Original Contribution

Relation of Blood Cadmium, Lead, and Mercury Levels to Biomarkers of Lipid Peroxidation in Premenopausal Women


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Exposures to cadmium, lead, and mercury are associated with adverse health effects, including cardiovascular disease, which may be promoted by lipid peroxidation. The authors examined cadmium, lead, and mercury in relation to plasma levels of F2-isoprostanes (isoprostane), 9-hydroperoxy-10,12-octadecadienoic acid (9-HODE), 13-hydroxy-9,11-octadecadienoic acid (13-HODE), and thiobarbituric acid reactive substances (TBARS) in 252 women from western New York State (2005–2007). Healthy premenopausal women were followed for 2 menstrual cycles, with biomarkers of lipid peroxidation being assessed 8 times per cycle. Metals were measured at baseline in whole blood. Linear mixed models were used to estimate the association between cadmium, lead, and mercury and lipid peroxidation biomarkers. Median cadmium, lead, and mercury levels were 0.30 μg/L, 0.86 μg/dL, and 1.10 μg/L, respectively. Blood cadmium, lead, and mercury were not associated with increases in isoprostane, TBARS, 9-HODE, or 13-HODE levels. Isoprostane levels decreased 6.80% (95% confidence interval: −10.40, −3.20) per 1% increase in mercury. However, after adjustment for a simulated strong confounding factor, such as precisely measured fish consumption, the observed association was attenuated, suggesting that this unexpected association could be attributable to unmeasured confounding. In this population of healthy premenopausal women with low exposure levels, cadmium, lead, and mercury were not associated with elevated lipid peroxidation biomarkers.

cadmium; hydroxyl-octadecadienoic acid; isoprostane; lead; mercury; oxidative stress; thiobarbituric acid reactive substances; women

Abbreviations: CI, confidence interval; EDTA, ethylenediaminetetraacetic acid; 9-HODE, 9-hydroxy-10,12-octadecadienoic acid; 13-HODE, 13-hydroxy-9,11-octadecadienoic acid; LOD, limit of detection; SD, standard deviation; TBARS, thiobarbituric acid reactive substances.
(16, 17). Epidemiologic evidence is limited, and studying oxidative stress biomarkers poses a particular challenge in premenopausal women because of fluctuating hormone levels, which may influence levels of oxidative stress (18, 19).

F₂₈ isoprostanes (hereafter called isoprostane), 9-hydroxy-10,12-octadecadienoic acid (9-HODE), 13-hydroxy-9,11-octadecadienoic acid (13-HODE), and thiobarbituric acid reactive substances (TBARS) are biomarkers of different components of the lipid peroxidation process and have been linked with chronic disease development. Isoprostane is derived from arachidonic acid and is stable (20, 21), whereas 9-HODE and 13-HODE are linoleic acid peroxidation metabolites (22). The TBARS test represents an index of systemic lipid peroxidation, albeit with methodological concerns (23).

Given the paucity of research among women with low levels of metals and the broad public health implications of lipid peroxidation-related effects, our aim was to evaluate the effects of metal exposure, specifically exposure to cadmium, lead, and mercury, in relation to 4 biomarkers of lipid peroxidation in a cohort of healthy premenopausal women.

MATERIALS AND METHODS

Study cohort

The BioCycle Study enrolled healthy, premenopausal women (ages 18–44 years) to evaluate the relation between biomarkers of oxidative stress and hormone levels over the course of a regular menstrual cycle. Health status was ascertained by self-report. Inclusion criteria comprised a self-reported menstrual cycle length of 21–35 days for the past 6 months, not actively trying to conceive, and no history of polycystic ovary syndrome; exclusion criteria included vitamin supplement use (24). Women were followed prospectively for 1 (n = 9) or 2 (n = 250) menstrual cycles. The 9 women followed for 1 cycle were recruited for a pilot substudy. A total of 252 women had blood measures of metals and lipid peroxidation and were thus included in this analysis.

Recruitment and data collection occurred from 2005 to 2007 at the University at Buffalo (State University of New York) in western New York State, under an Intramural Research Program contract from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. A sample size of 250 was needed to achieve 99% power to detect a change in slope from 0.11 under the null hypothesis to 0.00 under the alternative hypothesis when the standard deviation of the exposure was 1.00, the standard deviation of the outcome was 0.36, and the 2-sided alpha level was 0.05.

All participants provided written informed consent prior to participation. Under a reliance agreement, the National Institutes of Health depends on the designated institutional review board of the University at Buffalo for review, approval, and continuing oversight of its human subject research for the BioCycle Study.

Clinic visits

Women made clinic visits to the University at Buffalo Women’s Health Research Center to provide blood samples 8 times per menstrual cycle, corresponding to early menstruation, the mid- and late follicular phases, 2 days around the expected time of ovulation, and the early, mid-, and late luteal phases. Visits took place in the morning in order to obtain fasting blood samples and reduce diurnal variation. Clearblue Easy fertility monitors (Inverness Medical, Waltham, Massachusetts) aided in scheduling visits to appropriate menstrual cycle phases (25).

Analysis of metal exposure

Whole blood was collected at the screening visit in ethylenediaminetetraacetic acid (EDTA) purple-topped tubes (prescreened for trace metals) provided by the Centers for Disease Control and Prevention. Samples were analyzed for levels of cadmium, lead, and mercury in whole blood by inductively coupled plasma mass spectrometry at the Division of Laboratory Sciences of the National Center for Environmental Health, Centers for Disease Control and Prevention. The limits of detection (LODs) for cadmium, lead, and mercury were 0.20 µg/dL (25% < LOD), 0.25 µg/dL (0% < LOD), and 0.30 µg/dL (12% < LOD). Values below the LOD were reported by the laboratory. To minimize potential bias, values below the LOD were not substituted (26). The interassay precisions (relative standard deviations) for cadmium, lead, and mercury were 4.3%, 2.6%, and 3.2% at levels of 2.04 µg/L, 2.89 µg/dL, and 5.77 µg/dL, respectively.

Biomarker analysis

Blood serum and plasma collection and handling protocols were designed to minimize preanalytical variability (27). Specimens were collected at each of the clinic visits (up to 8 visits) during each menstrual cycle and were delivered to the processing laboratory, centrifuged at 1,500 × g for 10 minutes, portioned into cryotubes, and frozen at −80°C within 90 minutes of phlebotomy. Isoprostane, TBARS, 9-HODE, and 13-HODE levels were measured in EDTA (15% K₂ EDTA) anticoagulated plasma. Isoprostane samples were analyzed by gas chromatography-mass spectrometry at the Molecular Epidemiology and Biomarker Research Laboratory of the University of Minnesota (21, 28). An internal standard, [¹H₄]-isoprostane (>98% pure; Cayman Chemical Company, Ann Arbor, Michigan), was used. Total plasma 9-HODE, 13-HODE, and TBARS were measured at the Oxidative Stress Research Laboratory of the University at Buffalo. 9-HODE and 13-HODE were measured by high performance liquid chromatography with diode array detection at 234 nm following mild alkaline hydrolysis of lipid esters to yield total free fatty acids, including 9-HODE and 13-HODE (29, 30). HODE samples were delivered to the laboratory and analyzed daily. The TBARS test measures malondialdehyde, a 3-carbon aldehyde produced from hydrolysis of some lipid hydroperoxides (23). TBARS samples were measured at an excitation of 535 nm and emission of 552 nm with an RF-5000U spectrofluorometer (Shimadzu Scientific Instruments Inc., Columbia, Maryland), and TBARS levels are expressed in nmol/mL (malondialdehyde) equivalents (29, 31). Collection and handling protocols were designed to minimize variability in exogenous factors (27, 32).
The half-lives of biomarkers of lipid peroxidation are several hours in duration (17). The interassay coefficients of variation were 9.4%, 8.3%, 9.0%, and 9.2%, for isoprostane, TBARS, 9-ODE, and 13-ODE, respectively.

Collection of covariate data

At screening, women provided information on their health and reproductive history and lifestyle. Trained staff recorded height (meters) and weight (kilograms) to determine body mass index (weight (kg)/height (m)²). Physical activity was measured with the International Physical Activity Questionnaire (33). Intakes of whole foods (fish, shellfish, vegetables, and grains) and nutrients (dietary iron) were assessed using the general food frequency questionnaire developed by the Nutrition Assessment Shared Resource of the Fred Hutchinson Cancer Research Center (Seattle, Washington) at baseline (6-month recall). Women recorded supplemental vitamin use in daily diaries and according to the study inclusion guidelines were not meant to be taking vitamin supplements. Occupation was reported to determine possible occupational exposures that might be associated with oxidative stress or metals.

Statistical analysis

Demographic characteristics were compared according to tertiles of metals and isoprostane measured at the first menstrual cycle visit during the first cycle under study, and associations were assessed using t tests, chi-squared tests, or Fisher’s exact tests where appropriate. Distributions of oxidative stress biomarkers were checked for normality and log-transformed.

Linear mixed models were used to evaluate the association between exposure to metals and levels of oxidative stress, while accounting for nonindependence between individual measurements across the cycle and multiple cycles per woman. Each log-transformed metal was analyzed in a separate model; results are interpreted as the percent change in oxidative stress level per 1% increase in metal (exposure) level. Random intercepts accounted for variation in baseline oxidative stress levels between women. Covariate selection was determined by a review of the literature and included age (years; continuous), body mass index (continuous), smoking status (current/not current), and race (white, black, Asian, other). Models for mercury were additionally adjusted for fish consumption (servings/month; continuous). Measures of income, education, physical activity, parity, dietary iron, shellfish, vegetables, dietary selenium, dietary calcium, and total energy intake were considered as potential confounders but did not appreciably alter the effect estimates. Because reproductive hormones are potential mediators in the association between metals and lipid peroxidation, they were not considered as confounders. P values are 2-sided. Statistical analyses were conducted in SAS 9.2 (SAS Institute, Inc., Cary, North Carolina) and R (R Foundation for Statistical Computing, Vienna, Austria).

We conducted a sensitivity analysis to evaluate the impact of adjusting for fish consumption. We compared results of this analysis with our final models, which were adjusted for confounder variables, including age, smoking status, physical activity, parity, dietary iron, shellfish, vegetables, dietary selenium, dietary calcium, and total energy intake. We also adjusted for income, education, and race.

RESULTS

Median blood cadmium, lead, and mercury levels were 0.30 \( \mu \text{g/L} \) (interquartile range, 0.19–0.43), 0.86 \( \mu \text{g/dL} \) (interquartile range, 0.67–1.20), and 1.10 \( \mu \text{g/L} \) (interquartile range, 0.58–2.00), respectively. The demographic characteristics of the study population varied somewhat by metal level (Table 1). Older women had significantly higher levels of cadmium and lead. Body mass index did not differ significantly by metal level. Parity and education did not differ by metal level. White women had lower cadmium levels (mean = 0.32 \( \mu \text{g/L} \); standard deviation (SD), 0.30) than blacks (mean = 0.36 \( \mu \text{g/L} \); SD, 0.23) and Asians (mean = 0.46 \( \mu \text{g/L} \); SD, 0.28). Asian women had higher lead levels (mean = 1.50 \( \mu \text{g/dL} \); SD, 0.78) and mercury (mean = 2.37 \( \mu \text{g/L} \); SD, 2.12) levels than whites (lead mean = 0.95 \( \mu \text{g/dL} \); SD, 0.65; mercury mean = 1.33 \( \mu \text{g/L} \); SD, 1.14) or blacks (lead mean = 0.92 \( \mu \text{g/dL} \); SD, 0.30; mercury mean = 1.36 \( \mu \text{g/L} \); SD, 0.99)). Current smokers had higher cadmium levels, but smoking status was not associated with lead and mercury levels.

Table 2 shows unadjusted and adjusted percent change in lipid peroxidation level per 1% increase in metal exposure derived using linear mixed models. We observed that blood metal levels tended to be inversely associated with isoprostane levels, though generally without statistical significance. In unadjusted models, each 1% increase in metal level was associated with statistically significant decreases in isoprostane level. In adjusted models, cadmium and lead were no longer statistically significantly associated with decreases in isoprostane levels, while metal levels tended to be inversely associated with isoprostane levels, though generally without statistical significance. In adjusted models, mercury was associated with a statistically significant 6.80% decrease (95% confidence interval (CI): −10.40, −3.20) in isoprostane levels. TBARS levels were consistently but not statistically significantly increased in relation to metal exposure, although, in unadjusted models, each 1% increase in mercury level was associated with a statistically significant 3.49% (95% CI: 0.34, 6.64) increase in TBARS. 9-ODE and 13-ODE were not statistically significantly associated with metals.

The addition of a simulated confounder at various levels of modest correlation between mercury and isoprostane (approximately −0.8 to +0.8) shows that such an association could be observed (see Web Figure 1 (http://aje.oxfordjournals.org/)). However, the peak of Web Figure 1 demonstrates that after adjustment for a highly correlated confounding factor (approximately \( p = 0.4–0.7 \) with mercury and \( p = −0.4 \) to −0.7 with isoprostane), the association between mercury and isoprostane was approximately null or weakly positive. Specifically, additional adjustment for a simulated confounder that was correlated with mercury (\( p = 0.55 \)) and isoprostane
Table 1. Characteristics of the Study Population According to Category of Metal Exposure,\textsuperscript{a} BioCycle Study, Buffalo, New York, 2005–2007\textsuperscript{b}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cadmium Level, (\mu g/L)</th>
<th>Lead Level, (\mu g/dL)</th>
<th>Mercury Level, (\mu g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.03–0.29 (n = 122))</td>
<td>(0.30–3.10 (n = 127))</td>
<td>(0.00–1.09 (n = 120))</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>%</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Age, years</td>
<td>25.8 (7.7)</td>
<td>28.7 (8.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>Body mass index\textsuperscript{d}</td>
<td>24.0 (3.8)</td>
<td>24.2 (3.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>1,653 (400)</td>
<td>1,550 (391)</td>
<td>0.04</td>
</tr>
<tr>
<td>Selenium, (\mu g)</td>
<td>85.6 (37.6)</td>
<td>94.3 (45.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Vitamin E, mIU\textsuperscript{e}</td>
<td>9.4 (6.0)</td>
<td>10.6 (9.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Isoprostane, pg/mL\textsuperscript{f}</td>
<td>50.5 (17.7)</td>
<td>48.1 (17.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>13-HODE, (\mu mol/L)</td>
<td>0.26 (0.30)</td>
<td>0.26 (0.30)</td>
<td>0.99</td>
</tr>
<tr>
<td>9-HODE, (\mu mol/L)</td>
<td>0.22 (0.21)</td>
<td>0.20 (0.20)</td>
<td>0.42</td>
</tr>
<tr>
<td>TBARS, nmol/mL\textsuperscript{f}</td>
<td>0.92 (0.24)</td>
<td>0.86 (0.22)</td>
<td>0.04</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>70</td>
<td>48</td>
<td>0.002</td>
</tr>
<tr>
<td>Black</td>
<td>17</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>7</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Nonsmoker/former smoker</td>
<td>99</td>
<td>94</td>
<td>0.04</td>
</tr>
<tr>
<td>Physical activity level</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>9</td>
<td>58</td>
</tr>
<tr>
<td>Medium</td>
<td>28</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>High</td>
<td>61</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>76</td>
<td>71</td>
<td>0.35</td>
</tr>
<tr>
<td>More than high school education</td>
<td>89</td>
<td>87</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Abbreviations: 9-HODE, 9-hydroxy-10,12-octadecadienoic acid; 13-HODE, 13-hydroxy-9,11-octadecadienoic acid; SD, standard deviation; TBARS, thiobarbituric acid reactive substances.

\textsuperscript{a} Metal exposures were dichotomized at the median value.

\textsuperscript{b} Percentages may not sum to 100 because of rounding.

\textsuperscript{c} Difference between mean values (analysis of variance) for continuous variables; chi-squared test or Fisher's exact test for categorical variables. All \(P\) values are 2-sided.

\textsuperscript{d} Weight (kg)/height (m\textsuperscript{2}).

\textsuperscript{e} Selenium and vitamin E levels were determined by baseline food frequency questionnaire.

\textsuperscript{f} Lipid peroxidation levels as measured at the first clinic visit.
(ρ = −0.50), at levels of correlation similar to measured fish consumption but more strongly correlated to account for deattenuation, further diminished the association between mercury and isoprostane to −3.87% (95% CI: −7.18, −0.55). Since the simulated confounder was based on strong correlations, we calculated deattenuated correlation coefficients for the correlations between mercury, isoprostane, and the measured confounding factors to determine whether such strong correlations were plausible, given our observed data (34). We found that for reasonable amounts of error variance in the measurement of dietary fish consumption (fish-mercury: σ²ε = 3.24; fish-isoprostane: σ²ε = 1.56), in comparison with the observed variance of fish consumption (2,028.41 servings/year; mercury, 1.77 μg/dL; isoprostane, 0.14 pg/mL), the observed correlations between fish consumption and mercury (ρ = 0.30) and fish consumption and isoprostane (ρ = −0.14) approximated the simulated levels (deattenuated ρ = 0.50 and deattenuated ρ = −0.50, respectively). Further, 43% of participants reported no fish consumption.

**DISCUSSION**

Our findings indicate that low blood levels of mercury, lead, and cadmium were not associated with increasing levels of lipid peroxidation biomarkers (isoprostane, TBARS, 9-HODE, and 13-HODE) in healthy premenopausal women. However, we did observe a modest inverse association between mercury and isoprostane but more strongly correlated to account for deattenuation, further diminished the association between mercury and isoprostane to −3.87% (95% CI: −7.18, −0.55). Since the simulated confounder was based on strong correlations, we calculated deattenuated correlation coefficients for the correlations between mercury, isoprostane, and the measured confounding factors to determine whether such strong correlations were plausible, given our observed data (34). We found that for reasonable amounts of error variance in the measurement of dietary fish consumption (fish-mercury: σ²ε = 3.24; fish-isoprostane: σ²ε = 1.56), in comparison with the observed variance of fish consumption (2,028.41 servings/year; mercury, 1.77 μg/dL; isoprostane, 0.14 pg/mL), the observed correlations between fish consumption and mercury (ρ = 0.30) and fish consumption and isoprostane (ρ = −0.14) approximated the simulated levels (deattenuated ρ = 0.50 and deattenuated ρ = −0.50, respectively). Further, 43% of participants reported no fish consumption.

Population-based study in US adults over age 40 years found that inflammatory biomarkers were not increased with lead exposure, particularly among women (35). Biomarkers of lipid peroxidation were not elevated in relation to lead and cadmium in males with blood lead levels less than 40 μg/dL (36). Further, an occupational study found a strong increase in lipid peroxidation with blood lead levels above 35 μg/dL but only a weak association with lower blood lead levels (37). Most epidemiologic evidence that metals are positively associated with oxidative stress has come from occupational studies with higher exposure (38, 39), suggesting that our findings could be attributable to a threshold or nonlinear effect. The maximum measured blood lead and mercury levels in the BioCycle Study were 6.2 μg/dL and 9.9 μg/L, lower than mean levels reported for most occupational cohorts, though comparable to levels found among adults in the general US population (9, 40). Geometric mean blood cadmium, lead, and mercury levels were 0.29 μg/L (7), 1.78 μg/dL (8), and 1.02 μg/L (41), respectively, among reproductive-aged women in the National Health and Nutrition Examination Survey, as compared with 0.29 μg/L for cadmium, 0.91 μg/L for lead, and 1.04 μg/L for mercury in the BioCycle Study.

The association between isoprostane and mercury was unexpected but did not persist among persons with lead levels above the median. While it is possible that this finding represents a true association, as nonlinear associations or hormesis could explain such a finding at low levels of mercury, there are several alternative explanations. This association could be attributable to residual confounding resulting from our use of a somewhat crude instrument (i.e., a food frequency questionnaire) to assess fish consumption. Fish intake is a recognized source of mercury exposure, and n-3 fatty acids found in fish have been associated with decreased

<table>
<thead>
<tr>
<th>Biomarker and Model</th>
<th>Cadmium, μg/L</th>
<th>95% CI</th>
<th>Lead, μg/dL</th>
<th>95% CI</th>
<th>Mercury μg/L</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoprostane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>−7.48</td>
<td>−13.49, −1.48</td>
<td>−11.89</td>
<td>−20.37, −3.42</td>
<td>−9.22</td>
<td>−13.16, −5.28</td>
</tr>
<tr>
<td>Adjusted⁵</td>
<td>−2.48</td>
<td>−8.65, 3.68</td>
<td>−4.67</td>
<td>−13.35, 4.01</td>
<td>−6.80</td>
<td>−10.40, −3.20</td>
</tr>
<tr>
<td>9-HODE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>4.56</td>
<td>−3.49, 12.61</td>
<td>2.40</td>
<td>−8.94, 13.75</td>
<td>−1.25</td>
<td>−6.65, 4.14</td>
</tr>
<tr>
<td>Adjusted</td>
<td>3.90</td>
<td>−5.03, 12.83</td>
<td>0.35</td>
<td>−12.13, 12.82</td>
<td>−0.92</td>
<td>−6.56, 4.72</td>
</tr>
<tr>
<td>13-HODE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>7.78</td>
<td>−1.26, 16.82</td>
<td>1.75</td>
<td>−11.01, 14.51</td>
<td>−3.54</td>
<td>−9.62, 2.55</td>
</tr>
<tr>
<td>Adjusted</td>
<td>6.08</td>
<td>−3.96, 16.12</td>
<td>−4.36</td>
<td>−18.36, 9.64</td>
<td>−3.54</td>
<td>−9.86, 2.78</td>
</tr>
<tr>
<td>TBARS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.32</td>
<td>−2.40, 7.03</td>
<td>6.08</td>
<td>−0.57, 12.74</td>
<td>3.49</td>
<td>0.34, 6.64</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.32</td>
<td>−3.95, 6.59</td>
<td>5.24</td>
<td>−2.18, 12.67</td>
<td>3.08</td>
<td>−0.20, 6.37</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; 9-HODE, 9-hydroxy-10,12-octadecadienoic acid; 13-HODE, 13-hydroxy-9, 11-octadecadienoic acid; TBARS, thiobarbituric acid reactive substances.

⁵ For mercury, adjustment included adjustment for fish consumption (servings/month; continuous) as measured by baseline food frequency questionnaire.

⁶ Results were adjusted for age, race (black, white, Asian, or other), body mass index (weight (kg)/height (m)²), and smoking (yes vs. no/former).
isoprostane levels (42, 43). Most mercury exposure among non-occupationally exposed persons is likely to be methyl-mercury from fish (44), but we did not separately examine different forms of mercury (inorganic, methyl), which may exert different effects on oxidative stress biomarkers (45). Because of measurement error, residual confounding from fish consumption is likely; ideally, one would measure n-3 fatty acid and methylmercury concentrations to preclude this bias. Dietary intake data are subject to measurement error, which may attenuate the observed correlations between fish consumption, mercury, and isoprostane (46). Therefore, fish consumption (47) may be more strongly correlated with mercury and isoprostane than we observed, because of attenuation from measurement error. The observed correlation between fish consumption (measured at baseline by food frequency questionnaire) and mercury was 0.30 ($\rho = 0.30$), and the correlation between fish consumption and isoprostane was −0.14 ($\rho = -0.14$). The sensitivity analysis that accounted for possible measurement error by simulating a confounding factor at deattenuated levels of correlation demonstrated the plausibility of a strong confounding factor, measured with error, to account for the unexpected association between mercury and isoprostane.

The association between mercury and isoprostane represents the total effect, or any direct effect of mercury on isoprostane plus any indirect effects that are mediated by estradiol. In previous work, we observed an inverse association between estradiol and isoprostane (18) and a positive, though not statistically significant, association between mercury and estradiol (48), such that the indirect effect of mercury on isoprostane mediated by estradiol (the product of the estradiol-isoprostane and mercury-estradiol associations) would be inverse. Under assumptions of no interactions, linearity of effect, and no unmeasured confounding, the sum of the direct and indirect effects equals the total effects (49). It is plausible that the indirect effects are stronger than the direct effect between mercury and isoprostane, resulting in the total effect’s being inversely associated as well.

Our study had several strengths, including the timing of biomarker specimen collection, multiple measurements across the menstrual cycle, and state-of-the-art measurement techniques for both metals and measures of oxidative stress. It is unlikely that our findings are attributable to diurnal variation in oxidative stress levels, which are generally considered minimal (50). Our samples were processed rapidly and were shipped in batches to minimize batch variability that may occur when analyzing repeated samples among individuals. Finally, the BioCycle Study recruited healthy women who were selected to minimize known confounding factors, such as adherence to a specific diet, which may affect oxidative stress and metal levels.

However, our analysis had some limitations. Metal exposure was assessed only at the screening visit, and in some cases women did not complete the study protocol in consecutive menstrual cycles ($n = 23$). Thus, metals measured at screening may not have reflected exposure during the second cycle under study. We conducted subanalyses restricted to the first menstrual cycle and to women who completed the study protocol in consecutive cycles, and our findings were consistent (data not shown). BioCycle Study participants may have a narrower range of lipid peroxidation and metal levels than the general population, as the women were selected to be healthy. This could have hindered detection of effects, particularly if associations occur beyond a threshold level. The BioCycle Study had a low prevalence of current smoking ($n = 16$), and smoking contributes to both metal exposure and oxidative stress (51). Despite the lack of a statistically significant difference in isoprostane levels by smoking status, participants who reported never smoking had slightly lower mean isoprostane levels than those who reported smoking daily to weekly (50.33 pg/mL vs. 54.93 pg/mL). We observed expected trends in lipid peroxidation biomarkers. For example, obese women in BioCycle had higher mean isoprostane levels than normal-weight women, as expected (70.4 pg/mL (SD, 34.0) and 57.1 pg/mL (SD, 20.1), respectively) (52). Moreover, lipid peroxidation biomarkers in plasma may not represent oxidative damage in other tissues, thus limiting our ability to observe oxidative damage in other parts of the body. This is plausible, since mercury and cadmium are stored in the kidney and may exert localized effects.

We demonstrated that biomarkers of lipid peroxidation were not elevated in relation to increasing blood levels of cadmium, lead, and mercury at the low exposure levels experienced by the general population. Our findings suggest that isoprostane may decrease in relation to increasing levels of mercury, although this finding may be explained by measurement error or by indirect effects’ being stronger than the direct effect between mercury and isoprostane. To our knowledge, this was the first study to assess repeated measures of multiple biomarkers of lipid peroxidation in relation to low levels of metal exposure. Our data suggest that there is no association between low, environmentally relevant levels of cadmium, lead, and mercury and blood levels of TBARS, 9-HODE, and 13-HODE. However, in future studies, researchers may consider investigating these associations in other populations, as this was a highly selected, healthy population of premenopausal women, and little is known about potential effects of metals on oxidative stress biomarkers in older women or children.

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