Confounding of the Relation between Homocysteine and Peripheral Arterial Disease by Lead, Cadmium, and Renal Function

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Homocysteine levels are associated with peripheral arterial disease (PAD) in observational studies. Lead and cadmium are risk factors for PAD that affect thiol metabolism, and they may partly explain the association of homocysteine with PAD. To evaluate the roles of lead and cadmium exposure in confounding the association between homocysteine and PAD, the authors performed a cross-sectional study among 4,447 persons aged ≥40 years who participated in the 1999–2002 National Health and Nutrition Examination Survey (NHANES). PAD was defined as an ankle-brachial blood pressure index less than 0.90 in at least one leg. After adjustment for sociodemographic variables, the odds ratio for PAD in the highest quintile of homocysteine compared with the lowest was 1.92 (p_trend = 0.004). Adjusting for blood lead and cadmium levels reduced this odds ratio to 1.37 (p_trend = 0.13), and further adjusting for estimated glomerular filtration rate and smoking reduced it to 0.89 (p_trend = 0.87). Adjustment for other risk factors did not affect this association. In the general population, the association of homocysteine level with PAD can be completely explained by confounding due to smoking, increased blood lead and cadmium levels, and impaired renal function. The association of lead and cadmium with PAD risk deserves further investigation.

cadmium; glomerular filtration rate; homocysteine; lead; peripheral vascular diseases; smoking

Abbreviations: CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; PAD, peripheral arterial disease.

In observational studies, elevated homocysteine levels are associated with peripheral arterial disease (PAD) (1–4), often more strongly than with other cardiovascular disease endpoints (1). As a consequence, the possibility of preventing PAD through homocysteine-lowering interventions has received substantial attention, based on the assumption that the association between homocysteine and PAD is causal and not just a marker of the effect of other etiologic agents. Randomized trials, however, have shown no effect of homocysteine-lowering interventions on cardiovascular outcomes (5, 6). Although these trials did not assess PAD specifically, they suggest the possibility that previously unidentified confounders may account for the association of homocysteine with PAD.

Elevated lead and cadmium levels are risk factors for PAD (7) and other cardiovascular disease endpoints (8, 9). Lead and cadmium are divalent cations with high affinity for thiol groups (10–13), an action that could affect homocysteine metabolism. Schafer et al. (14) recently identified an association between homocysteine level and blood lead and cadmium levels, and impaired renal function. The association of lead and cadmium with PAD risk deserves further investigation.

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between lead, cadmium, and homocysteine as a potential explanation for the results of observational studies of homocysteine and PAD.

We used 1999–2002 data from the National Health and Nutrition Examination Survey (NHANES) to systematically evaluate the roles of traditional and nontraditional risk factors, including lead and cadmium exposure, in confounding the association between homocysteine and reduced ankle-brachial blood pressure index, a specific marker of PAD commonly used in epidemiologic studies (15, 16).

MATERIALS AND METHODS

Study population

NHANES is a series of cross-sectional surveys that have been conducted by the National Center for Health Statistics since the 1960s to obtain health and nutrition data in samples selected to represent the civilian, noninstitutionalized US population. In 1999, NHANES became a continuous survey, and the National Center for Health Statistics started to measure ankle-brachial index in men and women aged ≥40 years (17, 18). The response rate for the physical examination component of NHANES 1999–2002 was 76 percent. In the present analysis, we used data from NHANES 1999–2002 participants who were eligible for measurement of ankle-brachial index (n = 5,965). We restricted our study to the 4,874 participants with valid measures of ankle-brachial index, plasma homocysteine level, and blood lead and cadmium levels (81.7 percent of eligible participants). We further excluded 23 subjects with an ankle-brachial index value greater than 1.5 (often related to calcified, noncompressible vessels in the legs (15)) and 404 participants (8.2 percent) with missing information on at least one covariate of interest. This left 4,447 persons in the study sample.

Variables

Plasma homocysteine. Blood was drawn and processed in NHANES mobile examination centers according to standard protocols (17, 18). Plasma samples were frozen in the field, shipped on dry ice, and stored at −70°C. Total homocysteine concentration in plasma was measured using a fully automated fluorescence polarization immunoassay (Abbott Homocysteine Assay; Abbott Laboratories, Abbott Park, Illinois) at the NHANES Central Laboratory (Centers for Disease Control and Prevention, Atlanta, Georgia). Plasma levels were calculated using a machine-stored calibration curve (Abbott IMx analyzer in 1999–2001 and Abbott AxSYM analyzer starting in 2002; Abbott Laboratories). The fluorescence polarization immunoassay method is equivalent to high performance liquid chromatography (19). The analytical laboratory followed extensive quality control procedures (17, 18). Since standard reference materials are not available for homocysteine assays, bench quality control pools with low, medium, and high levels of homocysteine were prepared at the NHANES Central Laboratory. The pools were analyzed in duplicate during each run and used for calibration and calibration verification procedures. The interassay coefficients of variation ranged from 2.7 percent to 3.4 percent. Blood lead and cadmium. Blood for lead and cadmium measurements was collected in ordinary tubes after confirmation of no background contamination in all collection and storage materials. Lead and cadmium levels in whole blood were measured with a PerkinElmer model SIMAA 6000 simultaneous multielement atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Inc., Wellesley, Massachusetts) with Zeeman background correction (17, 18). The detection limits were 0.3 μg/dl for lead and 0.3 μg/liter for cadmium. There were 11 participants with levels below the detection limit for lead and 731 (16 percent) with levels below the limit for cadmium. For these participants, we imputed a level equal to the detection limit divided by the square root of 2 (20). National Institute of Standards and Technology whole-blood standard reference materials were used for external calibration. The interassay coefficients of variation ranged from 3.1 percent to 4.0 percent for lead and from 4.1 percent to 7.3 percent for cadmium. Peripheral arterial disease. A specific protocol was used to measure ankle-brachial index in NHANES 1999–2002 (17, 18). The measurements of blood pressure used for ankle-brachial index were different from the measurements of blood pressure used to define hypertension. Supine systolic blood pressure was measured on the right arm (brachial artery) and on both ankles (posterior tibial arteries) using a Doppler device (Parks Mini-Lab IV, model 3100; Parks Medical Electronics, Aloha, Oregon). If the participant had a condition that would interfere with blood pressure reading in the right arm, the left arm was used. Systolic blood pressure was measured twice at each site for participants aged 40–59 years and once at each site for participants aged 60 years or more. Measurements for left and right ankle-brachial index were obtained by dividing the mean ankle systolic blood pressure on each side by the mean brachial systolic blood pressure. PAD was defined as an ankle-brachial index less than 0.90 in at least one leg. Other variables. Information on age, sex, race/ethnicity, education, smoking, and alcohol consumption was based on self-report. Body mass index was calculated by dividing weight in kilograms by height in meters squared. Hypertension was defined as mean systolic blood pressure ≥140 mmHg, mean diastolic blood pressure ≥90 mmHg, a self-reported physician diagnosis, or self-reported medication use. Hypercholesterolemia was defined as total cholesterol level ≥240 mg/dl (6.2 mmol/liter), a self-reported physician diagnosis, or medication use. Diabetes was defined as fasting glucose level ≥126 mg/dl (7.0 mmol/liter), nonfasting glucose level ≥200 mg/dl (11.1 mmol/liter), a self-reported physician diagnosis, or self-reported medication use.

High-sensitivity C-reactive protein was measured using a Dade Behring Nephelemetry II analyzer (Dade Behring, Inc., Deerfield, Illinois). Serum creatinine concentration was measured by means of the modified kinetic method of Jaffé (21), using a Hitachi model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana). Estimated glomerular filtration rate was calculated from...
creatinine, age, sex, and race/ethnicity using the Modification of Diet in Renal Disease Study formula (21). Serum folate and vitamin B12 levels were measured at the NHANES Central Laboratory using the Bio-Rad Quantaphase II folate/vitamin B12 radioassay kit (Bio-Rad Laboratories, Hercules, California) (17).

Statistical analysis

We performed all statistical analyses using the survey weights and the svy commands in Stata, version 8.0 (Stata Corporation, College Station, Texas) to account for the complex sampling design and weighting in NHANES 1999–2002 and to obtain appropriate standard errors for all estimates. Data on homocysteine, lead, and cadmium levels were right-skewed and were log-transformed for statistical analyses. Quintile cutoffs were based on weighted distributions in the whole study sample.

First we investigated the association of homocysteine with lead and cadmium levels in a linear model with log homocysteine used as the dependent variable and quintiles of lead or cadmium used as independent variables. These models estimate the adjusted ratio of the geometric mean homocysteine value for a given quintile to the reference quintile. We obtained \( p \) values for linear trend by including log-transformed levels of homocysteine, lead, and cadmium as continuous variables in the regression models. Restricted cubic splines were also used to estimate nonlinear dose-response relations between levels of homocysteine and levels of lead and cadmium.

Second, we investigated the association between homocysteine and the prevalence of PAD using logistic regression. The primary objective of this analysis was to assess whether this association could be explained by confounding due to lead, cadmium, or other cardiovascular risk factors. We used four levels of adjustment: Model 1 adjusted for sociodemographic variables; model 2 further adjusted for body mass index, hypertension, hypercholesterolemia, diabetes, C-reactive protein, and serum folate and vitamin B12; model 3 additionally adjusted for smoking status (never/former/current), alcohol intake, body mass index, and C-reactive protein. Model 4 additionally adjusted for smoking and estimated glomerular filtration rate. The impact of adjusting for lead, cadmium, estimated glomerular filtration rate, and smoking was also assessed by adding each variable and each combination of variables to model 2. We obtained \( p \) values for linear trend by including log-transformed variables as continuous variables in the regression models.

RESULTS

The geometric mean levels of homocysteine, lead, and cadmium were 8.41 \( \mu \)mol/liter, 1.94 \( \mu g/dl \), and 0.47 \( \mu g/liter \), respectively. Men had higher homocysteine and lead levels than women. Homocysteine, lead, and cadmium levels all increased with age. Compared with Whites, Blacks and Mexican Americans had higher lead levels and Mexican Americans had lower homocysteine levels. Lead and cadmium were also lower with higher education. Compared with never smokers, current smokers had higher levels of homocysteine, lead, and cadmium (data not shown).
Lead and cadmium were positively associated with homocysteine (table 1). After adjustment for demographic variables, smoking, alcohol intake, body mass index, C-reactive protein, serum folate, serum vitamin B12, and blood cadmium, participants in the highest quintile of lead had 12 percent higher homocysteine levels than participants in the lowest quintile ($p_{trend} < 0.001$). Similarly, participants in the highest quintile of cadmium had 9 percent higher homocysteine levels than those in the lowest quintile ($p_{trend} < 0.001$). In dose-response analysis, the associations of homocysteine with lead and cadmium were progressive throughout the range of exposure (figure 1).

The weighted prevalence of PAD in the study sample was 4.8 percent (standard error, 0.3 percent). Homocysteine was significantly higher in PAD cases than in noncases (geometric mean values were 10.1 μmol/liter and 8.3 μmol/liter, respectively; $p < 0.001$ (table 2)). After adjustment for age, gender, race/ethnicity, and education, there was a progressive increase in PAD prevalence across quintiles of homocysteine (table 3, model 1). The odds ratio for PAD in the highest quintile of homocysteine as compared with the lowest quintile was 1.92 (95 percent confidence interval (CI): 0.95, 3.88). Adjustment for diabetes, hypertension, hypercholesterolemia, body mass index, C-reactive protein, alcohol intake, and serum folate and vitamin B12 did not materially affect this association (table 3, model 2). In contrast, adjustment for lead and cadmium reduced the adjusted odds ratio for PAD in the highest homocysteine quintile versus the lowest to 1.37 (95 percent CI: 0.58, 3.21) (table 3, model 3). After adjustment for smoking and estimated glomerular filtration rate, the odds ratio for PAD in the highest quintile of homocysteine compared with the lowest quintile decreased further to 0.89 (95 percent CI: 0.35, 2.26) (table 3, model 4). After adjustment, homocysteine was not associated with PAD in any subgroup of participants (figure 2).

When estimated glomerular filtration rate and smoking were each added individually to model 2, the respective odds ratios for PAD in the highest homocysteine quintile versus the lowest were 1.27 (95 percent CI: 0.50, 3.23) and 1.65 (95 percent CI: 0.71, 1.75), respectively. After adjustment for metals and estimated glomerular filtration rate, this odds ratio was 0.92 (95 percent CI: 0.36, 2.35), very similar to the results from model 4.

The association of lead or cadmium with PAD, however, was only modestly affected by adjustment for homocysteine (table 3, model 3). Adjusting for homocysteine changed the odds ratio for PAD in the highest quintile of lead compared with the lowest from 2.36 ($p_{trend} < 0.001$) to 2.20 ($p_{trend} = 0.002$). For cadmium, the odds ratio changed from 2.93 ($p_{trend} < 0.001$) to 2.77 ($p_{trend} < 0.001$). After further adjustment for smoking and estimated glomerular filtration rate, the PAD odds ratio for the highest quintile versus the lowest was 1.65 for lead ($p_{trend} = 0.045$) and 1.86 for cadmium ($p_{trend} = 0.085$).

**DISCUSSION**

In this large cross-sectional study in the general population, homocysteine, lead, and cadmium were each associated with the prevalence of PAD in a dose-response fashion. However, adjustment for blood lead and cadmium, estimated glomerular filtration rate, and smoking completely eliminated the association of homocysteine with PAD, while
the association of lead and cadmium with PAD persisted after adjustment for homocysteine. The associations of lead and cadmium with homocysteine and PAD are remarkable, since they occurred at low levels of both metals, well below current regulatory standards. For instance, only 54 participants (1.2 percent) had blood lead levels above 10 \( \text{g/dl} \) (23), and only two participants had levels above the Occupational Safety and Health Administration standard (<40 \( \text{g/dl} \)) (24). Similarly, only four participants had cadmium levels above the Occupational Safety and Health Administration standard (<5 \( \text{g/liter} \)) (25).

Lead and cadmium are widespread environmental toxicants. Since the ban on leaded gasoline was instituted over two decades ago, lead exposure in the United States has declined substantially (26), but it still occurs in many occupational and environmental settings. People can be exposed to lead through industrial and combustion sources, contact with lead dusts and soils, certain foods, smoking, and sometimes drinking water (27). Exposure to cadmium in the general population results from exposure to cigarette smoke, inhalation of ambient air near coal-fired power plants and municipal waste incinerators, and consumption of some foods (the highest levels are found in shellfish, liver, kidney meats, and crops grown in cadmium-rich soils) (28).

The association of lead and cadmium with homocysteine that we observed in NHANES 1999–2002 confirms the findings of the Baltimore Memory Study (14). The association was independent of well-known determinants of homocysteine level, including folate and vitamin B\(_{12}\). Because our study was cross-sectional, it does not provide information on the mechanisms involved in the association of homocysteine with lead and cadmium, and we can only speculate as to the causes. Lead and cadmium induce changes in glutathione metabolism (12, 29), which may result in increased demand for cysteine, the rate-limiting amino acid in the biosynthesis of glutathione. Lead also inhibits heme synthesis (12), which is essential to the activity of cystathionine-\(\beta\)-synthase, the rate-limiting step in the conversion of homocysteine to cysteine. In addition, lead and cadmium can affect absorption, carrier protein binding, and tissue distribution of iron and zinc—important metals needed for the metabolism of homocysteine (30).

Lead and cadmium were associated with PAD in this study even after adjustment for homocysteine level. Many investigators have reported that small elevations in blood lead are associated with increased blood pressure, particularly at low lead levels (31–33), and elevated blood lead has also been associated with cardiovascular endpoints in prospective studies (8, 9). Cadmium, although less well studied, has been associated with myocardial infarction (34, 35). Both metals increase oxidative stress (11), deplete glutathione and protein-bound thiol groups (11), and induce the

| TABLE 2. Characteristics of participants by peripheral arterial disease status, National Health and Nutrition Examination Survey, 1999–2002 |
|-----------------------------------------------|-------------------------------------------------|
| Cases \( (n = 310) \) | Noncases \( (n = 4,137) \) |
| % or mean | SE\(^*\) or 95% CI\(^*\) | % or mean | SE or 95% CI |
| Mean age (years) | 68.5 | 0.6 | 55.5 | 0.2 |
| Sex (% male) | 44.8 | 3.9 | 48.6 | 0.7 |
| Race/ethnicity (% White) | 80.0 | 2.4 | 78.7 | 1.7 |
| Education (% with more than high school) | 34.4 | 3.2 | 55.2 | 1.4 |
| Mean body mass index \(†\) | 27.7 | 0.4 | 28.3 | 0.2 |
| Smoking (% current smokers) | 25.4 | 2.2 | 17.9 | 1.2 |
| Alcohol intake (% current drinkers) | 46.2 | 0.3 | 59.9 | 2.2 |
| Hypertension (%) | 75.0 | 3.1 | 44.4 | 1.4 |
| Hypercholesterolemia (%) | 56.8 | 3.3 | 44.0 | 1.1 |
| Diabetes (%) | 20.6 | 2.9 | 9.9 | 0.6 |
| Mean C-reactive protein level (mg/liter) | 7.0 | 0.6 | 4.3 | 0.2 |
| Estimated glomerular filtration rate (% <60 ml/minute/1.73 m\(^2\)) | 39.2 | 4.1 | 11.4 | 0.9 |
| Mean serum folate level\(‡\) (ng/ml) | 14.3 | 13.2, 15.5 | 14.1 | 13.7, 14.5 |
| Mean serum vitamin B\(_{12}\) level\(‡\) (pg/ml) | 502 | 469, 538 | 464 | 457, 472 |
| Mean blood lead level\(‡\) (\(\mu g/dl\)) | 2.56 | 2.41, 2.72 | 1.91 | 1.86, 1.97 |
| Mean blood cadmium level\(‡\) (\(\mu g/liter\)) | 0.63 | 0.57, 0.70 | 0.47 | 0.44, 0.49 |
| Mean plasma homocysteine level\(‡\) (\(\mu mol/liter\)) | 10.13 | 9.71, 10.58 | 8.34 | 8.20, 8.47 |

\(\ast\) SE, standard error; CI, confidence interval. 
\(\ddagger\) Weight (kg)/height (m)\(^2\). 
\(\ddagger\) Geometric mean with 95% confidence interval. Other results shown in the table are percentages or arithmetic means with standard errors.
production of inflammatory cytokines (36) and endothelial dysfunction (37), but the precise mechanisms responsible for their cardiovascular effects at the low doses observed in this study are unknown. Mechanistic studies at low levels of exposure and prospective studies in humans using appropriate biomarkers of chronic exposure are needed to further characterize the role of metals in atherosclerosis.

A key causal issue is whether the association of lead or cadmium with PAD is itself a reflection of confounding by impaired renal function. The evidence indicates that increased levels of lead and cadmium are the cause, rather than the consequence, of declining renal function (38, 39). In several studies, patients with chronic kidney disease or end-stage renal disease of known etiology did not have increased levels of blood lead (40, 41), bone lead (40–42), or bone cadmium (42). In contrast, increased body lead burden has been associated with impaired renal function and nephropathy in subjects with occupational lead exposure (43–45), childhood lead poisoning (46, 47), gout (41, 48), and essential hypertension (40, 41) and in middle-aged and elderly men without previously known heavy lead exposure (49). In one study, high-normal levels of chelatable lead predicted renal function decline in patients with chronic renal insufficiency (50). Furthermore, chelation therapy improved renal function in these patients and slowed the progression of renal insufficiency (50). Thus, impaired renal function is likely to be an intermediate variable, rather than a confounder, for the association of lead or cadmium with PAD.

Smoking and impaired renal function are established causes of PAD and of increased homocysteine levels (51–53). Although adjusting for estimated glomerular filtration rate and smoking substantially reduced the association between homocysteine and PAD, additional adjustment for lead and cadmium further reduced this association. The importance of each of these confounders may differ across populations as a function of their prevalence. While previous studies of homocysteine and PAD have adjusted for smoking, few have adjusted for markers of renal function, and none (to our knowledge) have adjusted for lead or cadmium levels.

The hypothesis that homocysteine is a cardiovascular disease risk factor originated from the clinical observation that patients with genetic defects leading to very high

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**TABLE 3. Odds ratios for peripheral arterial disease according to quintiles of homocysteine, lead, and cadmium levels, National Health and Nutrition Examination Survey, 1999–2002**

<table>
<thead>
<tr>
<th>Quintile (Q)</th>
<th>No. of cases</th>
<th>No. of noncases</th>
<th>Model 1*</th>
<th>Model 2†</th>
<th>Model 3‡</th>
<th>Model 4§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/liter)</td>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Q1 (≤6.6)</td>
<td>22</td>
<td>867</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Q2 (6.6–7.8)</td>
<td>40</td>
<td>845</td>
<td>1.15 0.47, 2.93</td>
<td>1.14 0.45, 2.92</td>
<td>1.04 0.40, 2.74</td>
<td>0.94 0.36, 2.48</td>
</tr>
<tr>
<td>Q3 (7.8–9.1)</td>
<td>48</td>
<td>843</td>
<td>1.21 0.54, 2.70</td>
<td>1.18 0.50, 2.79</td>
<td>1.02 0.44, 2.40</td>
<td>0.87 0.36, 2.08</td>
</tr>
<tr>
<td>Q4 (9.1–11.0)</td>
<td>76</td>
<td>814</td>
<td>1.19 0.68, 2.85</td>
<td>1.40 0.60, 3.25</td>
<td>1.11 0.49, 2.53</td>
<td>0.87 0.37, 2.04</td>
</tr>
<tr>
<td>Q5 (&gt;11.0)</td>
<td>124</td>
<td>768</td>
<td>1.92 0.95, 3.88</td>
<td>1.87 0.79, 4.42</td>
<td>1.37 0.58, 3.21</td>
<td>0.89 0.35, 2.26</td>
</tr>
<tr>
<td>p trend</td>
<td>0.004</td>
<td>0.011</td>
<td>0.134</td>
<td>0.870</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead (µg/dl)</td>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Q1 (≤1.1)</td>
<td>43</td>
<td>939</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Q2 (1.1–1.6)</td>
<td>41</td>
<td>821</td>
<td>1.15 0.65, 2.15</td>
<td>1.16 0.64, 2.11</td>
<td>1.15 0.64, 2.08</td>
<td>0.99 0.53, 1.85</td>
</tr>
<tr>
<td>Q3 (1.6–2.2)</td>
<td>53</td>
<td>811</td>
<td>1.49 0.93, 2.40</td>
<td>1.50 0.94, 2.38</td>
<td>1.47 0.94, 2.31</td>
<td>1.19 0.74, 1.92</td>
</tr>
<tr>
<td>Q4 (2.2–3.2)</td>
<td>73</td>
<td>809</td>
<td>1.92 1.30, 2.84</td>
<td>2.11 1.44, 3.08</td>
<td>2.00 1.35, 2.98</td>
<td>1.64 1.02, 2.61</td>
</tr>
<tr>
<td>Q5 (&gt;3.2)</td>
<td>100</td>
<td>757</td>
<td>2.46 1.56, 3.88</td>
<td>2.36 1.53, 3.63</td>
<td>2.20 1.46, 3.33</td>
<td>1.65 1.07, 2.56</td>
</tr>
<tr>
<td>p trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood cadmium (µg/liter)</td>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Q1 (≤0.2)</td>
<td>45</td>
<td>1,135</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Q2 (0.2–0.4)</td>
<td>41</td>
<td>861</td>
<td>0.78 0.44, 1.38</td>
<td>0.76 0.42, 1.37</td>
<td>0.75 0.41, 1.37</td>
<td>0.72 0.40, 1.31</td>
</tr>
<tr>
<td>Q3 (0.4–0.5)</td>
<td>47</td>
<td>647</td>
<td>1.07 0.66, 1.73</td>
<td>1.05 0.65, 1.69</td>
<td>1.03 0.65, 1.64</td>
<td>0.95 0.58, 1.55</td>
</tr>
<tr>
<td>Q4 (0.5–0.8)</td>
<td>89</td>
<td>834</td>
<td>1.51 0.81, 2.79</td>
<td>1.37 0.72, 2.58</td>
<td>1.33 0.71, 2.50</td>
<td>1.06 0.54, 2.08</td>
</tr>
<tr>
<td>Q5 (&gt;0.8)</td>
<td>88</td>
<td>660</td>
<td>3.26 2.02, 5.26</td>
<td>2.93 1.74, 4.93</td>
<td>2.77 1.65, 4.65</td>
<td>1.86 0.98, 3.54</td>
</tr>
<tr>
<td>p trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.085</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results adjusted for age, sex, race/ethnicity, and education.
† Results further adjusted for diabetes, hypertension, hypercholesterolemia, body mass index, C-reactive protein, alcohol intake (never/former/current), and serum folate and vitamin B12.
‡ Homocysteine further adjusted for lead and cadmium; lead and cadmium further adjusted for homocysteine.
§ Results further adjusted for smoking (never/former/current and cigarettes day) and estimated glomerular filtration rate.
¶ OR, odds ratio; CI, confidence interval.
homocysteine levels in blood and homocystinuria had premature atherosclerosis. Our study does not contradict the possibility that high homocysteine concentrations contribute to atherosclerosis, but in our general population sample, homocysteine levels were low (the 90th percentile of homocysteine in the NHANES sample was 12.4 µmol/liter). In this context, our findings indicate that the homocysteine elevations observed among PAD cases were more likely to be a consequence of their association with lead and cadmium exposure, impaired renal function, and smoking rather than a cause of PAD. Thus, adjustment for these variables is needed in order to avoid confounding of the association between homocysteine level and PAD.

Perhaps the most significant limitation of our study is the cross-sectional nature of NHANES data. However, most studies of homocysteine and PAD are either cross-sectional or case-control in nature and share common design limitations with our study. Furthermore, cross-sectional and retrospective designs, if anything, tend to overestimate the association of homocysteine with cardiovascular endpoints (54). It is thus unlikely that the lack of association of homocysteine with PAD after multivariate adjustment is due to biases in cross-sectional study designs.

Interpretation of our findings is limited by a lack of understanding of the mechanism underlying the association of lead or cadmium with homocysteine. While lead and cadmium may affect homocysteine levels, it is also possible that homocysteine affects blood lead or cadmium levels. Homocysteine has a thiol group and is similar in structure to penicillamine and dimercaptosuccinic acid (55), two chelating agents used to enhance urinary excretion of lead. Homocysteine could shift the equilibrium of heavy metals across different compartments by such binding, and this could result in an association between serum homocysteine and blood lead. In fact, high metal concentrations in blood, hair, and urine have been observed in homocystinuric patients (56), although the relevance of this finding to the much lower homocysteine levels observed in the general population is unknown.

The strengths of our study include the rigorous sampling design and quality control of NHANES, the representative nature of the study sample, the large sample size, and the use of standardized measures of ankle-brachial index.

In conclusion, our findings indicate that previous studies may have overestimated the association between homocysteine and PAD because of confounding by lead and cadmium exposure. Even more important, our results agree with an increasing body of evidence indicating that exposure to even low concentrations of these metals may have important cardiovascular effects. Because of the widespread exposure to lead and cadmium at the levels that were associated with PAD in our study, identifying the precise mechanisms of effect and determining their role in other cardiovascular outcomes should be a research priority.

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