was prospectively associated with an increased risk of PAD. Our prospective findings in the SHS will add to the evidence base for cadmium-related PAD, but large studies in never-smokers with adequate evaluation of potential confounders, including vegetable intake, are needed to fully establish the cardiovascular effects of nonsmoking-related cadmium exposure. Atherosclerotic cardiovascular disease is a major cause of death, functional disability, and medical costs around the world.63,64 The implementation of population-based preventive strategies to decrease cadmium exposure could contribute to reducing the burden of cardiovascular disease, including PAD, in the population.

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Disclosures
None.

References


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SUPPLEMENTAL MATERIAL

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AUTHORS: Maria Tellez-Plaza, Eliseo Guallar, Richard R. Fabsitz, Barbara V. Howard, Jason G. Umans, Kevin A. Francesconi, Walter Goessler, Richard B. Devereux, Ana Navas-Acien

Supplemental Figure 1. Hazard ratios for peripheral arterial disease (ankle-brachial index <0.9) by urine cadmium concentrations, Strong Heart Study.

Supplemental Figure 2. Hazard ratios for peripheral arterial disease (ankle-brachial index >1.4) by urine cadmium concentrations, Strong Heart Study.
Supplemental Figure 1. Hazard ratios for peripheral arterial disease (ankle-brachial index <0.9) by urine cadmium concentrations, Strong Heart Study.

Abbreviations: PAD, peripheral arterial disease. Solid lines are adjusted hazard ratios and dashed lines are 95% confidence intervals based on restricted quadratic splines for log-transformed cadmium concentrations with knots at the 5th, 50th and 95th percentiles (0.33, 0.94 and 2.65 µg/g, respectively). The reference was set at the 10th percentile of cadmium distribution. Vertical bars represent the histogram of cadmium distribution. Models were adjusted for sex, age at baseline (restricted cubic splines with knots at 50 and 65 years), education, location, mass index, postmenopausal status, total cholesterol, estimated LDL-cholesterol, hypertension, diabetes, glomerular filtration rate, smoking status and cumulative smoking dose (restricted cubic splines with knots at 10, 20 and 30 pack-years).
Supplemental Figure 2. Hazard ratios for peripheral arterial disease (ankle-brachial index >1.4) by urine cadmium concentrations, Strong Heart Study.

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